EVALUATION OF THE EFFECT OF MARINATION PROCESSES ON THE QUALITY AND SAFETY OF PORK PRODUCTS

by

OLAMIDE TOLULOPE OYETUNJI

(Under the Direction of Karina Martino)

ABSTRACT

The first section of this study investigated how marination conditions including; tumbling time, presence/absence of vacuum and presence of 0.5% Cetylpyridinium chloride (CPC) affects quality characteristics of pork loin. Tumble time did not influence percent pick-up, cook yield or fat content. Absence of vacuum increased pick-up, with a 2.52% pick-up with vacuum compared to 5.76% without vacuum, at 15 min tumbling without CPC. Presence of vacuum improved cook yield. Cook yield was 85.10% and 79.25% with and without vacuum respectively, both at 15 min tumbling with CPC. The second section focused on the effects of post-injection tumbling and tumbling duration in the presence of a potassium lactate solution. At 0%, 1% and 2% (w/w) of Purasal Hipure P Plus® in final product, the growth of *Listeria monocytogenes* in ready-to-eat (RTE) marinated pork loin, cook yield and overall yield were significantly affected. Neither post-injection tumbling nor tumbling duration had an effect on growth of *L. monocytogenes,* cook yield or overall yield.

INDEX WORDS: marination, *Listeria monocytogenes*, tumbling, cook yield, pork loin

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DEDICATION

To my parents, who offered me love, support and encouragement to achieve this dream. To my beloved sisters, Adelugba Oluwaseun, Oyetunji Olufunmilayo and my sweetheart, Adeyemo Abimbola, who were always there to keep me going on, for their never-ending love and prayers.

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CHAPTER 1

INTRODUCTION

Marination is the process of incorporating salts, spices and herbs into foods such as meat, fish and shrimp to improve flavor and texture of the food product. Long before marination technology was implemented in the meat processing industry, it had been used in home cooking of different varieties.

Marination is a process that is thought to be applicable to muscle foods; however, its use has ranged from muscle foods to fishery products such as shrimps and oysters. Methods commonly used include immersion and soaking for many hours and rubbing (Alvarado and McKee, 2007). The immersion method is unreliable for the meat industry as it does not ensure uniformity in ingredient distribution and it requires long processing times. Marination has been employed for its ability to improve taste, tenderness, juiciness and palatability (Xargayo et al., 2001).

However, the benefits of marination has morphed from just being used for its palatability benefits to a wider range of applications in the meat processing industry including yield improvement as well as enhancing the safety of meat. There are different formulations used for marination, currently the principal/primary ingredients are phosphate, salt and water. Antimicrobial agents and flavoring are secondary ingredients and the level of concentration of each primary ingredient depends on the desired product. (Palang, 2004)

Just as benefits of marination have widened, so have the methods of application to enhance absorption and retention of marinade during processing. The efficiency of a marination application method is judged on the method's ability to give good absorption and more importantly retention of the marinade, thereby minimizing purge. The ability of marinade to

improve cook yield is also highly desirable. Marination technology has improved significantly from the simple immersion and soaking for long hours to more sophisticated methods such as tumbling and injection (Xargayo et al., 2001).

Injection and tumbling, as methods of marination, have been successful, and they are now a normal routine to meat processors. Xiong and Kupski (1999) have studied the effect of tumble time on marinade absorption and retention, cooking yield and other sensory properties of chicken fillets. Their results have shown that marinade absorption and retention is time-dependent with the highest uptake within the first five minutes of tumbling. As much as processors are interested in improving yield of marinated products, safety assurance of these products is also of great importance.

According to the Center for Disease Control and Prevention (CDC), an estimated 2,500 people in the United States become seriously ill with listeriosis each year with a total of 500 deaths. Food borne listeriosis occurs as a result of consuming food contaminated with *Listeria monocytogenes (L. monocytogenes)*. Symptoms of *L. monocytogenes* infection can be fatal in immuno-compromised subjects such as the aged, infants and ill patients; *L. monocytogenes* is also known to be a hazard to pregnant women as it may cross the placenta barrier leading to death of fetus, abortion or stillbirth (CDC, 2009). The 2008 CDC FoodNet data on the incidence of infection from foodborne pathogens in 10 states, reported that the percentage of persons hospitalized was highest in those aged 50 and above, with *Listeria* owning up to the highest share at 86.2% of this group (CDC, 2009). The CDC has also recommended that people at high risk of listeriosis should not to eat hot dogs, luncheon meats and RTE meat products without reheating until steaming hot. Immune-compromised individuals should also avoid handling other food items after handling hot dogs, luncheon meat and deli meats without proper hand washing.

Ready-to-eat (RTE) meat products are often incriminated in outbreaks of *L. monocytogenes* thereby making this organism a main concern for RTE meat processors. Glass and Doyle (1989) studied the fate of *L. monocytogenes* in processed meat products, and their findings showed that contamination of processed meat with *L. monocytogenes* after thermal processing is a potential hazard as significant growth was observed. Variations in growth occurred with sliced chicken, sliced turkey, ham, bologna, roast beef, wieners and bratwurst. *L. monocytogenes* contamination is a major concern in the ready-to-eat food industry, even though outbreaks of *L. monocytogenes* are not as frequent as some other pathogens, the severity of *L. monocytogenes* contamination is significant.

Most of the studies carried out on the effects of marination conditions on product quality and yield have been focused on poultry products. Since pork loins can be made into marinated ready-to-eat pork chops, the effect of processing variability of meat quality is of interest. Information on how marination variability affects the safety of pork loins is lacking. A scientific study to provide these information may help processors make informed decisions to improve the safety of their products, cut costs where necessary and optimize processing.

The overall goal of this study is to evaluate the effect of tumbling with and without vacuum, post-injection tumbling, presence of antimicrobial, and variation in tumbling duration on the quality and safety of ready-to-eat pork products. In order to fulfill this goal, the following objectives are proposed: 1) to evaluate the effect of tumbling and tumbling duration on quality aspects of marinated pork loin including percent pick-up, cook yield, color, pH and fat retention, with other variables such as presence/absence of vacuum and antimicrobial (CPC); 2) To assess the effect of post-injection tumbling and duration of tumbling on the growth of *L. monocytogenes* with variation in levels of antimicrobial; Purasal Hipure P Plus® (Purasal)

present in marinade; 3) To determine the effect of post-injection tumbling, duration of tumbling and presence of Purasal Hipure P Plus® on cooking yield, overall yield and percent moisture content of marinated pork loin.

REFERENCES

Alvarado, C., and McKee S. (2007). Marination to Improve Functional Properties and Safety of Poultry Meat. *J. Appl. Poult. Res.* 16(1): 113-120.

CDC website; Surveillance data. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5813a2.htm accessed October 2009.

Glass, K. A., and Doyle, M. P. (1989). Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol*. 55:1565–1569.

Palang, E. Y. (2004). The role of ingredients and processing conditions on marinade penetration, retention and color defects in cooked marinated chicken breast meat. Dissertation. Food Science Department. University of Georgia.

Xargayo M., Lagares, J., Fermandez, E., Ruiz, D., and Bornell, D.2001. Fresh meat spray marinating: The Influence of spray injection on the quality of marinated products. In; Alvarado, C. & McKee S. (2007). Marination to Improve Functional Properties and Safety of Poultry Meat. *J. Appl. Poult. Res.* 16(1): 113-120.

Xiong, Y. L., and D. R. Kupski (1999). Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. *Poult. Sci*. 78(7): 1053-1059.

CHAPTER 2

LITERATURE REVIEW

2.1. Marination Technology

Tumbling and injection methods are widely used in commercial marinade application. Tumbling subjects muscles and meat pieces to mechanical action or agitation in the presence of marinade (Hedrick et al., 1994). Tumbling causes meat samples to drop from the top to the bottom of the tumbler continuously as the tumbler is rotated. This dropping action is expected to cause some disruption in the muscle fibrils of the meat, due to mechanical (kinetic) actions (Xiong and Kupski, 1999a). Relatively, the mechanical action is a function of the quantity of a load within a particular drum size.

In tumbling, the size of the meat load relative to the size of the tumbler, the marinade to meat ratio, the length of marination cycle, the speed of the drum rotation, presence of vacuum and temperature are important parameters. Marinade uptake is faster with the mechanical action of tumbling when compared to a static soaking process. The presence of vacuum during tumbling is essential to allow for penetration of the marinade into the interstitial spaces of the meat more easily, causing the marinade to diffuse faster into the internal part of the meat (Palang, 2004).

The main structural component of meat is the myofibril which comprises of about 70% of volume of lean meat, myofibrils are about 20% protein and the rest is water (Offer and Trinick, 1983). The most important myofibril proteins associated with meat quality characteristics and water binding are actin (thin filament) and myosin (thick filament) and the combined structure, actomyosin. Anything that influences the spacing between the two filaments or the ability of the

proteins to bind water such as tumbling can affect the water holding properties of the meat (Alvarado and McKee, 2007).

Tumbling in the presence of phosphates improve water retention by shifting the ultimate pH further away from the isoelectric point of myofibrillar proteins and by unfolding muscle proteins, thereby exposing more charged sites for water binding (Alvarado and McKee, 2007). Tumbling improves meat yields because of improved water binding capacity. These treated products also are more tender because of the hydrated state of proteins and loss of structural integrity. The loss of normal muscle fiber structure and destruction of cellular barriers facilitates thorough and rapid distribution of marinade, thereby improving uniform color development and palatability (Hedrick et al., 1994).

Multi-needle injection delivers the marinade solution into the meat under pressure when the array of needles comes in contact with the surface of the meat. This delivery process disrupts the muscle fiber. It also causes a more even distribution and penetration of marinade because the marinade is injected directly into the muscle fibers. Injection methods give the benefit of injecting specified/desired amount of marinade into the meat. However, the speed of the conveyor belt may influence the amount of marinade injected into the meat.

Both injection and tumbling have an effect on the rheological property of the meat. Some processors employ both methods simultaneously to ensure even distribution of marinade while some use one or the other. When both methods are combined, the injection delivers the marinade into the meat while the tumbling helps distribute the marinade evenly through mechanical action which also helps in enhancing interaction of the salt soluble protein and marinade components. The combination of injection and tumbling may improve water holding capacity and cook yield due to an increase in protein and marinade interactions (Palang, 2004). According to Hullberg

and others, (2005), tumbled pork loin has lower shear force values leading to increased tenderness compared to non-tumbled pork loin.

2.1.1 Marinade Absorption and Retention

Absorption and retention of marinade in muscle food is an important aspect of marination. There are certain factors which affect the level of marinade absorption and most importantly retention in muscle foods. These include; salt and ingredient functionality, post-injection tumbling and presence/absence of vacuum during tumbling.

(a) Salts and Ingredient Functionality

The most common ingredients (generally referred to as primary ingredients) added to water to make marinades are salt (Sodium chloride) and phosphates. However, other ingredients (secondary) added include antimicrobials, flavorings, and starch to improve shelf life, taste/flavor and water holding capacity in the meat (Palang and Toledo, 2001). Sodium chloride (NaCl) helps increase moisture retention in marinated meat. Salt easily dissolves in water, therefore the ionic strength of the water increases. Ionic strength in muscle tissue fluid is lower than that of brine, and through the process of osmosis, the brine solution will be absorbed by the meat until a state of equilibrium is reached (Alvarado and McKee, 2007). Since too much salt will affect palatability, there is a limit to how much salt can be added to brine, salt levels can range from 1.5% to 3.0% depending on the product (Alvarado and McKee, 2007). However, considering restrictions on dietary intake of salt, phosphates and other high ionic strength compounds can act synergistically with salt to increase the water holding capacity while maintaining low sodium levels in meat.

Salt acts by unfolding myofibrillar proteins (actin and myosin), thereby exposing charged binding sites where water can be bound. Furthermore, the spaces between the actin and myosin

filaments are increased, thereby increasing the amount of water retained by the muscle (Alvarado and McKee, 2007). Similarly to salt's ability to expose charged binding sites, phosphates improve water retention by shifting pH further away from the isoelectric point of the myofibrillar proteins and by additional unfolding of muscle proteins thereby exposing more charged binding sites (Pearson and Gilley, 1996).

Alkaline phosphates, di and trisodium phosphates and tetrasodium phosphates are more likely to be used in marinade solutions to maximize water holding capacity (Alvarado and McKee, 2007). Phosphates also improve the oxidative stability of meat (Ang and Young 1987). A common salt and phosphate combination levels used in industry marinade composition is 1.5% NaCl and 0.45% STPP. Phosphate concentration is regulated by the United States Department of Agriculture (USDA) with a maximum percentage in finished product as 0.5% (9 CFR 318). As mentioned earlier, protein solubility of myofibrils increases with salt, however the protein solubility can be significantly improved by combining phosphates at low concentrations (Palang, 2004).

(b) Effect of Tumbling

Tumbling meat has often been associated with injection of brine into the meat. A major benefit of tumbling is that it extracts salt-soluble proteins from muscle fibers to form a binding agent. Extraction of the salt soluble proteins, actin and myosin, promotes cohesion during cooking, juiciness and improved slicing characteristics (Babji et al., 1982). The extraction of salt soluble proteins to the meat surface serves two main purposes; 1) coagulation of protein upon heating to improve binding properties; 2) acting as a sealer during cooking to enhance retention of moisture contained in the meat tissues (Rust and Olson, 1973). Xiong and Kupski (1999) have conducted several studies on the effect of tumbling on marinade absorption in poultry products

amongst many others. However, there is minimal information on the effect of post-injection tumbling of pork products and the effect of duration of tumbling on marinade absorption and retention.

(c) Presence/absence of Vacuum

Another factor affecting marinade absorption and retention is the presence/absence of vacuum during tumbling. Studying the effect of vacuum during tumbling on moisture absorption and retention in marinated broiler fillets, Young and Smith (2004), reported that the use of vacuum during marination increased moisture absorption, but after cooking, yields were similar to non-vacuum tumbled broiler fillets. Duration of tumbling is another factor affecting moisture absorption and retention. Xiong and Kupski (1999b), observed that marinade absorption rate was greatest in the first 5 min of tumbling. They explained that time-dependent marinade absorption during the initial 30 min of tumbling indicated that water entry into the muscle is a kinetic process.

However, duration of tumbling is dependent on muscle type and the percentage marinade absorption desired, nevertheless, tumbling time is limited by the adverse effect of excessive tumbling on the texture of the meat. The marinade must overcome physical barriers and constraints in muscle, (such as actomyosin cross-linkages), in order to diffuse into the fibers and myofibril matrices. The kinetic energy input, together with abrasion causes significant fiber disruption. In their study, initial high marinade gradient between muscle surface and inside tissue probably contributes to the fast absorption observed in the first 5 min (Xiong and Kupski, 1999b). Varying injection pressures can also affect absorption and retention. Increased injection pressure of brine solution increased overall yield and decreased shear value of injected loins (Detienne et al., 2001).

2.2 Listeriosis and *Listeria monocytogenes*

Listeriosis is a dangerous infection contacted by eating food items contaminated with the bacterium *L. monocytogenes*. Amongst the six species currently recognized within the genus *Listeria*, *L. monocytogenes* is the most important human pathogen, although some others have occasionally been associated with human illness. *L. monocytogenes* is a gram-positive, rod-shaped, catalase-positive, oxidase-negative, facultative anaerobe, non-spore forming bacterium. *L. monocytogenes* grows in a wide range of temperature between 0 – 42^{o}C with an optimum between 30 and 35^{o}C. Growth below 5^{o}C is slow and has extended lag and generation times (Ryser and Marth, 2007; CDC, 2009)

L. monocytogenes is a very difficult organism to control, it is ubiquitous in the environment and can be commonly found in soil and water, in and around food plants and in homes. Its prolonged survival in the environment has been demonstrated in a study where level of *L. monocytogenes* in sewage sludge sprayed on to agricultural land remained for more than 8 weeks (Adams and Moss, 2008). Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats, poultry and dairy products (CDC, 2009)

It has been recognized as an important public health concern in the United States. Aged people, pregnant women, newborns and adults who are immune-compromised are at higher risk of listeriosis. Listeriosis induces fever, muscle aches and sometimes gastrointestinal disorders including nausea and diarrhea. The organism has the capability of spreading to the nervous system. When infected, patients exhibit headaches, stiff neck, confusion, loss of balance and convulsions. This microorganism has the ability to cross the placenta barrier and when this occurs, infected pregnant women can have miscarriages, stillbirth, premature delivery or systemic infection of the newborn. (CDC, 2009)

According to the CDC, an estimated 2500 persons become seriously ill with listeriosis each year with 500 deaths. Pregnant women are about 20 times more prone to infection compared to healthy adults, and the newborn rather than the pregnant women suffer the serious effects of infection during pregnancy. Individuals with cancer, diabetes, kidney disease, those who take glucocorticosteroid medications and the elderly are also at increased risk while persons with HIV/AIDS are 300 times more susceptible to contacting listeriosis than people with normal immune system (CDC, 2009). *L. monocytogenes* has been found in raw foods such as uncooked meats and vegetables as well as in processed foods that become contaminated after processing, such as soft cheeses, ready-to-eat meat products or cold cuts at the deli counter. Even though *L. monocytogenes* is easily killed by cooking, in certain ready-to-eat foods such as hot dogs and deli meats, contamination may occur after cooking and before or during packaging. There is a need to focus on foods frequently implicated in outbreaks and illnesses such as RTE products..

Eradication of *L. monocytogenes* from processing environments and finished products has not been possible, as this organism is continuously reintroduced into the plant environment, but its reduction has been achievable through extensive efforts (Bernard and Scott, 1999). Even though the incidence of listeriosis is low, there is a need to investigate new strategies to control it in ready-to-eat foods as they are commonly incriminated and the results are severe and fatal. A well known multistate (n=24) outbreak in 1998/1999 involved luncheon meats and hot dogs, both multistate outbreaks in 2000 and in 2002 (n=11 and n=9 respectively) involved Delicatessen turkey meat (Ryser and Marth, 2007). A more recent outbreak occurred in 2008, this involved RTE products from Maple Leaf causing 22 deaths. Since mid 1980s, the meat industry has been actively seeking and implementing control strategies for *L. monocytogenes.*

Outbreaks of *L monocytogenes* are not common, case control studies by the US surveillance program has shown that most listeriosis infections occur without a clear connection to an outbreak and the cases were considered to be sporadic. This implies that even though no outbreak is been reported, infections do occur and its severity is a cause for concern. The ubiquity of *L. monocytogenes* in the environment suggests that human exposure to this organism is frequent, even though incidence is low, because invasive infection will only occur if a susceptible individual is exposed to a high dose of a virulent strain. Estimates of minimum infective dose are usually difficult to establish and so is the case with *L. monocytogenes.* The infective dose is thought to be relatively high since foods implicated in outbreak have been found to contain numbers in excess of 10^3 cfu/g (Adams and Moss, 2008).

The psychrotrophic nature of *L. monocytogenes* which enables its growth at low storage temperature makes it a nuisance. Post-processing contamination is probably low but can grow to a high level through the course of the storage and display. Beumer et al., (1996) and Glass and Doyle, (1989) have demonstrated the considerable growth of *L. monocytogenes* on sliced cooked meat products over 6 weeks shelf-life, and the fate of *L. monocytogenes* in ready-to-eat meat products in refrigerated storage respectively. Within the shelf-life of the investigated products low initial numbers of *L. monocytogenes* increased up to 10^8 cfu/g (Beumer et al., 1996). *L. monocytogenes* growth varies amongst different RTE products, while a $10^3 – 10^4$ cfu/g increase was observed on ham, bologna and bratwurst, a $10^3 – 10^5$ cfu/g increase was observed on sliced chicken and turkey (Glass and Doyle, 1989)

2.3 RTE Meat Products and Listeriosis

Luncheon meats are very popular in deli sales, a perfect choice for time conscious consumers as they have already been prepared and processed by the manufacturer and therefore require no cooking before consumption (US Deli Knowledge, 2009).

Despite stringent hygienic conditions employed during processing, it is almost impossible to avoid minor contamination in cooked meat products especially during the finishing, handling and slicing stages at the deli. Considering the storage conditions, there is a limit to the types of microorganisms capable of growing on ready-to-eat products. RTE products are generally vacuum packaged providing an anaerobic environment and stored at temperature below 7^{o}C. This storage condition encourages growth of specific organisms such as Lactic acid bacteria (LAB). Pathogens such as *L. monocytogenes* are capable of growth at refrigerated temperatures, they are also facultative anaerobes and will therefore grow well in this storage conditions. As mentioned earlier, *L. monocytogenes* is usually in low numbers after packaging (due to post-cooking contamination), but can increase to a significant level during storage which poses a health hazard. The growth of LAB and *L. monocytogenes* is largely determined by water activity and storage temperature. Water activity is dependent mainly on amount of salt added to the meat.

2.3.1. Intervention strategies for *Listeria monocytogenes* in meat products

Stekelenburg and Kant-Muermans (2001), observed inhibition of growth of *L. monocytogenes* by organic acid additives in cooked hams over a 40-day storage period. Their method of intervention was by injection of different compositions of sodium lactate, sodium diacetate, or buffered sodium citrate in the raw ham, which was then tumbled before cooking. The product was subjected to a challenge test by inoculating the product with 10^2 cfu/g *L. monocytogenes.* They observed that sodium lactate and sodium diacetate was able to reduce

growth of *L. monocytogenes* to an undetectable level. Lagan (2007), also studied shelf-life extension of ham products using LAB culture (otherwise known as bio-preservatives) and observed a 39-day shelf life.

Antimicrobials have been used to control growth of microorganisms in RTE meat products. Lim and Mustapha 2007, studied inhibition of *L. monocytogenes* on sliced roast beef using CPC and acidified sodium chlorite (ASC). This was done by applying solutions of these antimicrobials through spraying on the beef slices and on absorbent pads on which the RTE meat slices were placed and then sealed. Their results showed that 0.5% CPC reduced *L. monocytogenes* to an undetectable level through the storage period and concluded that CPC application could extend the shelf-life of sliced roasted beef.

2.3.2. Control of *Listeria monocytogenes* by Marination with Organic Acids

Typical marinades utilized for their antimicrobial properties include sodium lactate, potassium lactate, sodium citrate, sodium lactate combined with sodium diacetate, and combinations of sodium lactate with potassium lactate and diacetate (Alvarado and McKee, 2007). Sodium diacetate is a mixture of acetic acid and sodium acetate. Several researchers have noted that in culture media, acetic acid has more potent antilisterial effects than lactic acid which in turn is more inhibitory than hydrochloric acid (Doyle, 1999). Many poultry processors are using organic acids in marinades to control the growth of *L. monocytogenes* in the final cooked deli loaf but with no validation of these applications. Carroll et al., (2007) carried out a study using commonly used marinade ingredients (sodium tripolyphosphates (STPP), sodium lactate, sodium diacetate, and sodium citrate either alone or combined) to determine the effects of these organic acids on the growth of streptomycin-resistant *L. monocytogenes* (for selectivity amongst background microflora) and meat quality. They marinated turkey breast by vacuum tumbling and

discovered that these organic acids are capable of extending the lag phase of *L. monocytogenes*, however the acids may have negative effects on the quality and yield of turkey deli loaves including water holding capacity, marinade retention, cook loss, moisture content and bind ability. In addition, these acidic marinades have a tendency to cause off-flavors.

Doyle (1999) stated that sodium lactate and diacetate are thought to inhibit bacterial growth by extending the lag phase. Studies by Samelis et al., (2001) has showed that single use of organic acids provided inhibition of surface-inoculated (3 to 4 log cfu/cm^2) *L. monocytogenes* ranging widely from 20 to 70 days between treatments of vacuum-packaged frankfurters stored at 4^{o}C. Sodium lactate at 3% was the most effective and sodium acetate at 0.25% were the least effective and sodium diacetate at 0.25% was intermediate in anti-*L. monocytogenes* effectiveness.

The inhibitory effect of organic acids can be correlated with their dissociation constant (pK_a) and with the greater permeability of the cell membrane to weak acids in their undissociated form. Hydrochloric acid is completely dissociated in aqueous environments, meanwhile acetic acid (pK_a = 4.76) has the highest concentration of undissociated acid, and lactic acid (pK_a = 3.86) has the lowest. Acetic acid is more efficient against *L. monocytogenes* than a stronger hydrochloric acid used at the same pH. This can be explained by its ability to diffuse through cell membrane which is permeable to nondissociated, nonprotonated, and lipophilic weak acids. This leads to accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton-motive force and inhibition of substrate transport (Vausser et al., 1999; Alvarado and McKee, 2007). However, other studies have shown that an increase in anti-*L. monocytogenes* activity can be expected if the pH of the acetate or lactate containing system is

decreased even to a small extent such that it was closer to the pK_a of the acid (Buchanan et al., 1993).

Sodium lactate is often added to meat and poultry products, as an antimicrobial at levels of 2 to 3%, based on final weight. Sodium lactate has been used as a flavor enhancer in fresh and cooked meat and poultry products. Potassium lactate acts in a similar manner as sodium lactate but less preferred because of its slightly bitter taste (Weaver and Shelef, 1992). Shelef and Yang (1991) studied the effect of lactate salts on sterile strained chicken or beef and discovered that a concentration of either of the sodium or potassium salts suppressed growth of *L. monocytogenes* causing an extended lag phase of 1 to 2 weeks at 5^oC.

"PURASAL Hipure P Plus® is the potassium salt of natural L(+)-lactic acid, which is produced by fermentation from sugar. It has no taste impact on the end product, it possesses antimicrobial properties and is neutral by pH. PURASAL Hipure P Plus® is a clear liquid solution and is soluble in water. PURASAL Hipure P Plus® is a ultra pure food grade potassium-L-lactate with excellent organoleptic properties" (Purac, 2009). Purasal Hipure P Plus® is a commercially available organic acid salt of potassium lactate, as mentioned above. Mellefont and Ross (2007) has shown that Purasal Hipure P®, very similar in composition to Purasal Hipure P Plus®, is listeriostatic rather than listericidal. It is effective in reducing growth of indigenous microflora including aerobic microflora and lactic acid bacteria when 3% (wt/wt finished product) is present in modified atmosphere packaged sliced ham.

Given the following needs, a) finding new strategies to intervene on *L. monocytogenes* contamination in ready-to-eat meat products, b) the minimal information on effect of duration of tumbling of pork products on marinade retention, and c) the commercial use of Purasal Hipure P plus® by meat processing plants, coupled with the knowledge that marination incorporates

ingredients into meat, this work is designed to study how tumbling after injection of marinade (containing Purasal Hipure P Plus®), and variation in duration of tumbling effects anti-*L. monocytogenes* activity over storage at 4^{o}C for 8 weeks.

REFERENCES

Adams, M. R., and Moss, M. O. (2008). Food Microbiology (3rd ed.), The Royal society of Chemistry Publishing, Cambridge, United Kingdom.

Alvarado, C., and McKee S. (2007). Marination to improve functional properties and safety of poultry meat. *J. Appl. Poult. Res.* 16(1): 113-120.

Ang, C. Y. W., and Young L. L. (1987). Effect of marination with sodium pyrophosphate solution on oxidative stability of frozen cooked broiler leg meat. *Poult. Sci.* 66: 676-678.

Babji, A. S., Froning, G. W., and Ngoka, D. A. (1982). The effect of short-term tumbling and salting on the quality of turkey breast muscle. *Poult. Sci.* 61(2): 300-303.

Bernard, D. T., and Scott, V. N. (1999). *Listeria monocytogenes* in meats: New strategies are needed. *Food Tech.* 53(3): 124.

Beumer, R. R., te Giffel, M. C., de Boer, E., and Rombouts, F. M. (1996). Growth of *Listeria monocytogenes* on sliced cooked meat products. *Food Microbiol*. 13:333–340.

Buchanan, R. L., Golden, M. H., and Whiting, R. C. (1993). Differentiation of the effects of pH and lactic or acetic-acid concentration on the kinetics of *Listeria monocytogenes* inactivation. *J. Food Prot.* 56(6): 474

Carroll, C. D., Alvarado, C. Z., Brashears, M. M., Thompson, L. D., and Boyce, J. (2007). Marination of turkey breast fillets to control the growth of *Listeria monocytogenes* and improve meat quality in deli loaves. *Poult. Sci.* 86(1): 150-155.

CDC – Center for Disease Control and Prevention website. Last modified March, 2008 http://www.cdc.gov/nczved/dfbmd/disease_listing/listeriosis_gi.html Accessed October, 2009.

Detienne, N. A., Reynolds, A. E., and Wicker, L., (2003). Phosphate marination of pork loins at high and low injection pressures. *J. Food Quality* 26(1): 1-14.

Doyle, M . E. (1999). Subject: Literature review of various techniques used in *Listeria* intervention. www.wisc.edu/fri/briefs.htm accessed October 2009.

Glass, K. A., and Doyle, M. P. (1989). Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol*. 55:1565–1569.

Hedrick, H. B., Aberle, E. D., Forrest, J. C., Judge M. D., and Merkel, R.A. (1994). Principles of meat science (3rd ed.), Kendall/Hunt Publishing Company, Dubuque, IA.

Hullberg, A., Johansson, L., and Kerstin, L. (2005). Effect of tumbling and RN genotype on sensory perception of cured-smoked pork loin. *Meat Sci.* 69(4): 721-732.

Lagan R, (2007). Cooked ham with a 39 day shelf-life. College of Agriculture, Food and Rural Enterprise, Loughry Campus.

Lim, K., and Mustapha, A. (2007). Inhibition of *E. coli* O157:H7, *L. monocytogenes* and *Staphylococcus aureus* on sliced roast beef by cetylpyridinium chloride and acidified sodium chlorite. *Food Microbiol*. 24: 89-94.

Mellefont, L. A., and Ross T. (2007). Effect of potassium lactate and a potassium lactate-sodium diacetate blend on *Listeria monocytogenes* growth in modified atmosphere packaged sliced ham. *J. Food. Prot.* 70(10): 2297-2305.

Offer, G., and Trinick J. (1983). On the mechanism of water holding in meat - the swelling and shrinking of myofibrils. *Meat Sci.* 8(4): 245-281.

Palang, E. Y. (2004). The role of ingredients and processing conditions on marinade penetration, retention and color defects in cooked marinated chicken breast meat. PhD Dissertation. Food Science department. University of Georgia.

Palang E. Y., Toledo R. T. (2001). Optimizing combinations of chemical and biological microbial inhibitors in inhibiting psychotropic spoilage organisms in raw chicken meat in Palang E. Y. (2004). Reference No 19.

Pearson A.M., and Gillet, T.A. (1996). Processed meats, 3rd ed. Chapman & Hall, New York.

Purac America, www.purac.com

Rust, R. E., and Olson, P. G. (1973) Meat curing principles and modern practice. In: Babji, A. S., Froning, G. W. and Ngoka, D. A. (1982). The effect of short-term tumbling and salting on the quality of turkey breast muscle. *Poult. Sci*. 61(2): 300-303.

Ryser, E. T., and Marth, E. H. (2007) *Listeria*, listeriosis and food safety, 3rd edition. CRC Press, Boca Raton.

Shelef, L. A., and Yang Q. (1991). Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken, and beef. *J. Food. Prot.* 54(4): 283-287.

Stekelenburg, F. K., and Kant-Muermans M. L. T. (2001). Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int. J. Food Microbiol.* 66(3): 197-203.

U.S. Deli Product Knowledge. Accessed September, 2009. http://www.usdec.org/files/Deli/PDFs/3_US_Deli%20Meats.pdf

Vasseur, C., Baverel, L., Hebraud, M., and Labadie, J. (1999). Effect of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. *J. Appl. Microbiol.* 86(3): 469-476.

Weaver, R. A., and Shelef L. A. (1993). Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. *J. Food Safety* 13(2): 133-146.

Xiong, Y. L., and Kupski D. R. (1999a). Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. *Poult. Sci.* 78(7): 1048-1052.

Xiong, Y. L., and Kupski D. R. (1999b). Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. *Poult. Sci*. 78(7): 1053-1059.

Young, L. L., and Smith, D. P. (2004). Effect of vacuum on moisture absorption and retention on marinated broiler fillets. *Poult. Sci*. 83: 128-131.

CHAPTER 3

EVALUATION OF THE EFFECT OF TUMBLING DURATION AND VACUUM DURING TUMBLING ON QUALITY ASPECTS AND COOK YIELD OF PORK LOIN MARINATED WITH CETYLPYRIDINIUM CHLORIDE

ABSTRACT

In marination, the fundamentals of processing conditions, and the effect of ingredients on the final product quality are often not well known or understood by the industry. Quantification of quality changes in pork loin marinated with CPC as an antimicrobial, and comparison of quality changes based on marination processing variables were evaluated in this study. Pork loin cuts were marinated with 1.5% NaCl, 0.45% STPP , and with or without 0.5% CPC. Marination was conducted by tumbling with and without vacuum for 15, 30, 45 and 60 min separately. Presence of vacuum improved cook yield. Cook yield with and without vacuum was 85.10% and 79.25% respectively, both at 15 min tumbling in the presence of CPC. pH and color were measured before and after marination, and fat content analysis was done using a modified AOAC 1995 method. Weight gain and cook yield was determined. Meat marinated with NaCl, STPP and CPC by tumbling without vacuum for 45 min had the highest pH of 6.44, a 21.5% increase from the pH 5.3 of the fresh pork. L*, a* and b* values showed an increase in darkness (L*), slight decrease in redness (a*) in most cases and no significant difference in yellow/blue color appearance (b*). Fat analysis showed that the pork loin marinated with CPC had higher percentage fat content than those marinated with NaCl and STPP alone. The antimicrobial did not influence cook yield. Cook yield for NaCl and STPP marinated loins ranged from 73.9 to 85.65%. All statistical analysis were performed at a significance level of 0.05.

Keywords: Pork loin, quality, cook yield, color, vacuum

Practical Application: The meat processing industry employs marination as a means of enhancing quality and improving yield of products. Most of these industries however, do not have a scientific basis for their application process. Therefore, there is a possibility of over-processing or under-processing. Sometimes processors buy equipment and use it based on supplier's specifications or recommendations. However, this might not be the best processing conditions for specific product types. The objective of this study was to quantify changes in pork quality. One batch was marinated with 1.5% NaCl and 0.45% STPP, and another batch included 0.5% CPC as an antimicrobial, by tumbling with varying tumbling duration (15, 30, 45 and 60 min) in the presence and absence of vacuum. Quality determinants such as color, pH and fat content and cook yield were studied. The results of this study may provide the pork industry a scientific basis to design better marination process or to optimize their existing marination procedure.

3.1 INTRODUCTION

Marination is the process of incorporating salts, spices and herbs in to foods such as meat, fish and shrimp to improve flavor and texture of the food product. Marination technology is now a regular practice in the meat and poultry industry. It is desirable for its ability to improve taste, tenderness, juiciness and palatability. However, the benefits of marination have also included yield improvement and safety enhancement.

Tumbling and injection methods are widely used in marinade application in the meats industry. Tumbling subjects muscles and meat pieces to mechanical action or agitation in the presence of marinade (Hedrick et al., 1993). It causes meat samples to drop from the top to the bottom of the tumbler continuously as the drum is rotated. This dropping action is expected to cause some disruption in the muscle fibrils of the meat due to the mechanical (kinetic) actions

applied (Xiong and Kupski, 1999). The most important myofibril proteins associated with meat quality characteristics and water binding are actin (thin filament) and myosin (thick filament) and their combined structure actomyosin. Anything that influences the spacing between the two filaments or the ability of the proteins to bind water can affect the water holding properties of the meat (Alvarado and McKee, 2007).

Furthermore, tumbling in the presence of phosphates improves water retention by shifting pH away from the isoelectric point of myofibrillar proteins and by unfolding muscle proteins, thereby exposing more charged sites for water binding (Alvarado and McKee, 2007). Tumbling improves product yields because of improved water binding capacity. These treated products are more tender because of the hydrated state of proteins and loss of structural integrity. The loss of normal muscle fiber structure and destruction of cellular barriers facilitates thorough and rapid distribution of marinade, thereby improving uniformity of color development and palatability (Hedrick et al., 1993).

Another factor affecting marinade absorption and retention is the presence/absence of vacuum during tumbling. Young and Smith, (2004) studied the effect of vacuum during tumbling on the moisture absorption and retention in marinated broiler fillets and found that use of vacuum during marination increases moisture absorption, but after cooking, yields were similar. However, there is a need to investigate if the same applies with pork loins. Duration of tumbling is another factor affecting moisture absorption and retention. Xiong and Kupski (1999), explained that time dependent marinade absorption during the initial 30 minutes of tumbling indicated that water entry into the muscle is a kinetic process. The marinade must overcome physical barriers and constraints in the muscle, (such as actomyosin cross-linkages), in order to diffuse into the fibers and myofibril matrices. The kinetic energy input, together with abrasion

causes significant fiber disruption. An initial high marinade gradient between the muscle surface and inside tissue probably contributes to the fast absorption in the first 5 min (Xiong and Kupski, 1999).

Antimicrobials as marination ingredients have been studied to an extent. Tan and Ockerman (2006) studied applicability of nisin and tumbling in improving microbiological quality of marinated chicken drumsticks, and their results showed that adding nisin with tumbling decreased total microflora and psychrotroph counts of marinated chicken drumsticks. Carroll et al., (2007) also showed that marination in the presence of sodium diacetate, citrate and lactate/diacetate extended lag phase of *L. monocytogenes* on marinated turkey breast fillets. CPC has been shown to be an effective antimicrobial (Lim and Mustapha, 2007; Pohlman et al., 2002). Even though CPC is FDA-approved for decontaminating raw poultry carcass (21 CFR 173.375), it is not yet approved as an additive in meat or poultry.

Quality is of importance to meat processors; however, there is still basic information, such as interaction of marination factors and specific ingredients, which are not well understood. The objective of this study was to determine how tumbling duration, presence or absence of vacuum, as well as the use of CPC as an antimicrobial, affects the quality of marinated pork loin.

3.2 MATERIALS AND METHODS

3.2.1 Marination and Product Preparation

A total of 35 kg of pork loins were purchased from a large local meat retailer, with each pork loin piece weighing approximately 4 kg. Eight loins were cut into 13 pieces of 300g. One of each group of 13 was set aside as fresh treatment used for pH, color and fat measurement, while the remaining 12 were put into four treatments (each treatment with 3 pieces). The four treatments are as follows:

1. Vacuum tumbled with CPC
2. Vacuum tumbled without CPC
3. Tumbled without vacuum with CPC
4. Tumbled without vacuum without CPC.

The initial eight pieces of loins were thus used in replicates for all the treatments above with 15, 30, 45 and 60 min of tumbling, meaning two loins (replicates) for each time variable.

Brines used for this study had two different composition: one prepared to deliver 1.5% NaCl (Mallinckrodt Baker Inc., Phillipsburg, NJ), 0.45% STPP (Sigma-Aldrich, St Louis, MO) in the meat, and another with the same level of NaCl and STPP in addition to 0.5% CPC (Spectrum Chemical, Gardena CA). The former will be referred to as without CPC and the latter as with CPC.

Each sub-portions of 3 pieces explained earlier were tumbled in a vacuum tumbler (U-mec, Los Angeles, CA) for 15, 30, 45 and 60 min in a vacuum/no vacuum, with CPC/ without CPC combinations of treatments as shown in Table 3.1. All marinated loins were allowed to equilibrate overnight in a 2^{o}C cooler.

Pick-up percentage (marinade absorption) for each treatment was calculated as shown in Equation 3.1 before samples were pulled for pH and color measurements for each treatment and remaining samples cooked.

$$\%\ \text{Pick} - \text{up} = \frac{\text{marinated weight} - \text{green weight}}{\text{green weight}} \times 100 \quad \text{..... Equation 3.1}$$

3.2.2 Quality Analysis

(a) Cook yield

After overnight equilibration, loins pieces were weighed and placed onto metal racks, and cooked in a smokehouse (Alkar, Lodi, WI) programmed to cook the loin pieces to an internal temperature of 73.9^{o}C. After cooking was completed, the cooked meat was moved into a 4^{o}C room until cool. Weights of the loins were then measured and recorded to calculate cook yield as (Equation 3.2);

$$\text{Cook yield} = \frac{\text{cooked weight} - \text{uncooked weight}}{\text{uncooked weight}} \times 100 \quad \text{..... Equation 3.2}$$

(b) pH, Color measurement and Fat Analysis

pH values of fresh loins and marinated loins were measured using IQ 240 pH meter (IQ Scientific Instruments Inc, San Diego, CA), equipped with a piercing probe, by averaging two piercings. Color values L*, a*, b* of fresh and marinated loins were also measured using a colorimeter (Konica Minolta Chroma Meter CR-410, Minolta Corp, Ramsey, NJ) by averaging 2 readings taken at 90^{o} of each other.

Fat content of the *longissimus dorsi* (center cut pork loin) before marination and after marination was measured to quantitatively assess the effect of the marination treatments on total lipid concentration. This was done using soxhlet method of fat extraction. Approximately 3g of loin sample (anterior section) was weighed and placed in a cellulose thimble (Whatman Inc., Piscataway, NJ), 5g of sea sand (Fisher Scientific, Fair Lawn, NJ) was added. Defatted cotton was placed on top and the extraction thimble was placed in an oven for drying at 125^{o}C for 1h. Weight of the entire extraction thimble was recorded. A soxhlet extraction unit was set up and the thimble was placed into it. Extraction was carried out with petroleum ether (Mallinckrodt Baker Inc., Phillipsburg, NJ) following the soxhlet extraction method (continuous self siphoning

method). After 3 hours of extraction, the thimble and contents were dried at 125^{o}C for 30 min, cooled in a desiccator, and then weighed again to determine fat content. Percent fat content was calculated as shown in equation 3.3

$$\% \text{ Fat content} = [(B - C) \times 100] \div A \quad \text{Equation 3.3}$$

Where A – weight of meat sample

B – weight of extraction thimble and contents before extraction

C – weight of extraction thimble and contents after extraction

3.2.3 Statistical Analysis

Results of these experiments were analyzed using JMP 7 (SAS Institute Inc., Cary, NC), specifically employing Analysis of variance tests, student's t-test and Dunnett's test to compare between treatments for significant differences. For all significance tests, $\alpha = 0.05$ was used.

3.3 RESULTS AND DISCUSSION

3.3.1 Pick-up and Cook Yield

When pick-up percentage (Fig 3.1) was compared within time treatments, for example 60V NCPC and 60NV NCPC, those tumbled without vacuum have higher pick-up, in this paired example, 60NV NCPC had a higher pick-up. An interpretation of all treatments is shown in Table 3.1. Treatments 3 and 9 were significantly different from treatment 12, 14 and 16. There was no significant difference within the other eleven treatments.

Fitting a three-way full factorial model, the effect of tumbling duration, presence or absence of vacuum and presence or absence of CPC, as well as the interaction of these three factors on percent pick-up and cook yield was investigated (Table 3.2). From these results, it can be highlighted that vacuum had a negative effect on percent pick-up but a positive effect on cook yield. Tumble time was not a significant factor contributing to either pick-up or cook yield

($p>0.05$). Presence of CPC had a significant negative effect on cook yield, however, it did not have a significant effect on percent pick-up ($p>0.05$).

Xiong and Kupski (1999) reported that marinade pick-up in chicken filets was time dependent increasing relatively with 5, 15 and 30 min tumbling, they also reported that marinade uptake was the fastest in the first 5 min of tumbling. Tumbling time ranged in this study from 15 min to 60 min, also the muscles used in this study were pork loin compared to chicken fillets in the previous study. This difference in muscle type and tumble time range may influence differences in results in this study when compared to the results in Xiong and Kupski's (1999). However, non-significant differences in pick-up may suggest that the first fifteen minutes is the main uptake window and beyond this time frame, uptake may increase but not significantly. Water entry into muscle is a kinetic process; the time dependence of marinade absorption in the initial 30 min of tumbling indicates that marinade must overcome physical barriers or constraints in muscle (Xiong and Kupski, 1999).

Percent pick-up varied with absence or presence of vacuum (Fig 3.1), in the presence of vacuum, percent pick-up ranged from 2.39 to 5.17 and from 4.54 to 6.75 in the absence of vacuum. There was no significant difference ($p>0.05$) in percent pick-up in all treatments, except for treatment 2 which had a pick-up that was significantly different from other treatments. There was no significant difference ($p>0.05$) in cook yield, only 15NV CPC was significantly different from the other treatments. Since cook yield is an indication of cook loss and in turn and indication of water holding capacity of the marinated loins (Carroll et al., 2007), observed cook yield ranging from 79.25 to 85.65 gives an idea of moisture lost during cooking. This did not vary significantly between treatments. These results suggest that when pork loin is tumbled during marination, presence or absence of vacuum will be more important to processors that sell

uncooked products since absorption is favored in the absence of vacuum. However, presence of vacuum will be important to processors aiming to sell cooked products since retention is favored. A negative significant effect observed when tumble time interacts with vacuum (Table 3.2) suggests that if a longer tumble time should be employed, absence of vacuum may be more favorable for a better cook yield.

3.3.2 pH, Color measurement and Fat Analysis

L* values (Table 3.3) of marinated loins were neither significantly different from the fresh loins nor from each other (comparing all the treatments). In marinated loins, a* values also had no significant difference compared to the fresh loins. There was no significant difference in b* values of all treatments ($p>0.05$), and no significant difference between marinated loins and fresh loins. These results suggest that the treatments used in this study can be used without affecting the freshness appearance of the product to consumers.

However, within the treatments, tumble time and presence of vacuum have a significant effect on L*, as seen in Table 3.2. Tumble time is significant in increasing L* values thereby making the meat lighter, this in other words means as tumble time increases, product becomes pale. Presence of vacuum as a main effect is significant in yielding a lower L* value, suggesting that pale marinated loins can be avoided in the presence of vacuum as compared to in the absence of vacuum. The interaction of these two (tumble and vacuum) is also significant in L* values (Table 3.2), and this time, it has a positive effect which suggests that tumbling for a longer period in the presence of vacuum will yield a slightly pale meat. Presence of vacuum also has a significant positive effect ($p<0.05$) on both a* and b* values (Table 3.3). This means presence of vacuum will cause the loin color tending towards more redness than green (more + a*), and more yellow than blue (more + b*). Greenish appearance in loin is undesirable.

Comparing between pairs of loins with the same vacuum and tumbling time, but absence or presence of CPC, presence of CPC does not have a significant effect ($p>0.05$) on retention of fat in marinated loins (Table 3.3). Presence of vacuum and tumble time as main effects does not have a significant effect ($p>0.05$) on percent fat reduction (Table 3.2). A significant difference ($p<0.05$) was observed between loins tumbled for 15 min and 60 min (Table 3.3). Both were in the presence of vacuum and CPC. The loins tumbled for 60 min had a significantly lower fat reduction (higher fat retention) than loins tumbled 15 min.

Fitting a three-way full factorial model for interaction effect, tumble time and vacuum interacts significantly ($p<0.05$) to reduce percent fat reduction (increased fat retention) (Table 3.3). The reduction in percent fat content after marination suggests increased intramuscular fat marbling. However, there is a possibility that fat contents are extracted in brine during tumbling, and purge during tumbling may contain some fat residue. Further research needs to be carried out on this aspect. Fat content is important to RTE meat processors since there are some labeling classifications based on the percentage fat present in the meat and nutrient content claims also includes fat content.

pH changes show that pH after marination were generally around 5.7 to 6.2. In all replicates, loins with the highest pH after marination were those tumbled for 45 min in the presence of CPC, and were not significantly different ($\alpha = 0.05$) from pH of the fresh loins. The highest in pH value observed was 6.44 at 45NV CPC in one replicate. The pH of the muscle of live animal was usually just above pH 7, this may decrease after slaughter to pH 5.4 to 5.7 in normal meat. Low pH causes the meat to become pale, soft and exudative (PSE) while too high pH may cause dark, firm and dry (DFD) meat (Swatland, 2002). Aside low pH causing PSE which is highly undesirable in meat, pH also influences water holding capacity (WHC).

Decreased WHC occurs when actin and myosin are near their isoelectric point which is approximately 5.1. Also when meat conditions such as PSE exist, other quality characteristics are compromised due to extensive protein denaturation (Alvarado and McKee, 2007). The pH range observed in this study was not lower than 5.7 which shows that CPC in the brine does not make the brine acidic. pH values of vacuum tumbled loins were not significantly different ($p>0.05$) from those tumbled without vacuum, Young and Smith (2004) observed similar results. These results suggest that whether or not vacuum is present, pH is not affected which is important for quality of marinated pork. None of the factors either as a main effect or as an interaction had a significant effect on the pH of the meat (Table 3.2).

3.4 CONCLUSION

The results of this study showed that enhancement of pork loin with salt, phosphates and most importantly CPC does not have adverse effect on the quality of the meat. Processors need to be aware that tumble time and presence of vacuum may either work together positively or negatively to affect quality characteristics of marinated loins, and should therefore be well informed when combining these factors or using them alone. Future research may be needed to understand diffusion of marinade/brine inside the meat, and how this mechanism affects color, texture and brine retention.

REFERENCES

Alvarado, C., and McKee S. (2007). "Marination to Improve Functional Properties and Safety of Poultry Meat." *J. Appl. Poult. Res.* 16(1): 113-120.

Carroll, C. D., Alvarado, C. Z., Brashears, M. M. Thompson, L. D. and Boyce, J. (2007). Marination of turkey breast fillets to control the growth of *Listeria monocytogenes* and improve meat quality in deli loaves. *Poult. Sci.* 86(1): 150-155.

Hedrick, H. B., Aberle, E. D., Forrest, J. C., Judge M. D., and Merkel, R. A. (1994). Principles of meat science (3rd ed.), Kendall/Hunt Publishing Company, Dubuque, IA.

Lim, K., and Mustapha, A. (2007). Inhibition of *E. coli* O157:H7, *L. monocytogenes* and *Staphylococcus aureus* on sliced roast beef by cetylpyridinium chloride and acidified sodium chlorite. *Food Microbiol*. 24: 89-94.

Pohlman, F. W., Stivarius, M. R., McElyea, K. S., Johnson, Z. B. and Johnson, M. G. (2002). The effects of ozone, chlorine dioxide, cetylpyridinium chloride and trisodium phosphate as multiple antimicrobial interventions on microbiological, instrumental color, and sensory color and odor characteristics of ground beef. *Meat Sci.* 61(3): 307-313.

Tan, F. J., and Ockerman, H. W. (2006). Applicability of nisin and tumbling to improve the microbiological quality of marinated chicken drumsticks. *Asian-Aust. J. Anim. Sci.* 19(2): 292-296.

Swatland, H. J., (2002) in Kerry, J., Kerry J., and Ledward, D. Meat Processing: Improving quality. Woodhead Publishing Ltd & CRC Press LLC, Boca Raton FL.

Xiong, Y. L., and Kupski D. R. (1999a). Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. *Poult. Sci.* 78(7): 1048-1052.

Xiong, Y. L., and Kupski D. R. (1999b). Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. *Poult. Sci.* 78(7): 1053-1059.

Young, L. L., and Smith, D. P. (2004). Effect of vacuum on moisture absorption and retention on marinated broiler fillets. *Poult. Sci*. 83: 128-131.

TABLES AND FIGURES

Table 3.1: An interpretation of treatments applied during pork loin marination.

Treatment	**Interpretation**
a	Fresh[1] V[2] CPC[3]
b	Fresh NV[4] CPC
c	Fresh V NCPC[5]
d	Fresh NV NCPC
1	15[6] V CPC
2	15 NV CPC
3	15 V NCPC
4	15 NV NCPC
5	30[6] V CPC
6	30 NV CPC
7	30 V NCPC
8	30 NV NCPC
9	45[6] V CPC
10	45 NV CPC
11	45 V NCPC
12	45 NV NCPC
13	60[6] V CPC
14	60 NV CPC
15	60 V NCPC
16	60 NV NCPC

[1] Fresh refers to non-marinated loin portions from which the relative treatments were chosen.

[2] V = Vacuum tumbled, [3] CPC –CPC in brine, [4] NV = tumbled without vacuum, [5] NCPC – No CPC in brine.

[6] 15, 30, 45 and 60 are duration of tumble.

Table 3.2: Significance of processing factors in influencing the quality characteristics of tumbled pork loins

	Response Effects[1]						
Factor(s)	**Percent Pick-up**	**Cook Yield**	**Percent fat reduction**	**L***	**a***	**b***	**pH**
Main effects							
Tumble time[2]	-	-	-	+	-	-	-
Vacuum[3]	+	+	-	+	+	+	-
CPC[4]	-	+	-	-	-	-	-
Interactions							
Tumble time/ Vacuum	-	+	+	+	+	-	-
Tumble time/CPC	-	-	-	-	-	-	-
Vacuum/CPC	-	-	-	-	-	-	-
Tumble time/CPC/Vacuum	-	-	-	-	-	-	-

[1] The "-" indicates no significant effect ($p>0.05$), whereas a "+" represents a significant effect ($p<0.05$)

[2] Tumble time were 15, 30, 45 and 60. [3] Vacuum was applied up to 711mmHg when applied. [4] CPC was applied with an intended final content of 0.5% of product.

Table 3.3: Means of measurements of color, pH, percent pick-up and cook yield of marinated pork loins, and average percent fat reduction due to marination

(a)

Treatments[x]	**Color**			**pH**
	L*	**a***	**b***	
Fresh - A	$54.25^{a} \pm 4.51$	$21.20^{a} \pm 1.23$	$10.95^{a} \pm 2.30$	$5.84^{abc} \pm 0.12$
1 - 15 V CPC	$51.34^{a} \pm 3.03$	$19.81^{ab} \pm 0.52$	$9.22^{a} \pm 0.81$	$5.97^{ab} \pm 0.31$
5 - 30 V CPC	$51.15^{a} \pm 2.33$	$19.72^{abcd} \pm 1.51$	$9.64^{a} \pm 0.83$	$6.08^{a} \pm 0.21$
9 - 45 V CPC	$52.43^{a} \pm 2.21$	$18.91^{bcdef} \pm 0.63$	$9.36^{a} \pm 1.61$	$6.09^{a} \pm 0.07$
13 - 60 V CPC	$55.66^{a} \pm 1.17$	$19.57^{abcd} \pm 0.70$	$10.38^{a} \pm 0.03$	$6.00^{a} \pm 0.11$
Fresh - B	$58.49^{a} \pm 6.0$	$18.42^{bcdef} \pm 0.46$	$11.02^{a} \pm 1.59$	$5.66^{c} \pm 0.10$
2 - 15 NV CPC	$54.67^{a} \pm 0.11$	$17.41^{f} \pm 0.17$	$8.47^{a} \pm 0.86$	$5.92^{abc} \pm 0.01$
6 - 30 NV CPC	$56.62^{a} \pm 1.43$	$18.14^{bcdef} \pm 1.32$	$9.72^{a} \pm 0.78$	$5.97^{ab} \pm 0.01$
10 - 45 NV CPC	$53.56^{a} \pm 1.15$	$18.86^{bcdef} \pm 0.52$	$8.95^{a} \pm 0.83$	$5.87^{abc} \pm 0.06$
14 - 60 NV CPC	$54.93^{a} \pm 1.13$	$17.96^{def} \pm 0.42$	$9.02^{a} \pm 0.69$	$5.94^{ab} \pm 0.10$
Fresh - C	$57.66^{a} \pm 8.15$	$18.50^{bcdef} \pm 0.16$	$9.86^{a} \pm 3.88$	$5.72^{bc} \pm 0.24$
3 - 15 V NCPC	$51.64^{a} \pm 0.64$	$21.16^{a} \pm 1.24$	$9.01^{a} \pm 0.32$	$5.94^{ab} \pm 0.02$
7 - 30 V NCPC	$54.37^{a} \pm 0.17$	$19.47^{abcd} \pm 1.51$	$10.25^{a} \pm 0.60$	$6.04^{a} \pm 0.03$
11 - 45 V NCPC	$54.08^{a} \pm 0.92$	$19.30^{bcde} \pm 0.28$	$10.55^{a} \pm 0.23$	$6.04^{a} \pm 0.03$
15 -60 V NCPC	$55.58^{a} \pm 3.85$	$18.35^{bcdef} \pm 0.64$	$9.49^{a} \pm 1.62$	$6.02^{a} \pm 0.11$
Fresh – D	$57.12^{a} \pm 2.91$	$19.77^{abc} \pm 0.71$	$8.73^{a} \pm 3.80$	$5.82^{abc} \pm 0.24$
4 - 15 NV NCPC	$54.74^{a} \pm 2.28$	$18.00^{cdef} \pm 0.30$	$9.58^{a} \pm 1.24$	$5.93^{abc} \pm 0.08$
8 - 30 NV NCPC	$53.91^{a} \pm 1.46$	$18.35^{bcdef} \pm 0.45$	$9.33^{a} \pm 0.07$	$5.99^{ab} \pm 0.01$
12 - 45 NV NCPC	$55.27^{a} \pm 2.09$	$17.69^{ef} \pm 0.97$	$9.24^{a} \pm 0.00$	$5.96^{ab} \pm 0.00$
16 - 60 NV NCPC	$54.88^{a} \pm 2.74$	$18.40^{bcdef} \pm 1.01$	$8.66^{a} \pm 0.06$	$6.05^{a} \pm 0.08$

^{abcdef} Values not connected with the same superscript within each column are significantly different. (student's t means comparison. $\alpha = 0.05$)

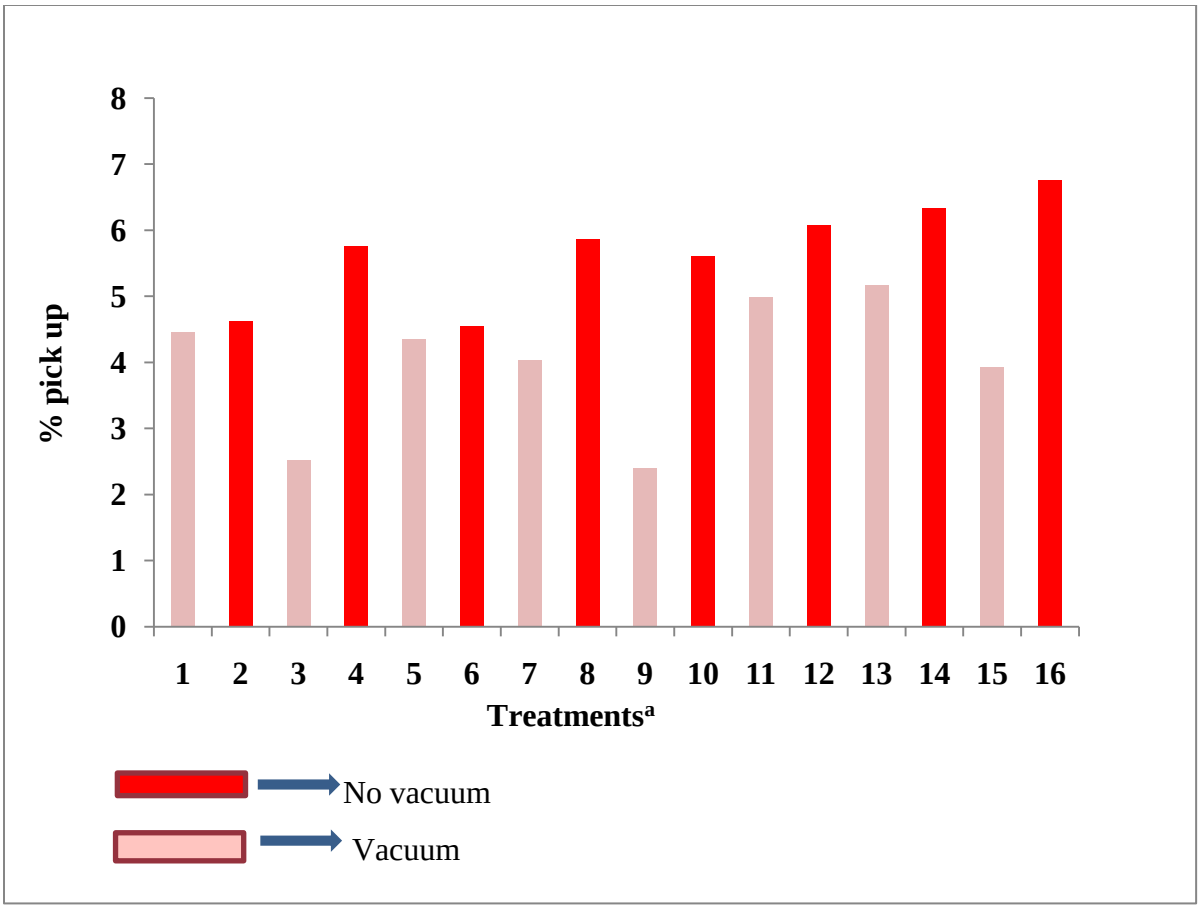
[x] Treatments 1 – 16 represent variations in marination processes (Table 3.1)

(b)

Treatments[x]	% fat reduction	Percent Pick-up	Cook yield
Fresh - A	-	-	-
1 - 15 V CPC	$57.68^{a} \pm 16.02$	$4.45^{abcd} \pm 0.22$	$85.10^{a} \pm 0.57$
5 - 30 V CPC	$35.27^{abcd} \pm 18.18$	$4.35^{bcd} \pm 1.81$	$84.95^{a} \pm 0.35$
9 - 45 V CPC	$41.76^{abcd} \pm 5.10$	$2.39^{d} \pm 0.91$	$84.75^{a} \pm 3.75$
13 - 60 V CPC	$25.42^{cd} \pm 10.69$	$5.17^{abc} \pm 0.01$	$84.75^{a} \pm 0.78$
Fresh - B	-	-	-
2 - 15 NV CPC	$42.77^{abc} \pm 9.33$	$4.63^{abcd} \pm 0.66$	$79.25^{b} \pm 1.06$
6 - 30 NV CPC	$27.00^{cd} \pm 0.20$	$4.54^{abcd} \pm 0.18$	$83.60^{a} \pm 0.28$
10 - 45 NV CPC	$36.27^{abcd} \pm 16.73$	$5.60^{abc} \pm 0.16$	$84.45^{a} \pm 0.92$
14 - 60 NV CPC	$53.72^{ab} \pm 3.34$	$6.34^{ab} \pm 0.25$	$83.80^{a} \pm 0.14$
Fresh - C	-	-	-
3 - 15 V NCPC	$33.24^{bcd} \pm 0.40$	$2.52^{d} \pm 0.06$	$85.15^{a} \pm 0.64$
7 - 30 V NCPC	$34.51^{abcd} \pm 19.43$	$4.03^{bcd} \pm 0.99$	$85.65^{a} \pm 0.21$
11 - 45 V NCPC	$21.98^{cd} \pm 5.88$	$4.98^{abc} \pm 0.03$	$85.35^{a} \pm 1.48$
15 -60 V NCPC	$43.36^{abc} \pm 1.72$	$3.93^{cd} \pm 3.16$	$84.70^{a} \pm 0.71$
Fresh – D	-	-	-
4 - 15 NV NCPC	$18.43^{d} \pm 7.49$	$5.76^{abc} \pm 0.83$	$84.65^{a} \pm 0.78$
8 - 30 NV NCPC	$25.41^{cd} \pm 17.95$	$5.86^{abc} \pm 0.03$	$84.50^{a} \pm 0.57$
12 - 45 NV NCPC	$52.64^{ab} \pm 5.24$	$6.08^{abc} \pm 0.02$	$84.75^{a} \pm 0.64$
16 - 60 NV NCPC	$31.82^{bcd} \pm 4.02$	$6.75^{a} \pm 1.69$	$84.75^{a} \pm 0.64$

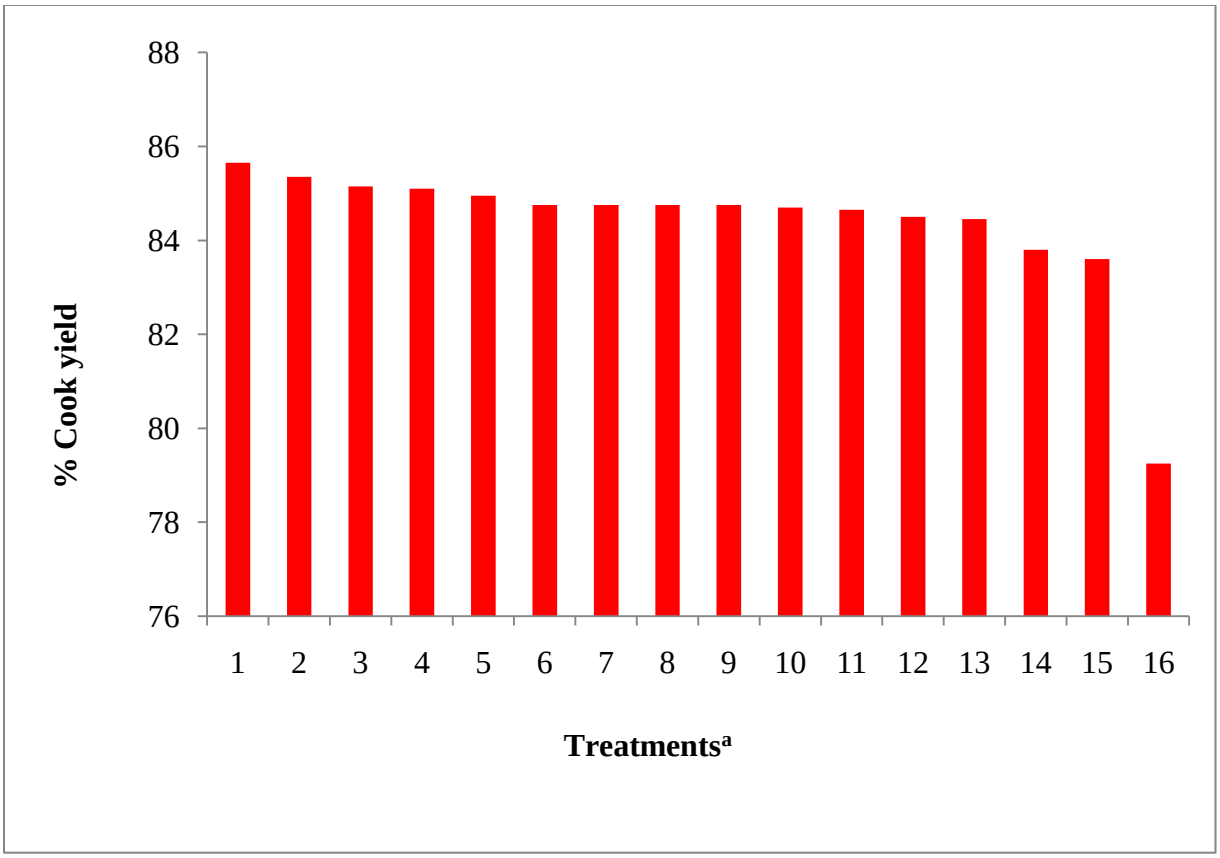
[abcdef] Values not connected with the same superscript within each column are significantly different. (student's t means comparison. $\alpha = 0.05$)

[x] Treatments 1 – 16 represent variations in marination processes (Table 3.1)



[a] Treatments 1 – 16 represent variations in marination processes (Table 3.1)

Figure 3.1: Effect of vacuum on % pick-up observed for the different treatments used in marination of pork loins.



[a] Treatments 1 – 16 represent variations in marination processes (Table 3.1)

Figure 3.2: % cook yield observed for the different treatments used in marination of pork loins.

CHAPTER 4

EVALUATION OF THE EFFECT OF POST-INJECTION TUMBLING AND DURATION OF TUMBLING ON GROWTH OF *LISTERIA MONOCYTOGENES* AND YIELD OF RTE MARINATED PORK LOIN

ABSTRACT

Effect of post-injection tumbling and tumble duration on growth of *L. monocytogenes* and some quality attributes of RTE marinated pork loin was studied. The results of this research showed that tumbling after injection is not statistically significant in the prevention of growth of *L. monocytogenes* in marinated pork loin. Without antimicrobial, with and without tumbling, a 2 log(cfu/cm^2) increase was observed in 7 days. However, the duration of tumbling did have an effect on the inhibition of *L. monocytogenes* growth. This trend was not the same when an antimicrobial, Purasal Hipure P Plus® (Purasal) was present. Purasal was used as an antimicrobial in two different concentrations (1% and 2%). At 1% Purasal content, without tumbling, a 2 log(cfu/cm^2) increase was observed in 28 days, while with tumbling, 2 log increase took 35 days. At 2% Purasal content, a 2 log increase in growth of *L. monocytogenes* was not observed during the entire 8 week storage period in this study.

Presence of Purasal significantly ($p<0.05$) reduced growth of *L. monocytogenes* in marinated pork loin. Post-injection tumbling did not have an effect on neither cook yield nor overall yield. Loins injected with marinade without Purasal and without tumbling had an average cook yield of 82.8, and overall yield of 101.5%. Loins tumbled 60 min had average cook yield of 82.0% and overall yield of 98.0%. Purasal content significantly affected cook yield and overall yield of the final product. Without antimicrobial, cook yield ranged from 71.9% to 89.3%, with 1% Purasal, range was 80.7% to 92.6%, while with 2% Purasal, range was 81.2 to 92.3%.

Keywords: Pork loin, marination, post-injection tumbling, *L. monocytogenes*, cook yield

4.1 INTRODUCTION

Cooked processed meats are commonly referred to as ready-to-eat (RTE) meat products, they are an important component of daily food consumption in the United States, and are staple in breakfast and lunch boxes. Ready-to-eat meat products are incriminated in listeriosis outbreaks. Foodborne listeriosis occurs as a result of consumption of food contaminated with the microorganism *L. monocytogenes*. As a part of response to outbreaks of listeriosis, the United States Food and Drug Administration worked with the United States Food Safety Inspection Service to assess the risk of listeriosis from a range of ready-to-eat foods, including "deli-meats". The report of this assessment (CFSAN/FSIS, 2003) states that, of 23 categories of RTE foods, deli meats represent the greatest contribution to listeriosis in the United States (Ross et al., 2009).

Indeed contamination of processed meat with *L. monocytogenes* after thermal processing is a potential hazard as significant growth has been observed with variations in different types of RTE meat products (Glass and Doyle, 1989). The Center for Disease Control and Prevention (CDC), reports that an estimated 2,500 people in the United States become seriously ill with listeriosis each year and of these 500 die. The hazardous nature of *L. monocytogenes* contamination in RTE meat products has raised concerns in the meat processing industry. Several studies have considered ways to combat its growth in meat products (Stekelenburg and Kant-Muermans, 2001; Samelis et al., 2002; Carroll et al., 2007; Lim and Mustapha, 2007; Mbandi and Shelef, 2001; Mellefont and Ross, 2007).

Since contamination of processed meat usually occurs post thermal-processing, incorporating salts and ingredients with antimicrobial properties in the meat before cooking may improve the safety of the RTE products. Marination has been used to describe the long-

established technique of immersion of meat cuts in a solution of ingredients to increase tenderness, juiciness and flavor, and recently safety. This traditional method slowly and passively incorporates ingredients into the meat by osmosis. Since this method is a slow process, injection of marinade into meat using mechanical injectors has become an option as equipment and technology develops (Sebranek, 2008). Tumbling is another method used to enhance uptake of marinade into meat. Presumably, post-injection tumbling will help improve cook yield, overall yield and moisture content of RTE meat products.

Since injection and tumbling delivers marinade ingredients deep into meat cuts, it is logical that these methods of marinade application may be employed to incorporate antimicrobial agents into meat during processing. Also, many processors employ injection and post-injection tumbling in their processing routines. Even though these practices have been applied for many years, there is still a need for a scientific study to better understand how variation in processing parameters affects the quality and safety of RTE marinated meat products.

The overall goal of this study is to investigate the effect of post-injection tumbling and tumbling duration on the safety and quality characteristics of RTE marinated pork loin. The first objective is to study the effect of post-injection tumbling and tumbling duration on the growth of *L. monocytogenes* over storage period, using the industry control brine (1.5% NaCl and 0.45% STPP). A potassium lactate solution, Purasal Hipure P Plus®, will also be used at 1% and 2% concentration in the marinated product to investigate how post-injection tumbling and duration affects growth of *L. monocytogenes* in the presence of a commercially employed antimicrobial. Another objective of this study is to investigate how these processing variables affect cook yield, overall yield and percent moisture content of RTE marinated pork loin.

4.2 MATERIALS AND METHODS

4.2.1 Loin Acquisition

Fresh pork loins were purchased from a local meat retailer, transported on ice to the laboratory and stored in a 2^{o}C room until the next day. Each loin weighing approximately 5 kg was cut into 5 equal pieces (about 1 kg each). The exact weight for each cut piece depended on size of the whole loin. Each of the pieces was weighed and the weights were recorded.

4.2.2 Brine Preparation

Three different types of marinades were prepared to deliver the compositions shown below as the final content in the processed meat.

Marinade 1: 1.5% NaCl, 0.45% STPP

Marinade 2: 1.5% NaCl, 0.45% STPP and 1% Purasal

Marinade 3: 1.5% NaCl, 0.45% STPP and 2% Purasal

To prepare the marinades, equation 4.1 was used to calculate the exact amount of STPP (Solutia Inc., St. Louis, MO), NaCl (Monarch table salt, Reid, Murdoch and Co., Columbia, MD), Purasal Hipure P Plus® solution (Purac America, Lincolnshire, IL) and water to be added for each of the three marinade composition.

$$C_m = C_p \left(\frac{1}{M} + 1\right) \quad \text{Equation 4.1}$$

Where,

C_m= concentration of component in marinade (%)

C_p= concentration of component in product (%)

M = expected marinade pick-up (%)

To prepare the brine, each component was weighed separately. To ensure proper dissolution of the salts in water, the salts were added to water in the following order; phosphate, salt and

finally, where applicable, the Purasal solution. The mixture was stirred vigorously to ensure complete dissolution of each marinade component before adding the next. Refrigerated water (with ice added when necessary) was used to prepare the brine. This was used to ensure that temperature of water before salt addition was at 0^{o}C, and that temperature of the prepared brine does not exceed 4^{o}C. Temperature of the brine was monitored with a monitoring thermometer (VWR Intl, West Chester, PA)

4.2.3 Processing

(a) Injection and Tumbling

A 3-row multineedle injector (Schroeder N40, Wolf-Tec Inc,. Kingston, NY) was calibrated to give a 20% pick-up to injected meat. Calibration of the injector was done by weighing a pork loin portion, injecting it with the brine to be used and weighing again after injection while adjusting injection pressure until the desired pick-up range was achieved. Fifteen pre-weighed pieces of loins were then injected with corresponding brine. 5 pieces were injected with the salt and phosphate only brine, another 5 pieces with the salt, phosphate + 1% Purasal brine and another 5 pieces with the salt, phosphate + 2% Purasal brine. The injected loins were then weighed. For each set of 5 loin pieces, the pieces were tumbled in a vacuum tumbler (Model 110.2, U-Mec, Los Angeles), for 15, 30, 45 and 60 min separately; the final piece was not tumbled at all. All the treated loin pieces were placed separately in labeled Cryovac® bags (Sealed Air, Duncan, SC), and stored in a 2^{o}C refrigerated room overnight.

(b) Cooking

The morning after marination, right before cooking, samples were collected from each of the marinated loins, for moisture analysis, by cutting a small piece of the chunk. The marinated loins were then placed on wire racks positioned on an oven truck, and racks were tagged ifor

easy identification of which loin belonged to a certain treatment after cooking. The truck was then pushed into a smokehouse (Alkar, Lodi, WI). The oven was programmed to cook the meat to an internal temperature of 73.9^{o}C and holding it for 15 min at this temperature. After cooking, the meat was moved into a cold room (approximately 7^{o}C) to enable fast cooling of the meat. Weights of cooked meat were recorded. Small section of the cooked loins was cut out for moisture analysis.

4.2.4 Quality Analysis

(a) Cook yield and Overall yield

Weights of the loins were measured before and after cooking and recorded to calculate cook yield (Equation 4.2); and overall yield (Equation 4.3)

$$\text{Cook yield} = \frac{\text{cooked weight} - \text{uncooked weight}}{\text{uncooked weight}} \times 100 \quad \text{..... Equation 4.2}$$

$$\text{Overall yield} = \frac{\text{cooked weight} - \text{green weight}}{\text{green weight}} \times 100 \quad \text{..... Equation 4.3}$$

In these equations, uncooked weight is weight after marination; green weight is weight of fresh non-marinated loin.

(b) Moisture Analysis

The samples taken before and after cooking were used to evaluate moisture content of the product to compare the processing methods. For moisture analysis, option (b) of the AOAC Official Method 950.46 (AOAC Official Method of Analysis, 39.1.02, 1995.) was used.

4.2.5. Microbiological Analysis

(a) Preparation of Inoculums

Five different strains of *L. monocytogenes* were used as a cocktail for this study. These are; strain 386 which was obtained originally from the drain of a chicken processing plant, strain

H7550 which is an isolate from an hot dog outbreak, strain YM112 (serotype $^{1}/_{2}$a), ATCC 19115 which is a human isolate and Scott A strain.

To prepare frozen strain stocks for inoculation onto cooked product, they were reactivated approximately a week before their use. Two beads were placed in 10 ml of BD™ tryptic soy broth (TSB, Becton Dickinson and Co., Franklin Lakes, NJ) and incubated for 18-24h at 37^{o}C, 0.1 ml of this was transferred to a fresh TSB tube and incubated for another 18-24 h. One more subsequent transfer from the resulting culture was made. This was carried out simultaneously for all five strains and resulting culture tubes were wrapped in parafilm and kept in a 4^{o}C storage refrigerator until used for inoculation. On the day before inoculation, 0.1 ml of the final transfer was placed in a 9 ml TSB tube and incubated overnight.

Level of *L. monocytogenes* in the overnight cultures for each strain was determined in triplicate to quantify cfu/ml of *L. monocytogenes* in overnight culture. To make the *L. monocytogenes* cocktail on the day of inoculation, 2 ml of overnight culture from each of the five strains were transferred to a sterile test tube, making a total of 10 ml. This was vortexed and diluted as needed to 5 log cfu/ml level using sterile water.

(b) Inoculation of Loin Product

Each cooked piece of loin was sliced using a slicer (Model 2612, Hobart, Troy, OH) into a minimum of 8 smaller pieces. These were 1 cm thick, 8 cm wide and 11 cm long. Each piece represented a sample for the 8 weeks of storage. Using a die cast for 25 sq cm, an impression approximately 2cm from the edge of the loins was made on the surface of each meat slice. Then, using a micropipette, 250 µl of the prepared *L. monocytogenes* cocktail (10^{5} cfu/ml concentration) was deposited on the 25 sq cm space (to deliver approx 2.2 log(cfu/cm^{2}) of *L. monocytogenes*) and spread evenly with a sterile glass rod. The pieces were then held in a

biological safety cabinet for 15-20 min to allow for drying of the liquid and attachment of the microorganisms to the meat surface. Each piece was placed into a pouch (Nylon/PE Vacuum Pouch, Prime Source, USA) and vacuum sealed. The sealed products were randomly labeled for storage over 8 weeks. A black colored marker was used to mark impression site on the pouch to ensure that inoculation site is recognizable throughout storage period. All inoculated and vacuum sealed products were then placed in a 4^{o}C refrigerator (Model 146E, Fisher Scientific, USA).

(c) *Listeria monocytogenes* Growth Check

At weekly intervals, a sample representative of each treatment (total of 15) was selected. Using a sterile scalpel, the inoculated portion was excised by removing approximately 0.5 cm of the upper surface, such that the whole inoculated part was aseptically excised and transferred to a stomacher bag. To the bag, 90 ml ± 5 ml of 0.1% peptone water was added and this was stomached at 230 rpm for 1 min in a stomacher (Model 400 circulator, Seward, England). Samples (with dilutions done where necessary) were plated using an automated spiral plater (Spiral Biotech Autoplate 4000, Advanced Instruments Inc., Norwood, MA) onto modified oxford agar (MOX) plates (Oxoid Ltd., Hants., England). The plates were incubated at 35^{o}C for 48h after which the number of observable colonies (characterized by a darkening around each cream colored colony) were counted and recorded.

4.2.6 Statistical Analysis

Experiments were in triplicates. The results of the experiments were analyzed using JMP 7, statistical software by SAS (SAS Institute Inc, Cary, NC). Statistical tests used in this study included Dunnett's test; a test which compares a set of means to another mean (control group), student's t-test, for comparison of means, analysis of variance and parameter estimates. All statistical tests were performed at $\alpha = 0.05$. Multiple regression models were fitted to investigate

the significance of main factors (tumbling time, Purasal content) on the growth of *L. monocytogenes*, cook yield, overall yield and percent moisture level responses. Also, full factorial models were fitted to investigate significance of interaction of these main factors on the stated responses.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of Post-injection Tumbling on Growth of *Listeria monocytogenes*

Dunnett's test performed across antimicrobial levels (Table 4.1), showed that tumbling following injection does not have a significant effect ($p>0.05$) on the growth of *L. monocytogenes* in the marinated loins. Non-tumbled loins without Purasal had an average of 3.47 log (cfu/cm^2) increase in growth of *L. monocytogenes* 21 days after inoculation. Tumbled loins had an average increase of 3.41 log (cfu/cm^2) in growth after the same storage duration.

To neglect possible effect of antimicrobial presence and variation on the growth response, Dunnett's test was repeated within the three antimicrobial levels; 0%, 1% and 2% Purasal concentration, (Table 4.1). These analyses similarly suggest that there is no significant difference ($p>0.05$) in the growth trend of tumbled and non-tumbled marinated loins. Growth response of tumbled loins were not significantly different ($p>0.05$) from that of non-tumbled loin at any given storage time with and without Purasal. For example, at day 42 after inoculation, loins injected but non-tumbled with marinade containing 1% Purasal had a 3.29 log (cfu/cm^2) increase in *L. monocytogenes* while tumbled loin had an average of 3.15 log (cfu/cm^2) increase in *L. monocytogenes* growth.

Tumbling increases distribution of curing and marinade solution (Ockerman and Organisciak, 1978; Fox et al., 1984). If tumbling causes an increase in distribution of marinade ingredients within muscle products during processing, it should enhance the distribution of

antimicrobial agents (Tan and Ockerman, 2006). This may help enhance microbiological safety of the product. Xiong and Kupski (1999a) pointed out that mobility of marinade depends on phosphate type and level as well as the salt treatment.

Tan and Ockerman (2006) studied tumbling as a factor in improving the microbiological quality of marinated chicken drumsticks. Their results indicated that at a given storage time, the samples tumbled for either 10 or 20 min had significantly ($p<0.05$) lower microbial counts when compared to the samples without the tumbling treatment. However, the microbial counts of the tumbled samples increased as storage time increased. In contrast, this study shows that at any given storage time, mean growth of *L. monocytogenes* on post-injection tumbled (15, 30, 45 or 60 min) pork loins was not significantly different from growth in the non-tumbled injected pork loins. Similar to Tan and Ockerman (2006)'s results, *L. monocytogenes* counts increased in all pork loin samples as storage time increased. An average of 1.96 log (cfu/cm^2) increase in growth was observed at day 7 in loins marinated without Purasal and this increased to 4.94 log (cfu/cm^2) after 56 days of storage.

At 0% Purasal content, tumbled and non-tumbled loins increased by 2 logs in 7 days, with the exception of the loins tumbled for 15 min which took approximately 14 days to reach a 2 log increase (Fig 4.1). However, by day 21 and beyond, the 15 min tumbled loin had very similar growth trends with all other treatments through the storage time. Statistical analysis of percent moisture level of the final product showed that the 15 min tumbled product had significantly ($p<0.05$) lower moisture content compared to the other tumble duration treatments. This may explain the longer lag phase in the 15 min tumbled loin treatments. A lower water activity level will increase lag phase. There was no significant difference in growth of *L. monocytogenes* between tumbled and non-tumbled loins (Fig 4.2 and 4.3)

The absence of significant difference between tumbled and non-tumbled treatments (Table 4.1) may be attributed to the fact that injection is a process that delivers marinade deep into the muscle. The tumbling procedures in this study were post-injection, suggesting that given the need to combat growth of *L. monocytogenes* only, post-injection tumbling may not be necessary and may be an added cost and time in processing. This also may explain the contrast with Tan and Ockerman's (2006) study as the tumbled chicken investigated were not pre-injected. However, post-injection tumbling may be desired for other palatability attributes including tenderness and juiciness.

4.3.2 Effect of Tumbling Duration on the Growth of *L. monocytogenes*

Tumbling duration was not significant ($p>0.05$) in growth trend of *L. monocytogenes* regardless of Purasal content. To neglect effect of antimicrobial levels on responses, a multiple regression model is fitted within the antimicrobial levels. At 0% Purasal content, tumbling time was not statistically significant ($p>0.05$). At 1% Purasal, tumbling time was statistically significant ($p<0.05$), and at 2%Purasal, tumbling time was not statistically significant ($p>0.05$) to *L. monocytogenes* growth response.

At any given storage time, duration of tumbling did not have a significant effect on growth of *L. monocytogenes*. Average increase in log (cfu/cm^2) observed in loins tumbled for different durations with 0%, 1% and 2% Purasal shows that tumbling duration is not a significant factor contributing to growth of *L. monocytogenes* (Fig 4.1, 4.2 and 4.3). For example, at day 35 for loins marinated without Purasal, non-tumbled loins had 4.84 log (cfu/cm^2) increase, while loins tumbled 15, 30, 45 and 60 min had 4.60, 4.77, 4.75 and 4.65 log (cfu/cm^2) increase, respectively.

Xiong and Kupski (1999b) studied time-dependent marinade absorption in chicken fillets and their results show that marinade penetration was most rapid in the initial 5 min of tumble and there was no significant increase in penetration in filets tumbled for longer periods. These findings agree with the results of this study in which tumbling time was not significant ($p>0.05$) in loins without Purasal, and those with 2% Purasal. However, in loins with 1% Purasal, tumbling time was significant ($p<0.05$), (Fig 4.2). This variation may be attributed to other factors in the processing. In the 1% Purasal set of samples, variation in salt and STPP contents in the products may have interacted with tumbling time to cause this variation.

4.3.3 Effect of Purasal Hipure P Plus® on Growth Trend of *L. monocytogenes*

The level of Purasal solution in the final product is significant ($p<0.05$) to growth response of *L. monocytogenes* in cooked RTE marinated pork loin. *L. monocytogenes* populations on loins marinated without Purasal increased by 1.47 to 5.04 log (cfu/cm^2) (Table 4.2), with 1% Purasal (Table 4.3), increase was 0.17 to 4.43, while with 2% Purasal (Table 4.4), increase observed was from -0.03 to 2.19 log (cfu/cm^2). These values show that as Purasal content increased, growth of *L. monocytogenes* in the meat decreased.

Purasal Hipure P Plus® is a potassium salt of natural lactic acid. It is approximately 78% lactate solution (Purac, 2009). Several studies have investigated the effect of lactic acid either in sodium lactate form or potassium lactate form on the growth of *L. monocytogenes* in RTE meat products (Carroll et al., 2007; Stekelenburg and Kant-Muermans, 2001; Shelef and Yang, 1991; Mellefont and Ross, 2007; and Doyle, 1999). These studies have shown that sodium lactate extends lag phase of *L. monocytogenes*, with varying extension duration depending on percentage lactate used. Stekelenburg and Kant-Muermans (2001) observed inhibition in growth of *L. monocytogenes* by sodium lactate.

The results from this study showed Purasal Hipure P Plus® (Purasal) to be significant ($p<0.05$) in the suppression of growth of *L. monocytogenes* in RTE marinated pork loins. Increased Purasal concentrations led to an increase in inhibition of growth of *L. monocytogenes*. Mellefont and Ross (2007), studied growth of *L. monocytogenes* on RTE hams using 3% Purasal Hipure P®, very similar to the product that was used in this study, but the P Plus solution is enhanced for better flavor. The results of their study showed that 3% Purasal Hipure P® inhibited growth of *L. monocytogenes* for up to 57 days at 8^{o}C storage while completely suppressing growth for 90 days in hams stored at 4^{o}C. The trend observed in this study suggests that growth of *L. monocytogenes* could have been suppressed if Purasal Hipure P Plus® in the marinated pork loins was up to 3%. However, at 2% Purasal content, growth of *L. monocytogenes* was slowed considerably, and it increased by less than 2 log (cfu/cm^2) until 56 days after inoculation.

4.3.4 Effect of Post-injection Tumbling on Cook Yield, Overall Yield and Percent Moisture Level of Product

Cook yield, overall yield and moisture content are characteristics important to processors. Non-tumbled loins were considered the control group, and the effect of post-injection tumbling during processing on these quality characteristics was determined. Statistical analysis showed that post-injection tumbling did not significantly affect cook yield and overall yield of RTE marinated pork loins (Table 4.5 and 4.6). To neglect the effect of antimicrobial levels, comparisons were made within each antimicrobial concentration data, (Tables 4.5, and 4.6). These results show that cook yield and overall yield of loins tumbled after injection are not statistically different ($p>0.05$) from those not tumbled. For example in loins marinated without Purasal, injected non-tumbled loins had an average cook yield of 82.8% and overall yield of

101.5% while loins tumbled 60 min had a cook yield of 82.0% and overall yield of 98.0% (Table 4.7). However, at 15 min of tumbling, percent moisture content of the product was significantly different from that of non-tumbled loins, the percent moisture content was lower in the 15 min tumbled loin (Table 4.7).

Yield of the final product determines profit in the RTE meat industry, which is an important issue to meat processors. This study shows that post-injection tumbling does not increase cook and overall yield (Table 4.7). Previous studies in this aspect have contradicting results. Pietrasik and Shand (2005) reported that injection on its own improved cook yield, while post-injection tumbling was not significant in increasing cook yield of beef roasts. Rejt et al., (1978) reported that cooking loss in massaged pork meat was reduced up to 50% compared to loss in control samples. They concluded that vacuum massage post-injection improves cooking yield to some extent. Babji et al., (1981) reported more cooking loss, (lower cook yield), in post-injection tumbled turkey breast. Detienne et al., (2003) reported an increase in overall yield with post-injection tumbling when compared to injection only samples.

While some of these contrasts may be due to variations in the muscle type involved, results from this study agree with a previous study (Pietrasik and Shand, 2005) which also used pork loin, while it disagrees with another (Rejt et al., 1978) using the same type of muscle. It is difficult to rationalize reason for these contrasts. Thus, more study needs to be done to examine other factors that may act either synergistically or not to influence yield of post-injection tumbled pork loins.

Aside from the 15 min tumbled loin treatment, post-injection tumbling (30, 45 and 60 min) did not have a significant effect ($p>0.05$) on moisture content compared to the non-tumbled loins (Table 4.8). The 15 min tumbled loins had lower moisture contents compared to all other

treatments. The reason for this seems ambiguous. Babji et al., (1981) did not observe a significant difference in percent moisture of post-injection tumbled and non-tumbled meat. They stated that tumbling did not significantly affect moisture content or water holding capacity of the turkey breast muscle. Alvarado and Sams (2004) reported higher moisture in tumbled broiler breast fillets, but their study was comparing tumbling in fillets that were not pre-injected, which makes the outcome of the study logical. The results of this study suggest that post-injection tumbling may not be effective in increasing moisture content of RTE marinated pork loins. When percentage moisture in RTE products is important to processors, especially considering labeling claims, it is very beneficial to know how post-injection tumbling affects percent moisture content of products.

4.3.5 Effect of Tumble Duration on Cook Yield, Overall Yield and Percent Moisture Level of Product

Using the data from all pork loin (regardless of antimicrobial presence), statistical analyses showed that tumbling time is not a statistically significant ($p>0.05$) contributor to cook yield, overall yield and percent moisture content. To neglect the effect of Purasal content, analysis within each Purasal content level (0%, 1% and 2%) was performed. This showed that tumbling time is not statistically significant ($p>0.05$) to cook yield, overall yield and percent moisture.

Xiong and Kupski (1999b) observed that retention of marinade generally reached its maximum after 15 min of tumble when salt was not in the marinade but continued to increase with tumble up to 30 min in the presence of 8% salt. The level of salt (8%) at which significant increase in marinade retention with tumble time was observed. Xiong and Kupski (1999)'s study is higher than the level of salt (~1.5%) used in this study. Since this study had 15 min as the

lowest tumble duration investigated as compared to 5 min lowest tumble time in the previous study, it is difficult to compare responses based on tumble time in the two studies. However, the fact that tumbling more than 15 min does not improve marinade retention in the previous study suggests that longer tumbling may not improve cook, overall yield or moisture content.

4.3.6 Effect of Purasal Level on Cook Yield, Overall Yield and Percent Moisture Level of Product.

Statistical analysis shows that Purasal content is a statistically significant contributor to cook yield ($p<0.05$), as well as to overall yield ($p<0.05$). However, Purasal content was not a statistically significant contributor to percent moisture level ($p>0.05$). At 60 min tumbling, loins without Purasal had cook yield, overall yield and percent moisture content of 82%, 98% and 65% respectively, while those with 1% Purasal had cook yield, overall yield and percent moisture content of 89.4%, 104.8% and 63% respectively (Table 4.7).

Purasal was statistically significant ($p<0.05$) in improving cook yield and overall yield of RTE marinated pork loins. Papadopoulos et al., (1991) reported that increased sodium lactate in injected beef top rounds resulted in increased cook yield. Potassium lactate functions in a similar manner as sodium lactate but is less preferred because of its slightly bitter taste. It is also soluble in water (Shelef and Yang, 1991). The results of this study agree with Papadopoulos and others (1991). In compensation for the slightly bitter taste in potassium lactate, which may make it undesirable to processors, Purasal Hipure P Plus® formula is enhanced for improved flavor by the manufacturer. (Purac, 2009) Purasal content, however, did not have a significant effect ($p>0.05$) in percent moisture content of RTE marinated pork loins.

4.3.7. Experimental Variation Effect on Growth of *L. monocytogenes*, Cook Yield, Overall Yield and Percent Moisture of the Product

During processing, some variations such as percent marinade injected in pork loins could not be avoided, thereby yielding variation in NaCl and Sodium tripolyphospate (STPP) content of the meat. Effect of these variations in salt and STPP levels in the meat on growth response were examined. The numbers showed that salt and STPP variations were not significant ($p>0.05$) in growth response in neither the whole data, nor in the 0% and 2% Purasal containing loins data. However, in 1% Purasal loins, both salt and STPP variations were significant ($p<0.05$) in the growth response.

Performing similar analysis on cook yield, overall yield and percent moisture content of the product, salt and STPP variations was not significant in cook and overall yield, but were significant ($p<0.05$) in percent moisture level. Neglecting antimicrobial content, within 0%, 1% and 2%, the salt and STPP variations did not have a significant effect ($p>0.05$) on cook, overall yield and percent moisture content.

These results show that variation in salt and STPP levels did have a significant effect only on moisture content of RTE marinated pork loins in the whole data (across Purasal levels). Salt is well known as a primary marination ingredient which helps improves water holding capacity of meat muscle. Alkaline phosphates are known to generally improve water retention by shifting pH away from isoelectric point of myofibrillar proteins, thus unfolding the proteins and exposing more charged sites for water binding (Pearson and Gillett, 1996; Cheng and Sun, 2008). Baublits et al., (2005) reported that increased phosphate led to a decrease in cook loss, in other words, increased cook yield. Similar results were reported by Carroll et al., (2007).

4.3.8 Interaction of Main Effects on Growth Response of *L. monocytogenes* and on Cook and Overall Yield

To check for possible interactions between independent variables in this experiment, a full factorial model was fitted. Some interaction effects were statistically significant ($p<0.05$) to growth response of *L. monocytogenes* (Table 4.9) including tumble time and salt level; and tumble time and STPP content.. For cook yield and overall yield, the only interaction that was statistically significant ($p<0.05$) was between salt, STPP and Purasal content.

This interaction effects shows that when processing factors contribute independently negatively or positively to affect response (growth of *L monocytogenes,* cook yield or overall yield), an interaction between these factors may contribute to response in a different way than they do independently. Processors therefore need to be aware of how each introduced processing variable interacts with another to affect quality and safety of their products.

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REFERENCES

Alvarado, C. Z., and Sams, A. R. 2004. Early postmortem injection and tumble marination effects on broiler breast meat tenderness. *Poult. Sci*. 83: 1035-1038.

Babji, A. S., Froning, G. W., and Ngoka, D. A. 1982. The effect of short-term tumbling and salting on the quality of turkey breast muscle. *Poult. Sci.* 61(2): 300-303.

Baublits, R. T., Pohlman, F. W., Brown, Jr A. H., and Johnson, Z. B. 2005. Effects of sodium chloride, phosphate type and concentration, and pump rate on beef biceps femoris quality and sensory characteristics. *Meat Sci.* 70(2): 205-214.

Carroll, C. D., Alvarado, C. Z., Brashears, M. M., Thompson, L. D., and Boyce, J. 2007. Marination of turkey breast fillets to control the growth of *Listeria monocytogenes* and improve meat quality in deli loaves. *Poult. Sci.* 86(1): 150-155.

CDC website; Surveillance data. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5813a2.htm accessed October 2009.

CFSAN/FSIS; Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. 2003. *In* Ross et al. 2009. Reference No. 21.

Cheng, Q., and Sun, D. W. 2008. Factors affecting the water holding capacity of red meat products: A review of recent research advances. *Crit. Rev. Food Sci. Nutr*. 48(2): 137-159.

Detienne, N. A., Reynolds, A. E., and Wicker, L. 2003. "Phosphate marination of pork loins at high and low injection pressures." *J. Food Qual.* 26(1): 1-14.

Doyle, M . E. 1999. Subject: Literature review of various techniques used in *Listeria* intervention. www.wisc.edu/fri/briefs.htm accessed October, 2009.

Fox, J. D., Langlois, B. E., Kemp, J. D., and Leak, F. W. 1984. Effect of tumbling and tumbling time on quality and microflora of dry-cured hams. *J. Food Sci.* 49(3): 695.

Glass, K. A., and Doyle, M. P. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol*. 55:1565–1569.

Lim, K., and Mustapha, A. 2007. Inhibition of *E. coli* O157:H7, *L. monocytogenes* and *Staphylococcus aureus* on sliced roast beef by cetylpyridinium chloride and acidified sodium chlorite. *Food Microbiol*. 24: 89-94.

Mellefont, L. A., and Ross T. 2007. Effect of potassium lactate and a potassium lactate-sodium diacetate blend on *Listeria monocytogenes* growth in modified atmosphere packaged sliced ham. *J. Food Prot.* 70(10): 2297-2305.

Mbandi, E., and Shelef, L. A. 2001. Enhanced Inhibition of *Listeria monocytogenes* and *Salmonella Enteritidis* in Meat by Combinations of Sodium Lactate and Diacetate. *J. Food Prot.* 64: 640-644.

Ockerman, H.W., and Organisciak, C. S. 1978. Influence of continuous versus intermittent tumbling on brine (salt, sugar and nitrite) diffusion in porcine tissue, *J. Food Prot.* 41: 878–880.

Papadopoulos, L. S., Miller, R. K., Ringer, L. J., and Cross, H. R. 1991. Sodium lactate effect on sensory characteristics, cooked meat color and chemical-composition. *J. Food Sci.* 56(3): 621-626.

Pearson A.M., and Gillet, T.A. 1996. Processed meats, 3rd ed. Chapman & Hall, New York.

Pietrasik, Z., and Shand, P. J. 2005. Effects of mechanical treatments and moisture enhancement on the processing characteristics and tenderness of beef semimembranosus roasts. *Meat Sci.* 71(3): 498-505.

Purac, 2009. Purac product datasheet http://www.purac.com/ufc/file2/purac_sites/monique/bb8a59dd383c205231973d6e2846b452/pu/HiPure_P_Plus.pdf accessed October 2009.

Rejt, J., Kubicka, H., and Pisula, A. 1978. Changes of physical and chemical properties and of histological structure of meat subjected to massage under vacuum. *Meat Sci.* 2(2): 145-153.

Ross, T., Rasmussen, S., Fazil, A., Paoli, G., and Sumner, J. 2009. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. *Int. J. Food Microbiol.* 131(2-3): 128-137.

Samelis, J., Bedie, G. K., Sofos, J. N., Belk, K. E., Scanga, J. A., and Smith, G. C. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4 degrees C in vacuum packages. *J. Food Prot.* 65(2): 299-307.

Sebranek, J. 2008. Best Practices: Determining ideal injection levels for fresh beef products. Meatingplace in print online. Accessed May, 2008.

Shelef, L. A., and Yang, Q. 1991. Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken, and beef. *J. Food Prot.* 54(4): 283-287.

Stekelenburg, F. K., and Kant-Muermans M. L. T. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int. J. Food Microbiol.* 66(3): 197-203.

Tan, F. J., and Ockerman, H. W. 2006. Applicability of nisin and tumbling to improve the microbiological quality of marinated chicken drumsticks. *Asian-Austr. J. Anim. Sci.* 19(2): 292-296.

Xiong, Y. L., and Kupski D. R. 1999a. Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. *Poult. Sci*. 78(7): 1048-1052.

Xiong, Y. L., and Kupski D. R. 1999b. Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. *Poult. Sci.* 78(7): 1053-1059.

TABLES AND FIGURES

Table 4.1: Statistical comparison of means of growth response of *L. monocytogenes* in post-injection tumbled pork loins with injected non-tumbled pork loins.

	Abs(dif) – LSD*			
Tumble time	**All treatments**	**No Purasal**	**1% Purasal**	**2% Purasal**
0	-0.81	-0.96	-1.19	-1.07
15	-0.74	-0.92	-1.15	-0.89
30	-0.75	-0.83	-0.97	-0.98
45	-0.56	-0.79	-0.72	-0.60
60	-0.66	-0.96	-0.89	-0.92

* If a value is positive, its mean is more than the least significant difference (LSD) apart from the control group mean and is thus significantly different, and vice-versa if negative.

Table 4.2: Increase in population of *L. monocytogenes* in RTE marinated pork loins without antimicrobial over storage period at different tumble durations.

Tumble time.	log (cfu/cm^2)[1]							
	Day7	**Day14**	**Day21**	**Day28**	**Day35**	**Day42**	**Day49**	**Day56**
0	2.17[a]	2.17[a]	3.47[a]	4.07[a]	4.84[a]	4.89[a]	4.58[a]	4.93[a]
15	1.47[a]	2.11[a]	3.37[a]	4.28[a]	4.60[a]	5.04[a]	4.96[a]	4.93[a]
30	2.17[a]	2.55[a]	3.51[a]	4.42[a]	4.77[a]	4.86[a]	4.86[a]	4.99[a]
45	2.07[a]	3.25[a]	3.44[a]	4.37[a]	4.75[a]	4.88[a]	4.78[a]	4.91[a]
60	1.94[a]	2.46[a]	3.32[a]	4.16[a]	4.65[a]	4.86[a]	4.73[a]	4.92[a]

[1] log(cfu/cm^2) is log(final – initial)cfu/cm^2

[a] Values with the same superscript were not significantly different ($p<0.05$), using student's t means comparison. Inoculum level is 2.2 log (cfu/cm^2)

Table 4.3: Increase in population of *L. monocytogenes* in RTE marinated pork loins with 1% Purasal over storage period at different tumble durations.

Tumble time.	log (cfu/cm^2)[1]							
	Day7	**Day14**	**Day21**	**Day28**	**Day35**	**Day42**	**Day49**	**Day56**
0	0.55[a]	0.74[a]	1.22[a]	2.11[a]	3.06[a]	3.29[a]	3.65[a]	3.89[a]
15	0.20[a]	1.22[a]	2.58[a]	1.94[a]	2.27[a]	2.97[a]	3.54[a]	3.89[a]
30	0.24[a]	0.17[a]	0.61[a]	1.66[a]	2.36[a]	3.30[a]	3.84[a]	4.43[a]
45	0.22[a]	0.51[a]	1.60[a]	0.69[a]	1.87[a]	2.63[a]	2.61[a]	4.38[a]
60	0.20[a]	0.18[a]	1.00[a]	1.56[a]	2.03[a]	3.71[a]	3.67[a]	3.78[a]

[1] log(cfu/cm^2) is log(final – initial)cfu/cm^2

[a] Values with the same superscript were not significantly different ($p<0.05$), using student's t means comparison. Inoculum level is 2.2 log (cfu/cm^2)

Table 4.4: Increase in population of *L. monocytogenes* in RTE marinated pork loins with 2% Purasal over storage period at different tumble durations.

Tumble time.	log (cfu/cm^2)*							
	Day7	**Day14**	**Day21**	**Day28**	**Day35**	**Day42**	**Day49**	**Day56**
0	-0.17[a]	0.01[a]	0.22[a]	0.88[a]	1.48[a]	1.87[a]	1.81[a]	2.12[a]
15	0.48[a]	0.67[a]	1.22[a]	1.24[a]	0.78[a]	0.60[a]	0.69[a]	1.32[a]
30	-0.05[a]	-0.22[a]	-0.19[a]	1.14[a]	1.37[a]	1.62[a]	1.73[a]	1.99[a]
45	-0.03[a]	0.14[a]	-0.04[a]	0.01[a]	0.57[a]	1.27[a]	1.13[a]	1.50[a]
60	-0.08[a]	0.17[a]	0.86[a]	1.14[a]	0.78[a]	1.08[a]	1.10[a]	2.19[a]

* log(cfu/cm^2) is log(final – initial)cfu/cm^2

[a] Values with the same superscript were not significantly different ($p<0.05$), using student's t means comparison. Inoculum level is 2.2 log (cfu/cm^2)

Table 4.5: Statistical comparison of means of cook yield of post-injection tumbled pork loins and injected non-tumbled pork loins, in all treatments, without antimicrobial, with 1% Purasal and with 2% Purasal

	Abs(dif) – LSD*			
Tumble time	**All treatments**	**No Purasal**	**1% Purasal**	**2% Purasal**
0	-5.1	-12.5	-8.35	-8.02
15	-3.04	-11.8	-4.99	-5.85
30	-1.01	-7.01	-5.02	-4.55
45	-1.52	-8.08	-5.35	-4.69
60	-3.07	-11.7	-4.89	-4.59

* If a value is positive, its mean is more than the least significant difference (LSD) apart from the control group mean and is thus significantly different, and vice-versa if negative.

Table 4.6: Statistical comparison of means of total yield of post-injection tumbled pork loins and injected non-tumbled pork loins in all treatments, without antimicrobial, with 1% Purasal and with 2% Purasal

	Abs(dif) – LSD*			
Tumble time	**All treatments**	**No Purasal**	**1% Purasal**	**2% Purasal**
0	-6.13	-15.5	-9.23	-11.5
15	-4.23	-13.0	-5.36	-7.19
30	-2.09	-10.7	-7.29	-6.19
45	-2.00	-12.7	-5.49	-5.72
60	-4.41	-12.0	-6.66	-5.45

* If a value is positive, its mean is more than the least significant difference (LSD) apart from the control group mean and is thus significantly different, and vice-versa if negative.

Table 4.7: Means of cook yield, overall yield and percent moisture content of RTE marinated pork loin.

Treatment	Tumble time (min)	Cook Yield (%)	Overall yield (%)	Moisture (%)
Salt and STPP	NT	82.8	101.5	64
Salt and STPP	15	83.4	99.0	58
Salt and STPP	30	88.3	106.4	63
Salt and STPP	45	87.2	104.4	61
Salt and STPP	60	82.0	98.0	65
Salt, STPP + 1% Purasal	NT	86.0	102.4	63
Salt, STPP + 1% Purasal	15	89.3	106.3	58
Salt, STPP + 1% Purasal	30	89.3	104.4	62
Salt, STPP + 1% Purasal	45	89.0	106.2	60
Salt, STPP + 1% Purasal	60	89.4	104.8	63
Salt, STPP + 2% Purasal	NT	86.1	100.0	63
Salt, STPP + 2% Purasal	15	88.3	104.3	61
Salt, STPP + 2% Purasal	30	89.6	105.3	61
Salt, STPP + 2% Purasal	45	89.4	105.8	64
Salt, STPP + 2% Purasal	60	89.5	106.1	61

Table 4.8: Statistical comparison of means of percent moisture of post-injection tumbled pork loins and injected non-tumbled pork loins in all treatments

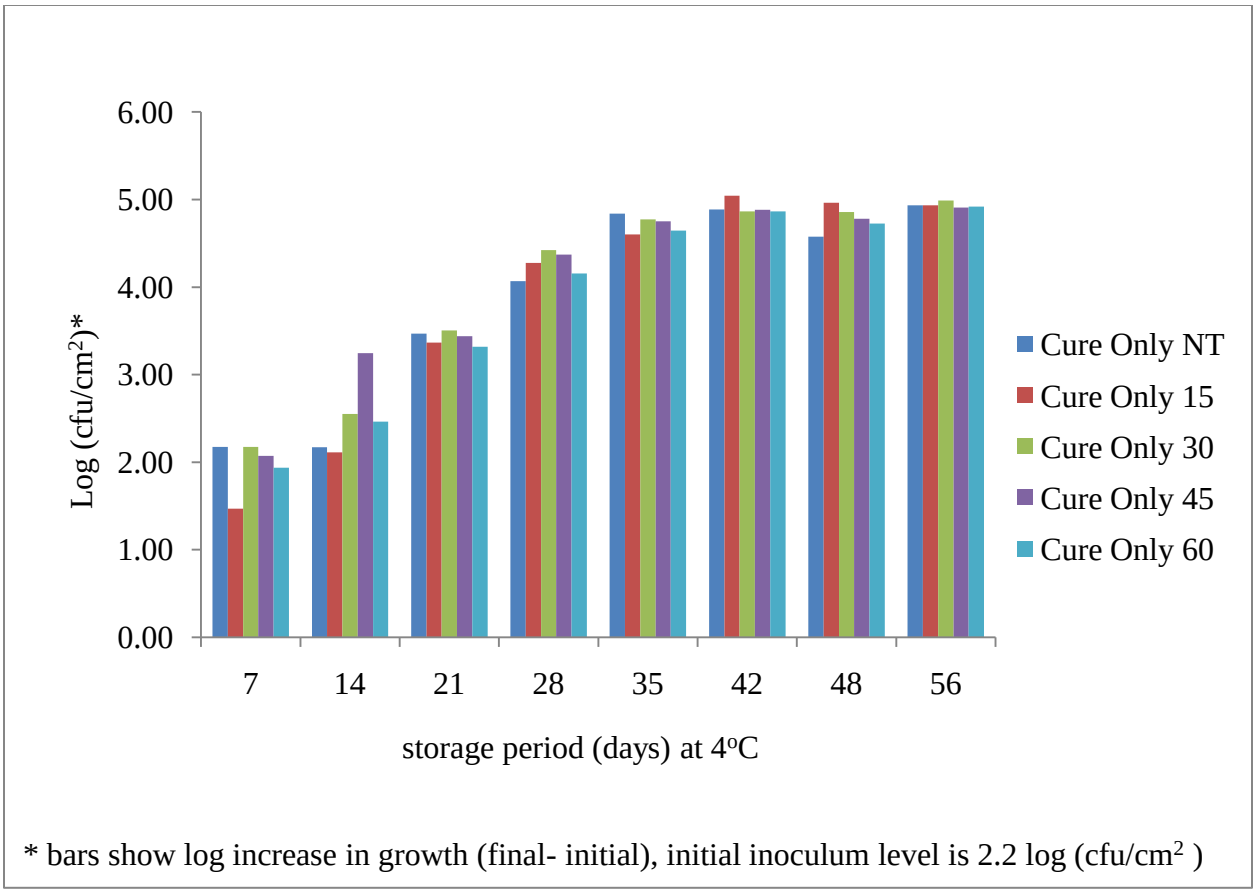
Tumble time	**Abs(dif) – LSD***	**p-value**
0 (control group)	-3.76	1.0000
15	0.58	0.0242
30	-2.42	0.7023
45	-2.09	0.5398
60	-3.42	0.9966

* If a value is positive, its mean is more than the least significant difference (LSD) apart from the control group mean and is thus significantly different, and vice-versa if negative. p-value less than 0.05 shows significant difference from control group.

Table 4.9: Significant interaction effects of tumbling time, salt, STPP, time of storage(days) and Purasal concentration on growth response of *L. monocytogenes*.

Interaction effect	**p-value***
Tumble time (min) x salt	0.0389
Tumble time (min) x STPP	0.0335
Salt x STPP	0.0001
Salt x Time (days)	0.0340
Purasal x Time (days)	<0.0001
Tumble time (min) x Purasal x Time (days)	0.0093
Salt x STPP x Purasal	0.0227
Tumble time (min) x salt x Purasal x Time (days)	0.0075
Tumble time (min) x STPP x Purasal x Time (days)	0.0076

* p-value less than 0.05 shows significant interaction effect between factors



* bars show log increase in growth (final- initial), initial inoculum level is 2.2 log (cfu/cm^2)

Figure 4.1: Effect of tumble duration on growth of *L. monocytogenes* over storage at 4^{o}C in inoculated RTE pork loins marinated with 1.5% salt and 0.45% STPP. NT: No tumble, 15: 15 min tumble, 30: 30 min tumble, 45: 45 min tumble, 60: 60 min tumble.

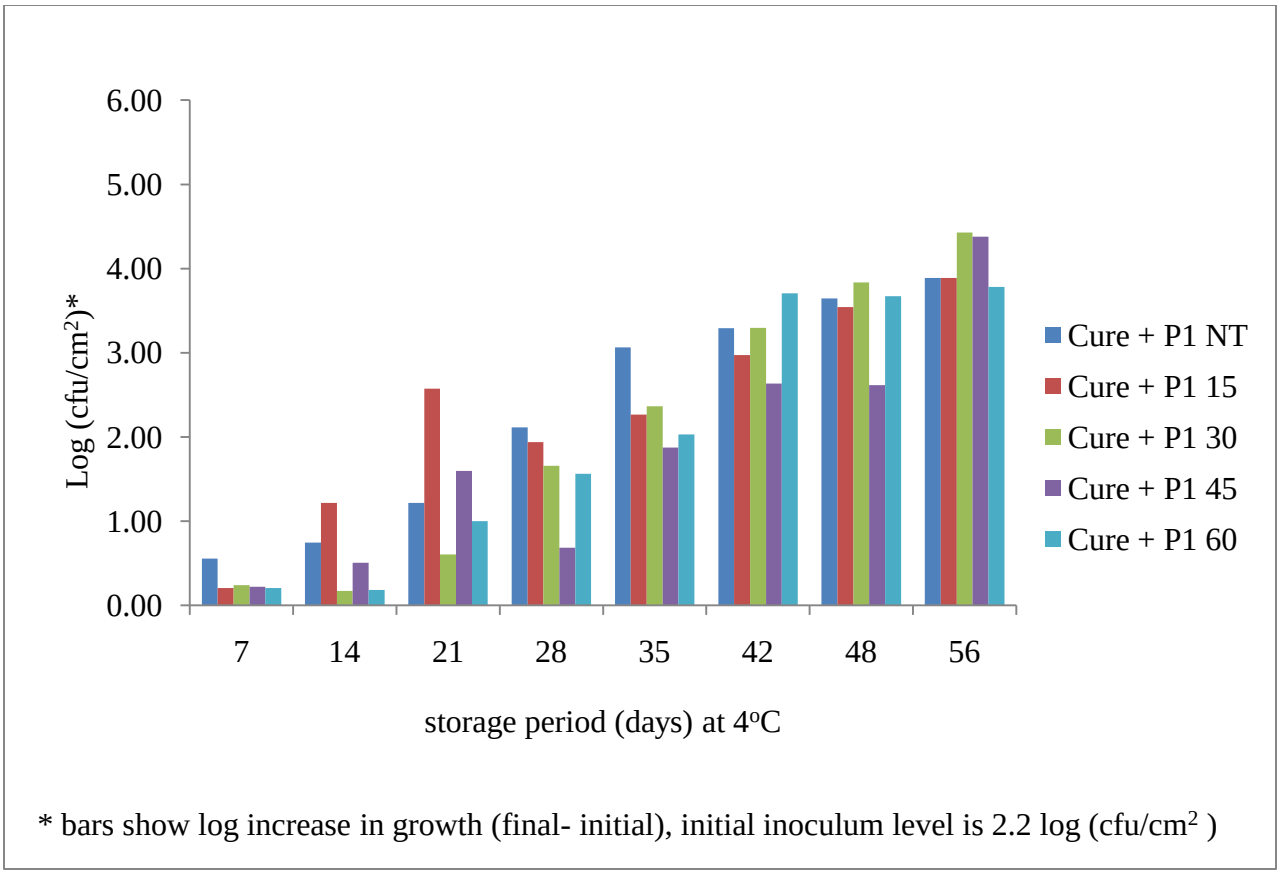


Figure 4.2: Effect of tumble duration on growth of *L. monocytogenes* over storage at 4°C in inoculated RTE pork loins marinated with 1.5% salt, 0.45% STPP and 1% Purasal. NT: No tumble, 15: 15 min tumble, 30: 30 min tumble, 45: 45 min tumble, 60: 60 min tumble.

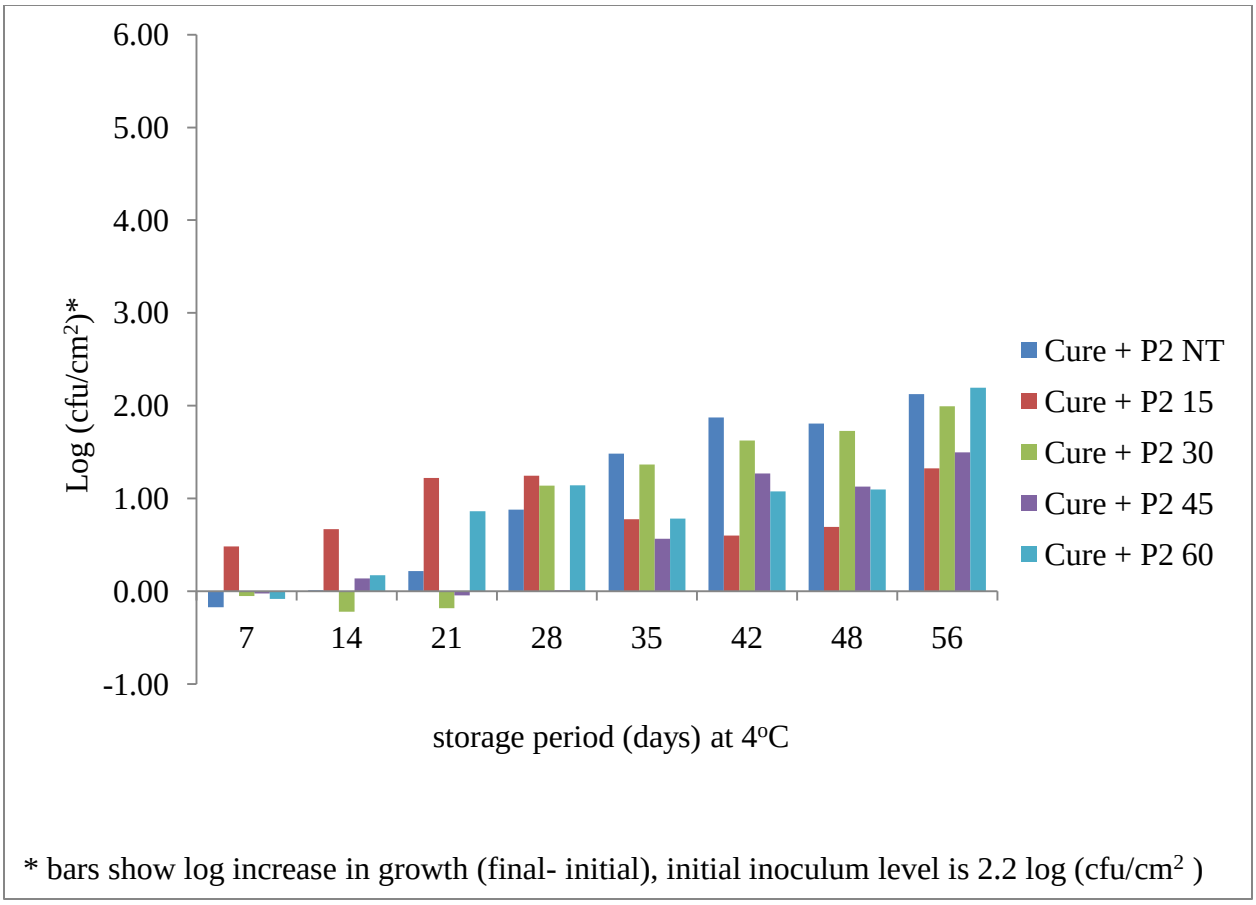


Figure 4.3: Effect of tumble duration on growth of *L. monocytogenes* over storage at 4°C in inoculated RTE pork loins marinated with 1.5% salt, 0.45% STPP and 2% Purasal. NT: No tumble, 15: 15 min tumble, 30: 30 min tumble, 45: 45 min tumble, 60: 60 min tumble.

CHAPTER 5

CONCLUSION

The results of this study showed that when employing tumbling (without pre-injection) as a means of incorporation of marinade ingredients and enhancing quality characteristics of marinated pork loins, meat processors need to be aware of the following; longer tumble time does not necessarily improve or influence cook yield, percent pick-up, fat content and pH of marinated pork loins. Vacuum application during tumbling may increase cook yield but not pick-up. Therefore, vacuum tumbling may not be necessary for processors selling fresh/raw marinated product, while processors aiming to sell RTE products may benefit more from vacuum tumbling. While presence of CPC does not have a significant effect on cook yield and fat content, when these factors are interacting, observations may be different.

Post-injection tumbling and duration of the tumbling does not significantly affect growth of *L. monocytogenes* in RTE marinated pork loin. Post-injection tumbling and its duration did not have a significant effect on cook yield and overall yield. Purasal Hipure P Plus® is an effective antimicrobial solution in inhibiting growth of *L. monocytogenes* in RTE marinated pork loins, as its concentration increases, growth inhibition increases. Purasal is significant in improving cook yield and overall yield of RTE marinated pork loin. Meat processors need to be aware that while a single factor/marination condition may or may not be effective in contributing to quality and safety of RTE meat products, an interaction of the different factors may give a significantly positive or negative effect to the quality and safety characteristics of the product.

Further research should focus on other quality aspects such as texture and sliceabililty of post-injection tumbled RTE marinated loins. Furthermore, similar studies on other commonly purchased muscles of pork meat such as the leg portion used in ham, will be beneficial.