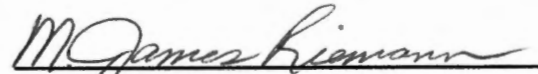


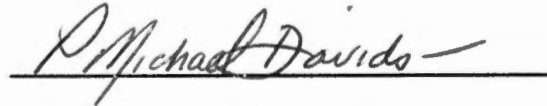
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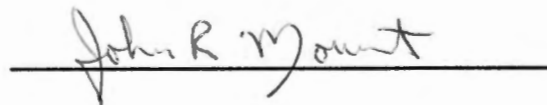
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THE USE OF SODIUM LACTATE AS AN ANTIMICROBIAL
AGENT IN PORK

A Thesis
Presented for the
Master of Science
Degree
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ABSTRACT

Sodium lactate (Na-L) was studied for use as an antimicrobial spray on pre-rigor (PRR) and post-rigor (POR) pork carcasses and as a direct additive to boneless pork for sausage production. Na-L was applied by spraying the carcass immediately post slaughter and/or adding it to the boneless meat. Spraying POR carcasses gave as much as log 1.3 reduction in aerobic plate count (APC), but APC's increased on two of the three PRR carcasses that were sprayed. The third PRR carcass had a 0.3 log reduction in APC. Sausage made from the POR carcasses had lower APC's than sausage from PRR carcasses through 8 days of display (2°C). Generally, the sausage samples that had the lower APC's during the ten-day display period had 3% Na-L added directly to the boneless pork.

Hunter Color Difference Meter "L" values showed the Na-L had a darkening effect on the sausage. Sausage from POR pork had lower "L" values than that from PRR pork. Hunter "a" values showed greater retention of redness in sausage made from POR pork with 3% Na-L only, although changes were not significantly different. Overall, Na-L had no significant effect on pork meat color.

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CHAPTER I

INTRODUCTION

Since prehistoric times, man has yearned for the flavor of meat in his diet. Fresh meat is one of the more perishable food items. Consequently, cave men had to consume a carcass as soon as possible before deterioration began. Whatever was not eaten was left for wild animal consumption or natural decomposition.

Healthy animals provide sterile, edible portions until slaughtering and handling (Price and Schweigert, 1987; Jay, 1986). Upon processing, the carcass becomes subject to microbial contamination by bacteria, yeasts and molds (Ayres, 1955). Shelf-life of meat is mainly dependent on the initial degree of contamination of the carcass surfaces (Woolthuis et al., 1984; Snijders et al., 1985; Woolthuis and Smulders, 1985). Since shelf-life is affected primarily by manufacturing practices, it is valuable to strictly monitor and control the entire process (Snijders et al., 1985; Woolthuis and Smulders, 1985). Critical hazard points should be identified throughout the slaughter line for microbiological evaluation. Previously, processing information was attributed mostly to final product satisfaction.

Porcine epidermis is invariably heavily contaminated. During the scalding and dehairing steps a substantial load of bacteria is removed. Yet, inadequate machinery cleaning and disinfecting lead to considerable carcass recontamination. Therefore, terminal decontamination of meat carcasses is a possibility despite hygienic practices (Snijders et al., 1985; Woolthuis and Smulders, 1985).

Several measures of meat preservation have been acknowledged. Ways of prolonging fresh meat spoilage include refrigeration, dehydration, thermal processing, irradiation and chemical treatment, with refrigeration being most widely utilized (Ingram and Ottoway, 1956). However, during prolonged refrigerated storage, microbial spoilage is still possible.

Consumers prefer purchasing fresh meats. Because fresh meats rapidly perish (Jay, 1986), it is necessary to establish a processing technique to extend the refrigerated shelf-life of these food items without

adversely affecting the quality. A few suggested physical methods involve the spraying of carcasses with cold or hot water or infra-red radiation. Also, chemicals can be beneficial as a post-slaughter line decontaminant. There are reports documented using chlorinated water, glucose, carbon dioxide (storage) and organic food acids (Woolthuis and Smulders, 1985).

Organic acids are chemical agents which provide bacteriostatic conditions when applied to meat due to a more acidic environment (Gill, 1986; ALS, 1982). Acetic, sorbic and lactic acids are some organic acids commonly applied to foods as chemical preservatives (Ingram and Ottoway, 1956).

This research project was developed to determine the effect of sodium lactate as an antimicrobial for pork when used as a carcass spray and as an additive to boneless pork.

CHAPTER II

LITERATURE REVIEW

1. MICROBIOLOGY OF FRESH AND PROCESSED MEATS

Fresh Meat

Postmortem changes. There are several steps in the conversion of muscle to meat. First, the animal is immobilized by a humane stunning technique (Leach, 1985; Price and Schweigert, 1987). Next, it is exsanguinated to remove a large proportion of the blood supply which leads to a change in muscle cells from aerobic to anaerobic conditions (Lawrie, 1979). Within a 24 to 48 hour period after slaughtering normal, rested, healthy animals, a sequence of events occurs at 35 - 40°F (holding room temperatures). Initially, there is circulatory failure to muscle and the inability for adenosine triphosphate (ATP) regeneration. In the absence of ATP, actomyosin is formed from the union of actin and myosin. This bonding contributes to irreversible contraction or stiffening. Secondly, there is a loss of oxygen to muscle. Without the oxygen supply, the oxidation-reduction potential weakens. Thirdly, there is a reduction of antioxidant and vitamin levels. (Jay, 1986). Without antioxidants, agents which retard fatty acid oxidative rancidity (Price and Schweigert, 1987), gradual progression of rancidity results. Next, nerves and hormones are no longer regulated; therefore, there is a reduction in the animal's temperature and this causes fat solidification. Then, respiration terminates, which stops the synthesis of ATP. During glycolysis glycogen is converted to lactic acid decreasing the pH from about 7.4 to about 5.6. The pH depression yields denaturation of protein and completeness of rigor mortis. The exchange of monovalent and divalent cations in muscle proteins accompanies protein denaturation. Finally, when the reticuloendothelial system ceases to sequester, there is potential for microbial growth and protein denaturing metabolites accumulate (Jay, 1986).

Pre-rigor versus post-rigor meat. After death, the composition of muscle tissue changes as rigor develops (Gill, 1986). Completion of rigor mortis is designated by muscle inextensibility and rigidity. Essentially rigor mortis and muscle contraction are the same mechanism, with the exception of rigor normally being irreversible. This "paralysis" causes shortening of the sarcomere (muscle subunit) length which tends to be associated with a decline in tenderness (Price and Schweigert, 1987).

Protein and fat quantities do not change upon rigor development, and are not used for nutrients by microorganisms. Instead soluble components (particularly glycogen and lactic acid of low molecular weight are consumed for bacterial growth. The concentrations of these components are altered considerably during rigor onset. The concentration of glycogen in pre-rigor beef (before completion of rigor) is 10 mg/gm and 1 mg/gm in post-rigor meat. On the other hand, the level of lactic acid (LA) in pre-rigor and post-rigor meat is 1.0 and 9.0 mg/gm, respectively (Gill, 1986).

When animal carcasses are hotboned, the musculature is removed immediately post-slaughter just prior to chilling (West, 1983). This technique allows for energy efficiency, reduced refrigeration costs, less labor, better trim utilization and faster marketing (Reagan, 1983; West, 1983). However, flaccid pre-rigor meat is somewhat more difficult to handle than sturdy post-rigor carcasses (Price and Schweigert, 1987). Yet, hot processing, in comparison to conventional processing, offers superior energy conservation and functional meat characteristics (West, 1983) as well as improved shelf-life (Reagan, 1983).

The microbiological status of hotboned meat has been studied, but the reported findings are extremely varied. Some data suggest lower bacterial levels for hotboned meat in comparison with meat processed in the conventional manner. Reagan (1983) suggests exactly the opposite.

Microbiology of Fresh Meat

Factors affecting growth. All nutrients required for growth of bacteria, yeasts and molds are contained in meats. pH values for red meat

carcasses (beef, 5.1--6.2; pork, 5.3--6.9; lamb, 5.4--6.7) are also within the growth ranges of most spoilage microorganisms (Jay, 1986).

During prolonged refrigeration, microbial spoilage begins. Moisture content, pH, initial bacterial content and temperature are factors influencing microbial spoilage in meats. As these determinants increase, there is a significant decrease in shelf-life (Jay, 1986).

Types of microorganisms. Psychrotrophic bacteria, particularly Pseudomonas, readily grow on surfaces of chilled and some hotboned meat (Price and Schweigert, 1987; Jay, 1986). Bacterial genera most frequently found to cause spoilage of fresh meats include Acinetobacter, Alcaligenes, Aeromonas, Moracella and Pseudomonas, all of which have a negative Gram reaction (Jay, 1986).

The fungal spoilage organisms are the molds Cladosporium, Mucor, Rhizopus, Geotrichum and Thamnidium, and the yeasts Rhotorula, Torulopsis and especially Candida (Jay, 1986).

Effects of microbiology on meat quality. Meat quality refers to the traits determining meat desirability by the consumer (Price and Schweigert, 1987), such as odor, color and texture. When Pseudomonas invades a meat product, it metabolizes amino acids to give meat its characteristic "spoiled" odor. Also, the product undergoes visual and physical alterations. The preferred bright red (oxymyoglobin) color becomes a brown (metmyoglobin) pigmentation and the surfaces become slimy (by protein degradation).

A common type of embedded (deep) spoilage (by Clostridium and Streptococcus) is located near the aitch bone in the hind quarters. Typically this spoilage is labeled "bone sour" (Jay, 1986).

There is an even greater problem associated with bacteria than spoilage--food poisoning. Red meat and poultry have been shown to harbor foodborne pathogens such as Salmonella and Campylobacter (Lammerding et al., 1988). It is estimated that 2 to 20 million Salmonellosis cases occur annually in humans in the United States involving poultry and red meat alone. Though both cause gastrointestinal diseases, Campylobacter occurs most frequently (Price and Schweigert, 1987). In homes, these outbreaks

can be somewhat controlled if proper storage and cooking temperatures are utilized (Jay, 1986).

Two methods of controlling spoilage microorganisms entail dehydration (physical) and lactic acid (chemical). The psychrotrophs can be eliminated by surface drying while hanging in a chill room of less than 80% relative humidity (RH). Bacteria are unable to grow, but after an extended period at these same conditions the fungi tend to predominate (Jay, 1986). Not only are bacteria a problem of surface contamination, but a problem of internal muscle, too.

Processing of Meats

Cutting of carcasses into primal and retail cuts allows for greater microbial contamination because more surface area is exposed. Grinding and chopping meat for pork sausage products greatly multiplies the meat's surface area and further distributes microbial contaminants. Generally, processed meats should be more stable than fresh due to the usual addition of salt and moisture reducing agents (Price and Schweigert, 1987).

Microbiology of Processed Meat

Factors affecting growth. The ideal environment for spoilage bacteria of processed meat is one of high moisture and oxygen content. Oxygen-permeable packages which retain moisture provide this environment. Fortunately some added constituents, such as spices, may reduce the desirability of specific microorganisms to grow (Price and Schweigert, 1987).

Types of microorganisms. Unlike carcass spoilage, processed meats are primarily spoiled by the Acinetobacter-Moraxella-Pseudomonas group. Often processed meats are distributed in oxygen-impermeable films as with pork sausage chubs. As the meat respire the small quantity of trapped oxygen is consumed and converted to carbon dioxide (CO₂). Thus, the aerobic (oxygen requiring) microbial group is inhibited and shelf-life is extended.

Now that conditions favor anaerobic bacterial growth lactic acid bacteria (LAB) dominate the vacuum packaged product.

Effects of microorganisms on meat quality. Only the aerobes produce strong undesirable odors. Consequently, higher levels of LAB are tolerated. In the absence of oxygen, meat takes on the purplish-red myoglobin pigmentation. The average consumer is not well educated on the subject of vacuum packaging and wants a product stored in an oxygen-permeable film (oxymyoglobin). With the production of CO₂ one achieves prolonged rancidity development during frozen storage (Price and Schweigert, 1987).

A common illness associated with pork consumption is trichinosis. The infestation occurs when infected pork is improperly cooked. Therefore thorough cooking eliminates the disease. Never-the-less, since 1966 there are records of 100 to 150 cases of trichinosis documented annually in the United States (Jay 1986).

2. PRESERVATION OF MEAT PRODUCTS

"Throughout history, the problem of food spoilage has plagued man" (Wagner and Moberg, 1989). In the beginning, food preservation involves a wide selection of additives and physical factors such as salts, organic acids, packaging and temperature. The best suited physical preservation technique or antimicrobial is determined by its properties, cost and safety as well as the properties of the food item (Wagner and Moberg, 1989).

Refrigeration

Effect on meat quality. Of all the physical techniques used refrigeration is the most common. Minimal moisture loss (shrinkage) is important for quality retention. The optimal RH lies between 88 and 92% for minimal shrinkage. The meat is less desirable when excessive water is removed due to the darkening and drying effect. To consumers, discoloration is the first sign of spoilage of ground and retail cut of fresh meat (Price and Schweigert, 1987).

Effect on microorganisms. Microbial injury can be induced through the use of chemicals, temperature (chilling, freezing or heating), dehydration and irradiation (Jay, 1986). At low temperatures, chemical and enzymatic reactions cease; thereby retarding microbial growth. However, the ideal RH to preserve meat quality actually promotes microbial development. Yet with high velocity cold air systems one attains both microbial minimization and high humidity. Presumably the rapid cooling of these type systems greater retard mold and slime production. Hence, the product is more stable (microbially) and does not shrink tremendously (Price and Schweigert, 1987).

Other Preservation Techniques

Irradiation. Irradiation is simply electromagnetic energy emission through a food item. Ionizing radiation, like gamma rays, is of importance in the food industry. The gamma rays are least expensive of preserving radiations and are able to penetrate deeply. Because these rays do not elevate temperature extensively in destroying microorganisms, this radiation process is called cold sterilization (Jay, 1986).

Radiated destruction of microorganisms is a result of chemical changes, particularly within the organism's genetic material (deoxyribonucleic acid -- DNA) (Price and Schweigert, 1987). Both yeasts and molds are less sensitive to radiation than bacteria, with yeasts being more resistant than molds. While Gram positive bacteria are most sensitive to radiation, Gram negative bacteria dominate the meat spoilage flora (Jay, 1986).

Packaging. Packages are designed to maintain quality of a product until consumption. In designing a packaging material, the moisture content, microbial load and color are factors taken into consideration. The factor most influential on consumer acceptability is meat color. For meat to express the oxymyoglobin pigment, the package must be highly oxygen permeable. Films of high oxygen permeability are low density polyethylene (LDPE) and plasticized polyvinyl chloride (PVC) (Price and Schweigert, 1987).

Moisture retention is an important factor in maintaining juiciness and flavor. During frozen storage, moisture loss results in freezer burn and off flavors. Yet, at refrigerated temperatures moisture loss decreases microbial spoilage. Meat spoilage depends on the microbial content, its environment and temperature. Therefore, the package determines the environment which determines the type of spoilage (Price and Schweigert, 1987). Vacuum packaging replaces oxygen with CO₂, thus restricting aerobic microorganisms. Now anaerobic LAB will predominate. Thus, the environment is unfavorable for pathogenic and spoilage microorganisms (Jay, 1986).

Antimicrobial Agents

Types. When only a single factor is used for a preservative measure, it must be utilized at an extreme level to be effective. Thus food product development is restricted. If antimicrobial agents are used in combination with physical preservation measures, greater product variety and development is possible (Wagner and Moberg, 1989). Some common antimicrobials that occur naturally in foods are used in food preservation and/or shelf-life extension because they are recognized as safe substances. These naturally occurring antimicrobials are proteins (enzymes), medium chain fatty acids, plant extracts, pigments and organic acids (Beuchat and Golden, 1989).

For years organic acids, whether naturally present or added, have been used as a control component for microbial spoilage. Researchers and scientists believe these acids inhibit microbial growth by pH reduction of the substrate due to the action of the undissociated acid molecule. Also there is probability that the mode of action includes NADH oxidation inhibition, which decreases the amount of reducing agents. Because the undissociated segment of the acid molecule establishes the antimicrobial activity, the effectiveness is determined by the dissociation constant (pK_a). Most organic acids are effective at low pH levels, because their pK_a 's are within the pH range of 3 to 5 (Beuchat and Golden, 1989).

Effect on microorganisms associated with meats. Citric acid is an organic acid found in fruits, particularly citrus fruits. Abian and Graham (1953) reported that thermophilic bacterial growth was more effectively inhibited by citric acid than by lactic or acetic acids. It is also more inhibitory to Salmonella than lactic or hydrochloric acids (Beuchat and Golden, 1989).

In 1945, sorbic acid and potassium sorbate were patented as antimicrobials (Robach and Sofos, 1982). Sorbic acid (sorbate) is used world-wide in a variety of foods including cakes, cheeses and fruit juices. Not only do sorbates have antimycotic activity, but they impart no additional flavor when used at the recommended levels. Since this compound is a metabolizable fatty acid, it is considered safe by GRAS (generally recognized as safe) guidelines. Also, sorbates are excellent antimicrobials for meat products in casings during the drying period (Perry et al., 1964; Robach and Sofos, 1982).

One widely used preservative produced by acetic acid bacteria is acetic acid (Jay, 1986). Its antimicrobial competency is pH dependent. A solution of acetic acid at pH 2.0 and 2.5, when used on pork carcasses, reduced aerobic plate counts (APC's) by 2 logs and 1 log, respectively. An application of pH 3.0 acetic acid was non-functional (Biemuller et al., 1973).

3. USE OF LACTIC ACID (LA) AND SODIUM LACTATE (Na-L) AS ANTIMICROBIAL AGENTS

Properties

Lactic acid is an organic acid produced by lactic acid bacteria (LAB) during the fermentation process for sauerkraut, olives, pickles, cheeses and some meats (Beuchat and Golden, 1989). Commercial lactic acid (2-hydroxypropanoic acid) is a white powder in the pure, dry state. It has a melting point range of 18^o-26^oC. A commercially prepared aqueous, edible form is available in a slightly yellow tint. At 22^oC lactic acid (LA) has a

pK_a of 3.857. LA can be corrosive to stainless steel, provided no resistant (plastic) sheeting is applied beforehand (Smulders et al., 1986).

Pure racemic LA is very seldom found. Yet, through hydrolysis of lactonitrile ($CH_3-CHOH-CN$), a synthetic 50/50 racemic form of L (+) and D (-) LA can be prepared. Glucose and sucrose are the main substrates used in commercial manufacturing of LA. Furthermore, racemization is induced by enzymes of some specific bacteria (Smulders et al., 1986).

LA is a natural constituent of several foods (Smulders et al., 1986). Naturally produced LA is identical to the physiological L (+) isomer of LA that builds up in muscle tissue during glycolysis (Stokes and Slabaugh, 1981). Because it is "natural" (Bacus, 1987; Roberts, 1989) and approved by FDA, it appears on the GRAS list (Woolthuis et al., 1985; Woolthuis and Smulders, 1985).

Production of LA in fresh meat is due to anaerobic metabolism (Smulders et al., 1986). This acid occurs in fermented foods as well. Such foods include breads prepared from sourdoughs, cheeses, sausages (Smulders et al., 1986) and sauerkraut (Jay, 1986). On the average, 0.9% LA content in fresh meats is a result of anaerobic carbohydrate degradation which constitutes the preservative qualities and the characteristic flavor (Smulders et al., 1986).

Homofermentative LAB yield predominantly LA by glucose fermentation. In commercial production of LA this method is most adequate. Lactobacillus delbruckii is the most common bacterium to catalyze the process (Smulders et al., 1986). Heterofermentative LAB produce equal quantities of lactate, ethanol and CO_2 (Jay, 1986). Smulders et al. (1986) claimed the three determinants for the amount of LA synthesized were: 1) the pH values, 2) the bacterial strain and 3) the bacterium's age.

Sodium lactate (Na-L) is simply a hygroscopic salt form of LA. It is available in aqueous, edible grades ranging from 50 to 80% purity. The lower the purity, the more likely the solution is to take on a yellowish tint. Pharmaceutical grades of 88% purity and higher are colorless. Recently developed and marketed, Na-L is available in a white 60% powder form. The pH range of Na-L is 6.5 to 7.5 (Weiner, 1989).

The primary factor affecting the antimicrobial activity of Na-L is water activity (a_w) (Weiner, 1989). The typical a_w of fresh meat is 0.99

(Roberts, 1989). In contrast, many bacteria have a minimum a_w of 0.91 for growth (Wagner and Moberg, 1989). Of all the organic sodium salts, Na-L has the greatest ability to lower a_w . It has also been documented that Na-L has no effect on binding capacity or color (Weiner, 1989), while LA, at >2.4% causes irreversible discoloration of pork (Smulders et al., 1986).

Effect of pH on Antimicrobial Activity

The antimicrobial action of LA is dependent upon three specific factors: 1) the effect of LA on pH; 2) the extent to which the acid dissociates; and 3) a specific form of the acid molecule (Ingram and Ottoway, 1956; Gill, 1986). Some researchers (Grau, 1981; Eklund, 1983; Ingram and Ottoway, 1956) consider the acid's form and environmental pH to be the main determinants of the antimicrobial activity. Gill and Newton (1982) favor only the pH decline effect. The undissociated form of LA is ten times more effective than the dissociated form. The undissociated acid enters a bacterial cell by diffusion and there produces an interior acidification. Dissociated LA simply acidifies the bacteria's environment (Smulders et al., 1986). When applied to meat, LA acts as an acidulant to lower and/or regulate pH and prolong the lag phase of microorganisms, thereby preserving the product (Bacus, 1987; Weiner, 1989).

Effect of Lactic Acid and Sodium Lactate on Microorganisms and Color

Carcasses. By following good manufacturing practices (GMP's), a substantial increase of contaminants onto beef, veal, pork and lamb carcasses should be prevented (Snijders et al., 1985). Unfortunately, this is not always sufficient. The addition of LA or Na-L spray following slaughter may further improve microbial destruction by pH and the Gram positive shift in the microflora (Snijders et al., 1985; Smulders et al., 1986; Wistreich and Olsson, 1981). Woolford (1975) reported that at pH 5.0, LA inhibited sporeforming bacteria, but did not inhibit yeast and mold growth. Aspergillus parasiticus growth was actually increased in the presence of LA up to a 2% concentration (Beuchat and Golden, 1989).

Studies have shown that beef and pork carcasses sprayed with a 1.5% LA solution post-slaughter, had an immediate decrease in the number of microorganisms and a delayed bacteriostatic effect was demonstrated which allowed for an extension of shelf-life.

Woolthuis and Smulders (1985) and Snijders et al. (1985) reported that 2% LA applied 40 to 45 minutes postmortem for 30 seconds resulted in unacceptable color scores for subcutaneous fat trimmings of red meat carcasses but no significant differences in lean meat tissue color or flavor. A 2.5% solution resulted in noticeable lean tissue denaturation. There was no effect on carcass discoloration when a 1.25% LA solution was applied, while providing a marked reduction of microbial impurities. Rusty brown spots appeared on surfaces of carcasses after treatment with high concentrations of LA if the carcass had not been rinsed beforehand. The pH of the carcass was unstable after treatment for two days; yet, within 72 hours the initial color was restored. On pork carcasses discolorations were found when 2.4% LA was used, particularly, in areas where the solution accumulated (Snijders et al., 1985; Smulders et al., 1986; Woolthuis et al., 1984). Snijders et al. (1985) reported that the optimal level of LA for spraying pork carcasses was 1%. These same researchers reported that Pseudomonas, Staphylococcus, coliforms and the pathogens were extremely sensitive to LA.

By-products. Similar results of acceptable color, pH range and decontamination were reported for porcine livers that were immersed in a 0.2% LA solution for 5 minutes then drained, vacuum packaged and stored five days (Woolthuis et al., 1984).

Hotboned pork bellies received 5% LA three hours postmortem and were stored eight days at 7°C. On the sixth day of storage Enterobacteriaceae counts were reduced significantly ($p < 0.01$) by 3 logs (Snijders et al., 1985).

Spraying of veal brains is not recommended. Snijders et al. (1985) sprayed 1.25% LA on brains only to attain small, insignificant microbial reductions. Furthermore, tremendous discolorations occurred after one week of storage.

Applications

Meats. There are three common methods of applying LA. Spraying is the method most often used for carcass treatment. Generally, the carcass is rinsed prior to LA application to remove blood. When LA and blood combine a rusty brown color develops, which may lower color scores.

Electrostatic dispersion is a second way to apply LA. This method utilizes higher concentrations and lower volumes of LA treatment of carcasses or primal cuts.

By-products are typically immersed into a LA solution. This process ensures a well distributed solution to better decontaminate the product. However, the solution gradually diminishes its antimicrobial ability by the buildup of lactate ion-peptide complexes in the immersion tanks (Smulders et al., 1986).

Poultry. Antimicrobials are just as important in poultry processing as they are in the red meat industry. Fresh refrigerated poultry has a relatively short shelf-life (Robach and Sofos, 1982). Several scientists have researched poultry carcass preservation through the use of lactic, sorbic, acetic, adipic, succinic, phosphoric, citric and hydrochloric acids (Mountney and O'Malley, 1964). Although spoilage is delayed at 3°C, at 4°C poultry parts undergo limited spoilage control when dipped in either sorbic or lactic acids (Patterson et al., 1984). Adipic, acetic and succinic acids were most effective at extending shelf-life, while lactic, sorbic and hydrochloric acids were best at bacterial growth suppression (Mountney and O'Malley, 1964). However, the best overall results are awarded to adipic and succinic acids with acetic being unacceptable (pungent odor and skin hardening) (Biemuller et al., 1973; Mountney and O'Malley, 1964).

Regulations

At this point, the only requirement is that lactic acid or sodium lactate be used at the recommended levels and be printed on the package (label) of the treated product (CFR, 1988).

CHAPTER III

MATERIALS AND METHODS

Experimental Animals and Treatments

Six slaughter hogs were acquired from The University of Tennessee-Knoxville, Experiment Station and slaughtered at the UT Meat Laboratory using the scalding and dehairing procedure for slaughter.

The carcasses were split completely so six sides could be used as pre-rigor (PRR) sides and six sides used as post-rigor (POR) sides (Figure 1). Three PRR and three POR sides were each totally sprayed with 500 ml of 1% (vol/vol) sodium lactate (Na-L) from a distance of 20 cm using a pressurized hand sprayer. The Na-L spray was prepared from a 60% (w/w) stock solution (TruMark, Inc., Linden, NJ). The remaining three PRR and POR sides were not sprayed. The six PRR sides were boned two hours after slaughter and the six POR sides were chilled (2.5°C) 24 hrs before boning. Boneless meat from each individual side was kept separated and ground through a 0.75 in. grinder plate. Aliquots (20 lbs) of boneless meat from each of the four rigor-spray combinations were chosen randomly and seasoned using a commercially prepared sausage seasoning blend. Each aliquot was divided in half and one portion (10 lbs) of each aliquot received 3% (by weight) of the 60% stock Na-L solution and the other portion (10 lbs) served as a control. After thorough mixing, each of the eight batches was ground through a 3/16 in. grinder plate. A 0.75 lb aliquot was then collected, placed in a tray, covered with a transparent film and used for microbiological evaluation.

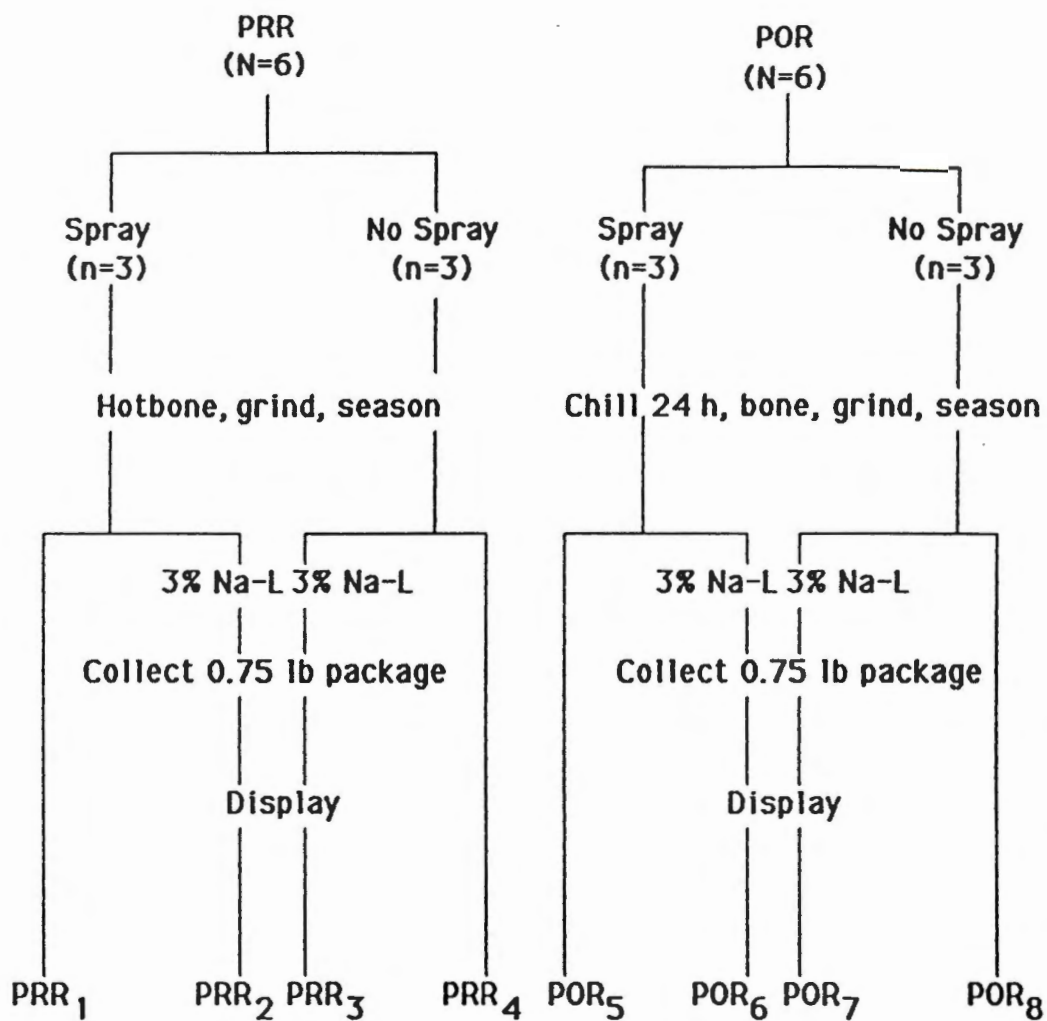


Figure 1. A Schematic Representation of the Experimental Procedure.

Microbiological Evaluation

Carcasses. To determine effectiveness of the spray as an antimicrobial, samples were obtained before and after Na-L application to the sides for microbial examination. The three sampling sites on the chine side of each pork side were: 1) posterior to the aitch bone including ham fat collar and ham face, 2) lean and fat area of the dorsal process of the third thoracic vertebra and 3) an area anterior to the first rib and ventral to the last cervical vertebra. The sampling sites were outlined by a 4 cm² template and swabbed aseptically with a peptone diluent moistened sterile cotton tipped applicator. The unhandled swab portion was transferred into a test tube of 4 ml sterile peptone, vortexed 30 second, stored and transported on ice to the laboratory. Aliquots (0.1 and 1.0 ml) were dispensed into petri dishes to which melted (46°C) Standard Methods Agar (SMA) was added. Plates were inverted and incubated 48 hrs at 32.0°C. Aerobic plate counts (APC's) were recorded as log CFU per cm² per location. The POR "after" samples were not taken until after the 24 hour chill period. The PRR "after" samples were obtained 30 minutes after treatment.

Sausage. Each sausage sample was displayed at 20°C for 10 days. On alternate days (day 0, 2, 4, 6, 8 and 10) of display, a microbial analysis was conducted on a single package of sausage. A 25gm aliquot of ground sausage was placed into a sterile stomacher bag containing 225 ml sterile 0.1% peptone diluent and blended (Stomacher 400, Trekmar, Inc., Cincinnati, OH) 2 minutes. The APC's were determined using SMA pour plates incubated at 32°C for 48 hrs.

Color Evaluation

Color of the sausage was studied using a HunterLab D25-2 Color-Difference Meter (Hunter Associates Lab, Inc.) which was standardized with a pink colored calibrated standard (L=68.4; a=16.0; b=9.4) tile to most closely match the color of the sausage. This allowed for increased sensitivity to differences among samples that were essentially the same in color. Treatment samples representative of each display period (day 0, 2, 4, 6, 8 and 10) were measured and recorded. The Hunter L, a and b values of each were compared.

Identification of Treatments

Individual treatments will be identified as PRR1, PRR2, PRR3, PRR4, POR5, POR6, POR7 and POR8. They are defined as follows:

Treatment 1 is PRR1 - - pre-rigor, 1% Na-L spray only.

Treatment 2 is PRR2 - - pre-rigor, 1% Na-L spray + 3% added Na-L.

Treatment 3 is PRR3 - - pre-rigor, 3% added Na-L only.

Treatment 4 is PRR4 - - pre-rigor, no Na-L (control).

Treatment 5 is POR5 - - post-rigor, 1% Na-L spray only.

Treatment 6 is POR6 - - post-rigor, 1% Na-L spray + 3% added Na-L.

Treatment 7 is POR7 - - post-rigor, 3% added Na-L only.

Treatment 8 is POR8 - - post-rigor, no Na-L (control).

Statistical Evaluation

Differences in APC and color due to sodium lactate treatments were assessed using Analysis of Variance. Significance among the means of each treatment was determined using Student Newman Keuls (SNK) tests.

CHAPTER IV

RESULTS AND DISCUSSION

Microbiological Evaluation

Carcasses. Results of the effect of 1% Na-L spray on the bacterial load of PRR carcasses are shown in Table 1. Log colony forming units (CFU's) for PRR carcass 1 increased significantly 30 minutes following the spray application. PRR 2 also had an increase in CFU's but the variability in counts at the three sample sites was so large that the difference was not significant statistically ($P > .05$). Other researchers reported an immediate lethal effect of LA on aerobic bacteria when sampled 45 minutes after treatment (Woolthuis and Smulders, 1985; Snijders et al., 1985; Smulders and Woolthuis, 1985; Smulders et al., 1986). Apparently, 30 min was not sufficient time for the Na-L spray to take effect. In immediate action studies performed by Smulders and Woolthuis (1985) the "after" samples were obtained 2.5 hours post-mortem. For PRR 3 there was a decline in log APC; however, it was not significant ($P > .05$). When bacterial loads are initially low like these APC's Na-L does not strongly reduce their growth (Woolthuis et al., 1984; Smulders and Woolthuis, 1985).

For POR carcasses 1 and 2, significant reductions in log CFU's ($P < .05$) occurred (Table 2). Although the log CFU of carcass 3 was reduced, the reduction was not significant ($P > .05$).

Sausage. Throughout the ten-day display period, the CFU's for each of the eight treatments increased steadily (Figure 2). This most likely occurred because Na-L is bacteriostatic not bactericidal (Gill, 1986). The lactate treatment had a greater influence on POR sausage than on PRR sausage. Figure 2 represents the PRR mean log APC's along with their standard deviations. PRR sausage containing both 1 and 3% Na-L and 1% Na-L treated sausage displayed faster growth rates than the control between days 4 and 6. At 6 days of display, PRR2 (1% + 3% Na-L) began to level off and slightly decrease in log APC while PRR1 (1% Na-L only) did not stabilize until day 10. However, the 1% Na-L sausage sample was at spoilage (log 7

Table 1. The Effect of Sodium Lactate Spray on Reduction of Aerobic Plate Count^a (log CFU/cm²) on Pre-rigor Pork Carcasses

Carcass	Before	After (30 min)	Difference
PRR 1	2.5	3.0	-0.5 ^b
PRR 2	2.4	3.0	-0.6
PRR 3	2.5	2.2	0.3

^aMeans of the three sampling locations.

^bDenotes significant difference ($P < .05$) between before and after aerobic plate counts

Table 2. The Effect of Sodium Lactate Spray on Reduction of Aerobic Plate Count^a (log CFU/cm²) on Post-rigor Pork Carcasses

Treatment	Before	After (24 hr)	Difference
POR 1	2.8	1.5	1.3 ^b
POR 2	3.1	2.0	1.0 ^b
POR 3	2.7	1.7	0.9

^aMeans of the three sampling locations.

^bDenotes significant difference ($P < .05$) between before and after aerobic plate counts.

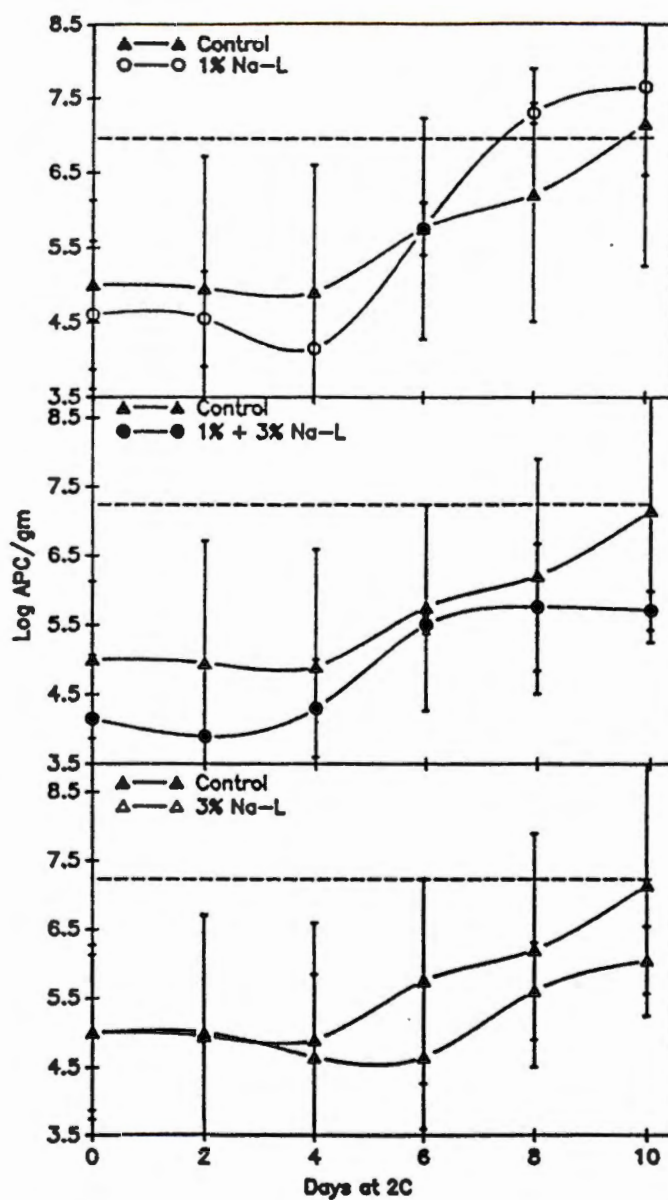


Figure 2. Aerobic Plate Count (APC) of Pork Sausage for Each Pre-rigor (PRR) Treatment Over a Ten-day Display Period.

CFU/gm) before day 8. Sausage receiving 3% Na-L (only) was most responsive to the treatment in terms of APC reduction. That is, counts were actually lowered beyond the initial ones. Yet, PRR2 (1% +3%) maintained the lowest level of microbial contamination throughout the 10-day display period for pre-rigor pork sausage. Both PRR2 (1+3% Na-L) and PRR3 (3% only) had an extended shelf-life beyond 10 days when stored at 2°C.

Initial APC's for PRR sausage did not differ much from that of POR sausage. All APC's for the POR sausage treatments were reduced below the control due to Na-L application (Figure 3). Yet, the POR sample containing the most Na-L (POR6) was less sensitive to Na-L's antimicrobial activity. Its curve closely followed that of the control until the final sampling period when there is nearly a 1 log APC difference. Shortly after 6 days of display, the 1% Na-L sausage (6.8 CFU/gm) nearly reached spoilage levels. By day 10 a drastic decline in APC was seen, as the control followed an upward trend. The sausage sample containing 3% Na-L only (POR7) produced the best curve. POR7 and the control started with similar CFU's and continued on a parallel pattern until day 8, when the APC's were, again, essentially one in the same. From that point on, the curves moved in opposite directions at relatively the same rates. The 3% Na-L sample decreased in APC to approximately 4.5 log CFU/gm (lower than its initial count), while the control (about 6.7 CFU/gm) headed towards microbial spoilage. Thus, 3% Na-L significantly reduced the APC of post-rigor sausage and extended the shelf-life (at least 10 days) of fresh ground pork.

The overall mean APC's for the sausage from each treatment over the ten-day display period are shown in Table 3. The values in the control column for the PRR (6.8) and POR (6.6) treatments represent the overall means of the sausage that had no Na-L which were PRR4 and POR8. Of the PRR treatments, PRR2 (spray and 3% added Na-L) had the lowest overall mean APC across the total display time. When compared to PRR4, both PRR1 (spray only) and PRR3 (3% added Na-L) had much lower mean APC's. The POR treatments had less reduction in mean APC's than the PRR treatments over the ten days of display. POR7 (3% added Na-L) had the largest reduction (1.0) in mean APC followed by POR6 (spray and 3% added Na-L). The spray only treatment (POR5) showed the smallest (0.2) reduction in mean APC.

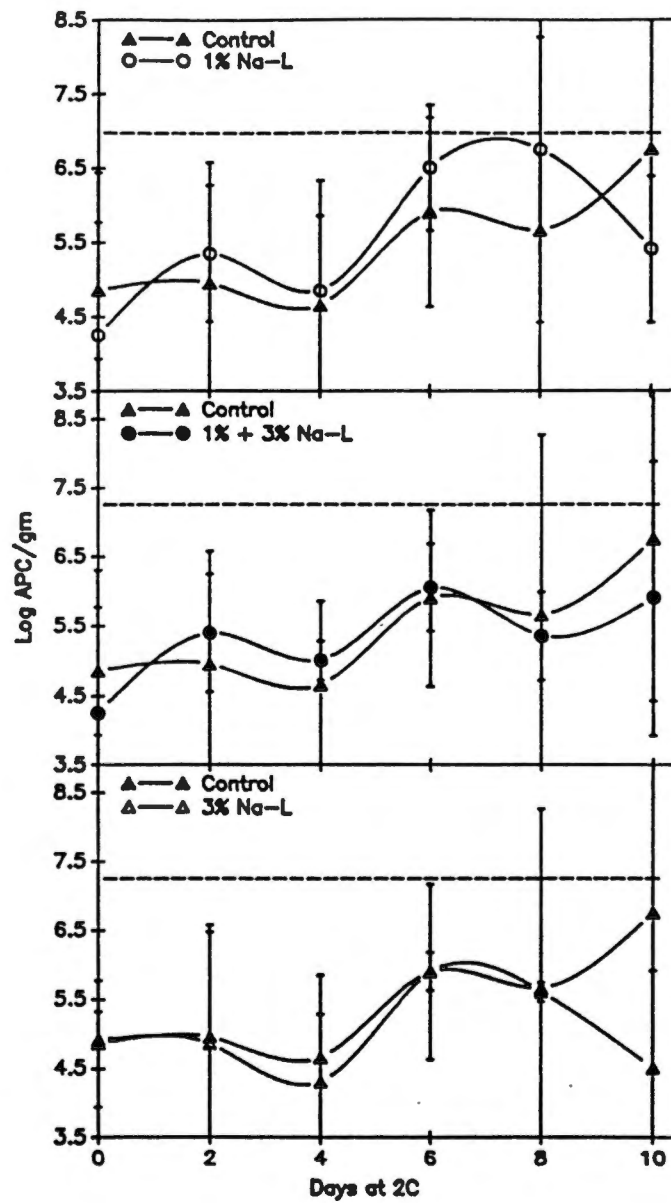


Figure 3. Aerobic Plate Count (APC) of Pork Sausage for Each Post-rigor (POR) Treatment Over a Ten-day Display Period.

Table 3. Overall Mean APC for Sausage Displayed (20°C) Ten Days in Overwrapped Trays.

Treatment	Na-L		Mean Log APC		Difference
	Spray	Add	Control	Experiment	
PRR1	1%	-	6.8	5.6	1.2
PRR2	1%	3%	6.8	4.5	2.3
PRR3	-	3%	6.8	5.9	0.9
POR5	1%	-	6.6	6.4	0.2
POR6	1%	3%	6.6	5.8	0.8
POR7	-	3%	6.6	5.6	1.0

Color Evaluation

Carcasses. Initially, it appeared visually as if the carcasses showed slight lean tissue discoloration during initial Na-L application, but the color returned to normal. Weiner (1989) reported that Na-L had no effect on color.

Sausage. Hunter "L", "a", and "b" values for combined PRR and POR sausage samples at each display time are given in Table 4. Lightness ("L" values) of the sausage samples did not change during the ten days of display. The "L" value indicates the degree of lightness/darkness, with 100 representing white and 0 representing black. The redness ("a" values) of the sausage decreased ($P < .05$) greatly over the ten days which indicated the lean portion became oxidized and developed more metmyoglobin or brownish color with each successive display period. The yellowness ("b" values) of the combined PRR and POR samples was greatest ($P < .05$) at the beginning (0-day) of the display period and differed from the 8- and 10-day samples.

The means of Hunter values across all display times for each treatment are given in Table 5. The Hunter "L" values show the sausage from PRR treatments was lighter than sausage from the POR treatments. This was an obvious difference and occurred as a result of fat smearing during grinding of the PRR meat. However, there were also differences among the PRR and POR treatments. Sausage samples from PRR1 (spray only) and PRR4 (control) were lighter ($P < .05$) than both PRR2 (spray and 3% added Na-L) and PRR3 (3% added Na-L). This data indicates that the 3% added Na-L may have darkened (lowered "L" values) the lean and fat of the PRR2 and PRR3 samples. There is some support for this conclusion in the POR data. The lightest colored sample was POR8 (control). The other three POR samples all received Na-L as a carcass spray and/or as a direct additive and were darker ($P < .05$) colored than POR8. The POR samples were more ($P < .05$) red ("a" values) than the PRR samples which showed no differences between the treatments. Of the POR treatments, POR7 had the most redness ($P < .05$) and POR8 the least, although values did not differ significantly for the other two treatments. The "b" values were higher for the PRR than the POR sausage samples. The PRR1 sausage sample was more ($P < .05$) yellow than any of the other PRR samples. The POR8 sample, which was the lightest

Table 4. Mean Hunter Color Values by Display Time for Pork Sausage From PRR and POR Pork.

Display (days)	Hunter color value		
	L	a	b
0	57.1 ^a	8.8 ^a	14.0 ^a
2	57.5 ^a	6.6 ^b	13.6 ^{ab}
4	57.4 ^a	5.4 ^c	13.4 ^{ab}
6	57.6 ^a	4.5 ^d	12.9 ^{ab}
8	56.9 ^a	3.4 ^e	12.4 ^b
10	56.5 ^a	3.0 ^e	12.6 ^b

a,b,c,d,e Column means with the same letter are not different ($P < .05$).

Table 5. Mean Hunter Color Values for Pork Sausage From PRR and POR Pork Over a Ten-day Display (2C) Period.

Treatment	Na - L		Hunter color value		
	Spray	Add	L	a	b
PRR1	1%	-	62.3 ^a	4.6 ^c	14.5 ^a
PRR2	1%	3%	60.4 ^b	4.5 ^c	13.6 ^b
PRR3	-	3%	59.2 ^c	4.8 ^c	13.7 ^b
PRR4	-	-	62.4 ^a	4.2 ^c	13.6 ^b
POR5	1%	-	52.1 ^f	6.1 ^{ab}	12.1 ^e
POR6	1%	3%	52.4 ^{ef}	6.1 ^{ab}	12.3 ^{de}
POR7	-	3%	53.3 ^e	6.2 ^a	12.6 ^{cd}
POR8	-	-	55.4 ^d	5.6 ^b	12.9 ^c

a,b,c,d,e,f Column means with the same letter are not different (P<.05).

colored, also had the highest "b" value of the POR samples, although it was not different from POR7.

CHAPTER V

SUMMARY

The use of sodium lactate (Na-L) as an antimicrobial proved to be advantageous for both pre-rigor and post-rigor pork. Spraying 1% Na-L on carcasses resulted in a decrease in APC for POR carcasses but not PRR carcasses. Sausage made from either PRR or POR carcasses, that had been sprayed with 1% Na-L, generally did not differ much in APC's in the first eight days of display than sausage from the control carcasses. APC's were also lower for sausage to which 3% Na-L was added directly to the boneless pork before grinding. Thus 3% Na-L significantly reduced the APC of post-rigor sausage and extends the shelf-life of fresh pork sausage for at least 10 days.

Na-L treated sausage maintained its (lean) red content better than untreated sausage, although not significantly. The Hunter "L" values, which measure lightness of the sample, indicate that Na-L may cause some darkening of the lean. Also, sausage made from POR pork was darker than sausage made from PRR pork. Overall Na-L had no significant effect on pork meat color.

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