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# EFFECTS OF SODIUM LACTATE AND ACETIC ACID DERIVATIVES ON THE QUALITY AND SENSORY CHARACTERISTICS OF HOT-BONED PORK SAUSAGE PATTIES

By

Emily McFall Bradley

A Thesis Submitted to the Faculty of Mississippi State University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science, Nutrition and Health Promotion in the Department of Food Science, Nutrition and Health Promotion

Mississippi State, Mississippi

May 2010

# EFFECTS OF SODIUM LACTATE AND ACETIC ACID DERIVATIVES ON THE QUALITY AND SENSORY CHARACTERISTICS OF HOT-BONED PORK SAUSAGE PATTIES

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Sodium lactate and acetic acid derivatives were evaluated for their effects on color retention, microbial growth (TPC), oxidation (TBARS), and sensory attributes of hot-boned pork sausage patties that were stored under retail store display conditions over time. Treatments included: (a) 2.5% sodium lactate 60% solids (L), (b) 2.5% buffered vinegar pH 6.5-8.0 (V), (c) 2.5% sodium lactate and vinegar 52/48% mixture (LV), (d) control with 0.02% BHA/BHT (C), and (e) negative control without additives (NC). Overall acceptability of day 17 LV and L treatments were not different (P>0.05) from day 14 treatments. These results revealed that the L and LV sausage patties retained sensory acceptability and microbial quality from day 14 through day 17 as opposed to other treatments. Additionally, sausage patties with 2.5% LV maintained color (redness) and overall acceptability throughout 17 days of shelf-life when held in retail conditions, when compared to other treatments.

### DEDICATION

I would like to dedicate this research, and the education required to reach this level to my late great aunt, Emma Porter Armstrong. It is through her will and support that I was given a chance for higher education.

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## TABLE OF CONTENTS

DEDI	CATION	ii
ACKI	NOWLEDGEMENTS	iii
LIST	OF TABLES	vii
LIST	OF FIGURES	ix
CHAI	PTER	
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	4
	History of Fresh Pork Sausage	4
	Production of Fresh Pork Sausage	5
	Microbiology of Fresh Sausage	
	Contamination	
	Microbiological Flora	
	Pseudomonas spp	
	Listeria monocytogenes	
	Salmonella spp	
	Lactobacillus spp.	
	Escherichi coli and coliforms	
	Molds and Yeasts	14
	Trichinella spiralis	14
	Spoilage Deterioration	15
	Antimicrobial Additives	16
	Sodium Lactate	17
	Acetic Acid	19
	Oxidative Rancidity	20
	Addition of Antioxidants	21
	2-Thiobarbituric Acid Reactive Substances (TBARS)	22

	Sensory Descriptive Analysis of Fresh Pork Sausage	24
	Relationship of Antimicrobials and Antioxidants to Sensory Descriptors	
	and Consumer Acceptance	27
	Principal Components Analysis (PCA)	
	Cluster Analysis	
	Product Development	28
III.	MATERIALS AND METHODS	29
	Materials	29
	Sample	29
	Retail Display	30
	Microbial Supplies	31
	2-Thiobarbituric Acid Reactive Substance	31
	Chroma-Meter	32
	Methods	32
	Microbial Analysis	32
	Instrumental Color Analysis	33
	Thiobarbituric Acid Reactive Substance Values	
	Sensory Sample Preparation	
	Descriptive Sensory Analysis	
	Consumer Acceptability	
	Statistical Analysis	38
IV.	RESULTS AND DISCUSSION	39
	Microbial Analysis	39
	Instrumental Color Analysis	
	Thiobarbituric Active Reactive Substances Values	47
	Sensory Evaluation	48
	Consumer Acceptability	56
V.	SUMMARY AND CONCLUSIONS	58
REF	ERENCES	60
APP	ENDIX	67

## LIST OF TABLES

3.1	Prepared standard solutions with increasing concentrations of tetramethoxy- propane that were used to evaluate the oxidative rancidity of fresh pork sausage samples by measuring absorbance 532 nm using a UV-VIS spectrometer
3.2	Fresh pork sausage sensory descriptors and definitions
4.1	Mean TPC (log CFU/g), over time of fresh pork sausage patties40
4.2	CIE $L^*a^*b^*$ color values, over time for fresh pork sausage patties stored up to 18 days at 1-2°C
4.3	Thiobarbituric acid reactive substances (TBARS) values over time for fresh pork sausage patties stored up to 18 days at 1-2°C46
4.4	Significant sensory descriptive attributes for aroma and oral texture, over time, of cooked fresh pork sausage49
4.5	Significant sensory descriptive attributes for basic tastes and flavors, over time, of cooked fresh pork sausage
4.6	Overall acceptability over time of cooked fresh pork sausage
4.7	Sensory descriptive attributes of cooked fresh pork sausage showing no significant difference (P>0.05) between treatments over time
4.8	Consumer panel means for fresh pork sausage C and LV treatments
4.9	Cluster analysis of consumer panel for fresh pork sausage C and LV treatments
A.1	Coliform log CFU/g for each replication75
A.2	Mean TPC's log CFU/g for treatments over time for each replication of fresh pork sausage patties

A.3	Mean values of CIE $L^*a^*b^*$ over time for each replication	78
A.4	Mean TBARS values over time for each replication	37
A.5	Means of sensory descriptive attributes for each replication	<del>)</del> 0

## LIST OF FIGURES

4.1	Principal components analysis biplot of sensory descriptive analysis data over time and treatments, control, and negative control of	
	fresh pork sausage	54
A.1	Informed consent form	68
A.2	Score sheet for descriptive sensory analysis	69
A.3	Score sheet for consumer acceptability tests	73
A.4	Dendrogram showing grouping of panelists into clusters based on dissimilarity of consumer acceptability scores between 2.5% sodium lactate/acetic acid and control fresh pork sausage patties	

# CHAPTER I

#### INTRODUCTION

Fresh pork sausages are typically uncured, comminuted, seasoned with salt, pepper, and sage, and stuffed into casings: Examples are bratwurst, bockwurst, or breakfast sausage. Breakfast sausage is common to the Southern U.S. and common seasonings are salt, pepper, and sage. The Code of Federal Regulations stipulates that fresh pork sausage is limited to 50% fat and 3% added moisture (9 CFR §319.141).

Due to the lack of antimicrobial additives, semi-processed meats are limited in shelf-life. According to the American Meat Institute (1987), the production of fresh pork sausage makes up about 90% of all sausage production in the United States. Off-odors, loss of color, and any other unsightly characteristics that develop while under retail conditions are responsible for a decline in consumer acceptability and a finite shelf-life. These characteristics predominately occur due to microbial growth and lipid oxidation of the product. Romans et al. (2001) stated that components of meat provide an ideal environment for microbial growth due to inherent high moisture content, mineral supply, and favorable pH. Surface area availability is also a factor in the proliferation of microbial growth. Large surface area allows for greater growth; especially in ground meats. Ground meats, like pork sausage, expose the maximum amount of surface area, and can have greater incidence of microbial contamination (Romans et al., 2001). During production, programs like Hazard Analysis and Critical Control Points (HACCP) are utilized to identify possible contamination points and to provide steps to reduce incidence of contamination. Most research on microbial spoilage in comminuted meats has been done on beef (Price and Schweigert, 1978). Microbial flora in ground meat products, such as beef, are predominately psychrophilic organisms; the most common being *Pseudomonas, Achromobacter, Flavobacterium*, and *Lactobacillus* (Price and Schweigert, 1978; Papadopoulous et al., 1991c; Liu et al., 2006).

Antimicrobials and antioxidants are added by some processors to fresh processed meats to extend shelf-life and in some cases replacing nitrite. Many studies have been conducted on the decontamination properties of sodium lactate and acetic acid in the form of spray washes on carcasses. Antimicrobial spray washes have the capability of reducing bacterial growth and extending shelf-life. Sodium lactate is an undissociated acid believed to pass through the microbial membrane to acidify the cellular interior (Hunter and Segel, 1973). Lamkey et al. (1991) produced fresh pork sausage chub packs with 3.0% sodium lactate which extended shelf-life up to two weeks over control pork sausage chubs. An extensive study by Brewer et al. (1991) reported that sodium lactate (2-3%) is responsible for reducing microbial growth, pH deline, and uncharacteristic off-flavors by 7-10 days compared to a control. Acetic acid use has demonstrated the ability to inhibit *Listeria monocytogenes* more so than with the use of lactic acid (Ita and Hutkins, 1991). Use of acetic acid was capable of a 4 log reduction at a pH of 3.5, while lactic acid reduced growth by only 1 log (Ita and Hutkins, 1991). It is believed that the

inhibitory ability of acetic acid on *Listeria monocytogenes* is due to its higher pKa value (4.76) compared to lactic acid (3.86) (Ita and Hutkins, 1991).

Oxidative rancidity is the second major cause of reduced quality for fresh meat. Rancidity occurs through the reaction of unsaturated fats with oxygen (Kramlich et al., 1973; Cheng et al., 2007). This reaction is influenced by heat, light, and pro-oxidant compounds found in the ingredients (Price and Schweigert, 1978). Control of oxidative rancidity is provided through the use of antioxidants. The more common antioxidants which are widely used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate. However, synthetic antioxidants that are heavily regulated and usage levels are found in the Code of Federal Regulations. Naturally occurring antioxidants can be found in rosemary, borage, green tea, or Pu-erh tea (Martinez et al., 2006). The use of these antioxidants have become more frequent over the last 5 -10 years due to the demand for all natural products.

The objective of this study was to use sodium lactate, acetic acid, and a combination of sodium lactate and acetic acid in fresh pork sausage to determine the extent of shelf-life capabilities while maintaining color and sensory acceptability. The objective was met by measuring the amount of bacterial growth over time, deterioration of color in simulated retail conditions, oxidative rancidity over time, and identifying and describing sensory characteristics. Principal components analysis was utilized to explain the relationship of descriptors over time, and cluster analysis was used to determine consumer preference.

3

#### CHAPTER II

#### LITERATURE REVIEW

#### **History of Fresh Pork Sausage**

Sausage is a comminuted, seasoned meat product whose origins predate written records. The term sausage is rooted in the Latin term *salus*, which means salted or preservation by salting (Price and Schweigert, 1978). From the beginning, sausage mixtures have been characteristically cylindrical in shape, differentiating them from other meat products. With advances in refrigeration, the volume of sausage production has drastically increased over time.

Differences in geological regions and availability of resources led to the creation of a wide variety of sausages, and they are best classified according to processing procedures. There are four main categories of sausages: dry, semi-dry, fresh, and cooked. Warmer climates of Italy and Spain led to the development of dry and semi-dry sausages, while cooler climates such as Germany, Austria, and Denmark developed fresh and cooked sausages (Romans et al., 2001). Availability of spices from the Far East, and sausage makers called *wurstmachers*, were responsible for the creation of distinct sausage types throughout Europe. These sausages were commonly named after the cities and towns in which they originated (Romans et al., 2001). Meat processing in the United States developed from the emigration of peoples, railroad construction, the Civil War, and the development of refrigeration (Price and Schweigert, 1978). These events allowed for centralizing processing sites, raising capital, and providing a means for more effective transportation of fresh meats. Mainstreaming meat processing over time has allowed for increased volumes of fresh meat available, thus increasing the amount of sausage production in the United States.

It has been estimated that one out of each ten pounds of fresh meat is used in the production of sausage (Price and Schweigert, 1978). Sausage production in modern times has become diversified in the manufacture and operation, and most production combines the original art of sausage making with scientific understanding. Most production plants have become highly mechanized to handle large volumes of production much more efficiently than in the past. The science of muscle foods has become a mainstay in the formulation of sausage, working to minimize variation from batch-to-batch (Romans et al., 2001).

#### **Production of Fresh Pork Sausage**

Fresh pork sausage accounted for 15% of pork consumption in 2001 (National Pork Board). Fresh pork sausage production has grown exponentionally and in 2008 more than \$900 million in sales was recorded (Annual Sausage Report, 2009). Fresh pork sausages are typically uncured, comminuted, seasoned with salt, pepper, and sage, and stuffed into casings; examples are bratwurst, bockwurst, or breakfast sausage. Breakfast sausage is very common to the Southern U.S. with common seasonings of salt, pepper, and sage. The Code of Federal Regulations stipulates that fresh pork sausage is limited to 50% fat and 3% added moisture (9 CFR 319.141).

Fresh pork sausage can be made using whole hogs or trimmings. Whole hog sausage is produced using butcher hogs ranging from 240- to 250- pounds (Romans et al., 2001) Fresh sausage today is made mostly from sows that have been removed from breeding herds. Fat levels from whole hogs vary significantly, and leaf fat can be used to raise fat levels if the hogs are too lean (Romans et al., 2001). Pork trimmings come from primal cuts and trimmings from processing hams, shoulders, loins, butts, and bellies (Kramlich et al., 1973). Processors can use chilled or hot-boned meat. Hot-boned meat is removed from the carcass prior to chilling and development of rigor mortis. Extraction of solubilizing proteins, actin and myosin, are most readily extracted prior to the onset of rigor. Hot-boned pork is used to produce fresh pork sausage because it retains good color, enhances flavor, and stability (Kramlich et al., 1973).

Pork sausage is prepared by coarse grinding hot-boned pork trimmings through a 0.48 cm grinder. Grinding hot-boned pork trimmings has a greater incidence of fat smears. Fat smear is reduced by chilling pork trimmings to 0°C prior to grinding (Romans et al., 2001). It is common for lean trimmings to be ground through a 0.32 cm plate and fatter trimmings through the 0.48 cm plate (Romans et al., 2001). This also helps to minimize fat smear. Initial grinding of meat trimmings allows for easier mixing with added spices, flavorings, and additives.

Salt is considered a critical ingredient for sausage production. Salt serves as a preservative, protein extractor, and flavor enhancer (Romans et al., 2001). Fresh pork

6

sausage contains an average of 1.5% - 2.0% salt (Price and Schweigert, 1978). The salt forms a brine with available water and works to retard microbial growth. However for fresh pork sausage, salt is more important as a flavorant and for extracting proteins. Sodium chloride (NaCl) is most commonly used, providing the most flavor, and is widely available. Other salts such as potassium chloride (KCl) and calcium chloride ( $CaCl_2$ ) can be used. These salts help to reduce sodium content but are known to have a more bitter taste (Romans et al., 2001). However, salt promotes rancidity in fats which can decrease the shelf-life of fresh sausage products. Grinding and chopping of meat also accounts for fatty acid oxidation due to greater surface area exposure to oxygen. Antioxidants are used to help prevent rancidity from occurring in the fat particles (Price and Schweigert, 1978). The more common antioxidants that are widely used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate. The Code of Federal Regulations permits the use of BHA or BHT in meat products at a level of 0.01 percent based on fat composition, or a level of 0.02 percent in combination based on fat composition with other antioxidants (9 CFR 424.21). Seasoning for fresh pork sausage is typically pepper and sage. Black and white pepper are common fresh sausage ingredients; white being used when black pepper specks are not desired.

Trimmings or whole carcass pork are mixed with salt, pepper, sage, and antioxidants prior to regrinding through a 0.48- or 0.32- cm grinder plate (Romans et al., 2001). Water regulated to 3% maximum can be added during mixing to help maintain meat temperature and reduce mechanical heating (Rust, 1977). Excessive mixing after addition of salt can produce a tough product due to a high quantity of salt soluble protein extraction (Romans et al., 2001). After mixing, the fresh sausage is stuffed into chubs or casings. Chubs are usually opaque or white hued plastic casings that are used to produce 0.45- or 0.91- kg extruded sausage links and that make up the majority of fresh pork sausage production (Romans et al., 2001). Specialty fresh pork sausages can be stuffed into narrow or medium sheep casing, narrow pork casing, or collagen casings (Romans et al., 2001). The appearance of pork sausage stuffed into casings and links is more important than in chub production. Producers want, and consumers desire fresh pork sausage to have a bright, reddish pink color, referred to as *bloom* (Price and Schweigert, 1978). Chub or casing fresh pork sausage is sold as frozen or refrigerated, and should be kept at a temperature of 0°C throughout distribution channels (Rust, 1977; Kramlich et al., 1973).

#### **Microbiology of Fresh Sausage**

Fresh pork sausages are typically sold in a raw state, are not heat treated and do not contain curing additives; i.e., nitrite. Because of this, fresh pork sausages are more perishable than other sausage types with a shelf life up to 10 days (Cocolin et al., 2004). The rapid decline in shelf life is due to microbial spoilage and oxidative rancidity. During refrigeration, microbial spoilage occurs due to the proliferation of psychrotrophic bacteria (Romans et al., 2001).

#### Contamination

Meat components are a breeding ground for microbe growth because meatis high in moisture, supplies minerals and accessory growth factors, and has a favorable pH (Romans et al., 2001). The surface area of meat products also impacts microbial growth. The larger the surface area, the more potential for microbial contamination. Ground meats, such as pork sausage, expose the maximum amount of surface area, and can have greater incidence of microbial contamination (Romans et al., 2001). Microbial contamination of surface area can occur due to possible contamination during slaughter, processing, and storage. Hazard Analysis and Critical Control Point (HACCP) programs are aimed at prevention or reduction of contamination during processing. Animal hides and exterior surfaces are contaminated with soil, air, and water-borne microorganisms. Skeletal muscle is mostly sterile, while one theory of contamination during slaughter is believed to occur by microorganisms using the circulatory system to reach the muscle tissues (Price and Schweigert, 1978). Pearce et al. (2006) studied the occurrence and distribution of airborne contamination and concluded a correspondence of the types and amounts of bacteria aerosolized in the air with types and amounts of bacteria adhering to surfaces. On a larger scale, contamination is believed to occur during removal of the hide and from dirty processing equipment contaminated from hide removal from carcass to carcass (Gill and Newton, 1980). S. Typhimuium has been detected in aerosol samples at evisceration and dehairer locations (Pearce et al., 2006). Acuff et al. (1988) studied the role of intramuscular and subcutaneous fat of pork carcasses in microbial contamination and growth. In the study they determined that pork, when compared to beef, had less

microbial growth due to processing practices of retaining the skin of pork carcasses until carcass cutting. They postulated the presence of skin protected the pork fat from initial contamination during slaughter and chilling (Acuff et al., 1988). Another contaminate is the parasite *Trichinella spiralis*, which has been found in pork around the world except for Australia (Scriven and Singh, 1986).

#### **Microbiological Flora**

Mixes of microbial flora have been determined to be predominately psychrophilic organisms; the most common being *Pseudomonas*, Achromobacter, Flavobacterium, and Lactobacillus (Price and Schweigert, 1978). Acuff et al. (1988) reported at day 0 and at day 6, *Pseudomonas* species were the dominant microflora at >50% and 71.9-87.1%, respectively. Measures of *Lactobacillus* gained dominance starting at day 7 through day 14 (Acuff et al., 1988). Salmonella is a widely studied bacteria, and has been the cause of many food poisoning and fresh meat recalls, especially in Mexico (Escartin et al., 1995). Escartin et al. (1995) tested 61 pork samples from Mexican butchers. Incidence of Salmonella species were found in almost all samples ranging from 0.04 - 9000 MPN g<sup>-1</sup> (Most probable number), and more than 70% of samples showed more than one type of species (Escartin et al., 1995). Scriven and Singh (1986) were unable to isolate Salmonella cultures that were known to grow in previous studies. However, Liu et al. (2006) reported that the predominant spoilage organisms and bacteria found on pork were *Pseudomonas* and *Salmonella* with a maximum count of 7.271 and 7.414  $\log_{10}$ . respectively. *Listeria monocytogenes* has also been found in *linguica*, a popular pork

sausage from Brazil (Miyasaki et al., 2009). The study conducted by Miyasaki et al. (2009) concluded 90% of *linguica* samples tested positive for *Listeria* species. Yeasts and molds are also a big issue with unpackaged fresh meats. Air humidity during storage is the main factor in production of mold in fresh pork sausage. A humidity of 75 to 80%, held at a temperature of 6° to 8°C is capable of preventing moisture loss and prolonging mold formation (Savic, 1985).

#### Pseudomonas spp.

*Pseudomonas* spp. are psychrophiles, capable of growing at temperatures between 0° to 20°C (Romans et al., 2001). *Pseudomonades* are not considered harmful organisms, and are generally used as an indicator that conditions are favorable for harmful bacteria growth. They are present in soil and water and are found on most fresh foods (Jay et al., 2005). Once considered to be the largest genus of foodborne bacteria, *Pseudomonas* spp. are now separated into several new genuses (Jay et al., 2005).

#### *Listeria monocytogenes*

*L. monocytogences* is found in soil, vegetation and water. It is a gram-positive, motile rod responsible for listeriosis, a foodborne infection. It is capable of growth at a variety of temperatures ranging from 0°C to 45°C and is not killed by freezing (Romans et al., 2001). *L. monocytogenes* is also able to withstand high percentages of salt (as high as 30.5 percent) and can survive in water activity as low as 0.92 (Romans et al., 2001). Listeriosis can cause septicemia in children, elderly, and pregnant women and can lead to

infection of the central nervous system with meningitis, encephalitis, or abscesses (Romans et al., 2001). *L. monocytogenes* has become a large concern in the food industry with raw-meat and fermented raw-meat sausages. Ready-to-eat (RTE) products also cause concern for potential *L. monocytogenes* growth due to lack of heating after initial processing (Nunez de Gonzalez et al., 2004). *L. monocytogenes* is capable of surviving in these types of products due to its adaptability to environmental stresses (Farber et al., 1989).

#### Salmonella spp.

There are several species and types of *Salmonella. Salmonella* spp. are gramnegative, nonspore-forming rods able to ferment glucose and produce gas. Optimum temperature for growth is 36.9°C in non-acid foods. *Salmonella* can be killed by pasteurization, 71.7°C for 15 seconds (Romans et al., 2001). *Salmonellosis* infection is caused by ingesting contaminated foods with a high *Salmonella* count (Rust, 1977). Over 1200 species are capable of causing the foodborne infection (Price and Schweigert, 1978). Children and elderly adults are more susceptible to the infection and symptoms can appear from 7-72 hours after ingestion (Price and Schweigert, 1978). Prevention or reduction in numbers can be obtained with safe handling during processing of fresh meats, and proper cooking.

#### Lactobacillus spp.

*Lactobacillus* spp. are responsible for souring of fresh meat products since it is a lactic acid bacteria. *Lactobacillus* are gram-positive, catalase-negative rods (Jay et al., 2005). They ferment lactic acid by oxidizing carbohydrates in food products and can be separated into three subgenera: 1) obligate homofermentative; 2) facultative heterofermentative; 3) obligate heterofermentative (Jay et al., 2005). They are considered mesophilic and have optimum growth at temperatures between 20°C and 45°C, though some species are capable of growing at temperatures below 5°C and up to temperatures of 45°C (Jay et al., 2005). Bacterial production of lactic acid is capable of reducing pH of foods with fermentable carbohydrates to a 4.0 (Jay et al., 2005).

#### Escherichia coli and coliforms

*E. coli* was first discovered during the quest to find the agent of cholera (Jay et al., 2005). It was determined that *E.coli* is a coliform indicative of the presence of fecal pollution. All forms of *E. coli* are coliforms, but not all coliforms are *E. coli*. Other types of coliforms are *Citrobacter, Enterobacter, and Klebsiella* species (Jay et al., 2005). Coliforms are gram negative rods that ferment lactose and are capable of growing at temperatures as low as -2°C and as high as 50°C (Jay et al., 2005). Elimination from fresh and frozen foods is considered impossible, and low numbers are permitted in foods at levels of 1 to not over 100/g or 100 ml (Jay et al., 2005).

#### Molds and Yeasts

Molds and yeasts generally only become a problem in fresh pork sausages during storage. Mold spores are present in the air and without proper air filtration, mold contamination and formation on products can quickly become hard to control (Rust, 1977). The more common molds known to form on sausages are *Aspergillus, Penicillium,* and *Mucor* (Savic, 1985). Molds form filamentous fungi on the sausage, giving a fuzzy appearance (Jay et al., 2005). Savic (1985) also states that molded sausages are not spoiled or unhealthy and can be consumed after treatment with vinegar or salt solution. Yeasts form on sausages in the presence of high moisture and are linked to sanitation during packaging (Rust, 1977). Yeasts are thought of as unicellular fungi, unlike molds, and are differentiated from bacteria due to their large size and shape varying from oval to elongate, elliptical, or spherical (Jay et al., 2005).

#### Trichinella spiralis

*Trichinella spiralis* is a parasite associated with pork that can cause trichinosis if contaminated pork is consumed. Contamination of the pork muscle occurs through the hog's ingestion of garbage that has not been properly processed (Price and Schweigert, 1978). Encysted larvae are eaten by the swine and complete their life cycle within the hog's digestive system (Romans et al., 2001). Young larvae are carried through the blood stream and deposited in the muscle (Romans et al., 2001). Human infection occurs from consuming larvae in improperly or undercooked pork products (Price and Schweigert, 1978). Due to state and federal legislation it is rare to have a reported case or an

occurrence of trichinosis, but in the United States 0.013 percent of swine are infected with *Trichinella spiralis* (Romans et al., 2001). Proper cooking (>62.2°C instantly) and/or proper freezing (-37.2°C in ½ hour) will destroy the trichina larvae.

#### **Spoilage Deterioration**

Sausage spoilage and deterioration are a direct link between bacterial growth in the mixture and molds on the surface. Deterioration from the processes used by the molds and bacteria can create souring, gas formation, off-odors, and color changes of the product (Savic, 1985). Fresh meats with a pH of 5.6, adequate glucose and simple carbohydrates are capable of supporting 10<sup>8</sup> organisms/cm (Jay et al., 2005). During storage at refrigerated temperatures, fresh meats are more prone to bacterial spoilage than mold growth, and the bacterial growth is indicated by slime production (Jay et al., 2005). The slime produced by the various bacteria species can take on a white or yellow hue and a combination of surface colonies gives a tacky consistency (Jay et al., 2005; Rust, 1977). Fresh pork sausage is more susceptible to this kind of bacterial degradation. The bacteria organisms cause a rise in pH, rather than a drop as in lactic acid production, and induce a slime with a "wet dog" odor (Rust, 1977). Degrading odors from bacteria growth begin to be detected when counts reach  $7.0 \log/cm^2$ , and slime appearance closely follows when bacteria levels reach 7.5  $\log/cm^2$  (Jay et al., 2005). After reaching a bacterial load of  $10^8$ CFU/cm<sup>2</sup>, most simple carbohydrates have been used and *pseudomonas* begin to break down amino acids and simple nitrogenous compounds for energy (Jay et al., 2005). Production of spoilage odors and off-flavors occur after amino acids are utilized for

energy (Jay et al., 2005). The surfaces of fresh meats also undergo color changes from bacterial growth. Common off-color compounds are ammonia, H<sub>2</sub>S, indole, and amines (Jay et al., 2005). Greening is associated with fresh sausage production and can occur as surface greening or create green cores. The *Lactobacillus* species, *lactobacillus viridescens*, is responsible for the greening color created (Rust, 1977). The bacterium produces hydrogen peroxide, an oxidizing agent which gives of a green color (Rust, 1977). The same bacterium is also responsible for creating green hue cores in stuffed pork sausage. Degradation begins in the center of the sausage but does not alter the color until the sausage is sliced and exposed to oxygen (Rust, 1977). Fresh pork sausage is also susceptible to souring, which includes production of a sour odor, discoloration, sour flavor, and possible gas formation (Rust, 1977).

#### **Antimicrobial Additives**

Preservation of fresh meats from microbial proliferations is generally controlled by processors through freezing and refrigeration. However, refrigeration and freezing temperatures do not kill microorganisms, they only slow the growth. Another way to help reduce microbial population, in the case of fresh pork sausage, is with the addition of ingredients with antimicrobial properties. It is important that the addition of antimicrobials, while reducing microbial counts, does not cause potential harm through consumption and is generally heavily regulated (Price and Schweigert, 1978). The use of organic acids in fresh meat formulations has been studied over the past 20 years (Jensen et al., 2003). Acetic, proprionic, sorbic, and lactic acid are common organic acids that have been studied and used as antimicrobial agents due to their bacteriostatic or fungistatic properties (Price and Schweigert, 1978). Anderson and Marshall (1990) used mixtures of 2% acetic, 1% lactic, 0.25% citric, and 0.1% ascorbic acids on beef carcass samples previously inoculated with bacteria. Results of the acid mixtures showed a 2.3log<sub>10</sub> reduction in *S. typhimurium* at 3%, and a reduction of *E. coli* at 0.3 log<sub>10</sub> at 1% acid mixture (Anderson and Marshall, 1990). Acetic and lactic acid have been used more commonly as carcass decontaminating spray washes. Hardin et al. (1995) conducted a similar experiment with the use of 2% acetic acid or 2% lactic acid. They determined that use of organic acid spray washes were capable of reducing microbial loads and in this particular study reduced indicator organisms by up to 5-log<sub>10</sub> (Hardin et al., 1995). However, they reported no statistical difference between the lactic acid and acetic acid treatments, but lactic acid reduced numbers below the minimum detection level more often than acetic acid washes (Hardin et al., 1995).

#### Sodium Lactate

Sodium lactate ( $C_3H_5O_3Na$ , MW 112.06, pKa 3.86) is considered an antimicrobial agent and is approved for use in meat products up to 4.8% by weight of total formulation, while acetic acid ( $C_2H_4O_2$ , MW 60.05, pKa 4.76) is labeled as an acidifier and used sufficient for purpose (9 CFR 424.21). It has been used in baked goods for several years as a humectant to help retain moisture (Papadopoulous et al., 1991b). It is considered a normal component of muscle tissue, and demonstrates an antimicrobial characteristic when found at high levels (Bacus and Bontenbal, 1991). Choi and Chin (2003) reported

that 3.3% sodium lactate in frankfurters reduced *Listeria monocytogenes* growth 2-3 weeks longer than a control manufactured with potassium sorbate and sodium benzoate. Sodium lactate increased the lag phase of food spoilage bacteria, thus extending the shelflife of the product (Satz, 1991). Hunter and Segel (1973) suggest that sodium lactate as an undissociated acid is capable of passing through the bacterial cell membrane and dissociating thereby effectively acidifying the cell interior. Lamkey et al. (1991) produced fresh pork sausage chub packs and extended the shelf-life by 2 weeks over control pork through utilization of 3.0% sodium lactate. In addition, cooked beef roasts treated with sodium lactate had a lower percentage of graved surfaces than untreated roasts (Papadopoulous et al., 1991b). Bradford et. al. (1993) also determined that the addition of 3.0% potassium lactate was capable of reducing psychrotrophic organisms in typical pork sausage and increasing 'a' (redness) color values. Brewer et al. (1991) reported that along with protection of red color, use of sodium lactate at 2-3% is capable of delaying microbial growth, pH decline and development of sour and off-flavors by 7 to 10 days when compared to a control. Bacus and Bontenbal (1991) attribute the increase in lag phase to sodium lactates ability to interfere with the bacterial cell's feedback inhibition, crossing of protons across the cellular membrane and creating intracellular acidulation. Studies conducted by Hammer and Wirth (1985) on cooked liver sausage hypothesize that sodium lactate reduced water activity  $(a_w)$ . Locin (1975) also attributes sodium lactate's effectiveness to reduction of a<sub>w</sub>. However, studies conducted by Papadopoulos et al. (1991c) reported that a<sub>w</sub> of meats were not lowered with the addition of sodium lactate.

#### Acetic Acid

The effects of acetic acid spray washes on beef carcasses have been studied in depth. Acetic acid is commonly known as vinegar and like sodium lactate has been shown to have antimicrobial properties. Vinegar is a concentration of 4-8% acetic acid generally made from the pressing of apples, but can be made from most vegetation containing sugar (Kovacic, 1998). Numbers of Escherichia coli 0157:H7 and Salmonella *Typhimurium* were reduced by 0.1 log CFU (colony forming units) to 4.67 log CFU/cm2 using an acetic acid spray wash (Cutter et al., 1997). The use of acetic acid has been demonstrated to inhibit the growth of *Listeria monocytogenes* (Ita and Hutkins, 1991). Ita and Hutkins (1991) reported a 4 log reduction of *Listerria monocytogenes* cells held at a low pH (3.5) in acetic acid, while bacteria cells in lactic acid were only reduced by one log. Acetic acid has a higher pKa value (4.76) than lactic acid, and it is thought that inhibitory effects of acetic and lactic acids are correlated to their pKa value (Ita and Hutkins, 1991). Acetic acid is thought to inhibit *Listeria monocytogenes* in two ways: acidifying the bacterial cell pH and disruption of physiological or metabolic activities of the cells (Jensen et. al., 2003). Ita and Hutkins (1991) hypothesized that not only did acetic acid work to acidify the cell interior, but is thought to interrupt bacterial proton pumps in the cell membrane responsible for ion regulation. While use of acetic acid may reduce microbial load there are instances of development of meat discoloration. Beef cubes dipped in varying levels (0.6, 1.2, 1.8 and 2.4%) of acetic acid solution for 10 minutes resulted in a difference of color as compared to the control (Bell et al., 1986).

Stivarius et al. (2002) also reported ground beef discoloration while reducing bacterial loads when using 5% acetic acid solution.

#### **Oxidative Rancidity**

In addition to microbial spoilage, fresh pork sausage is susceptible to spoilage by oxidative rancidity. Rancidity occurs through the reaction of unsaturated fats with oxygen (Kramlich et al., 1973). Fresh pork sausage is allowed to contain up to 50% fat (9 CFR §319.141). This amount of fat allowance, if used, allows for a high potential of oxidative rancidity. The reaction of oxygen and unsaturated fat has been known to produce rancid odors and flavors, and is influenced by heat, light, and pro-oxidant catalysts (Price and Schweigert, 1978). Double bonds in the fatty acid chain are susceptible to oxygen breaking the bond and the formation of aldehydes (Romans et al., 2001). A free radical chain mechanism has been determined to proliferate the hydroperoxides (Price and Schweigert, 1978). Creation of shorter fatty acid chains and aldehydes produce the rancid odor and flavor (Romans et al., 2001). Ingredients added to meat products can be prooxdiants, which act as catalysts for oxidation (Kramlich et al., 1973). Salt is considered a pro-oxidant, and all sausage contains salt (Kramlich et al., 1973). Cheng et al. (2007) produced a study on the use of KCL in reduction of rancidity as compared to a control of fresh sausage using salt (NaCl). The study showed that rancidity was reduced with the use of KCl in combination with NaCl or when used alone (Cheng et al., 2007). A previous study by Rhee and Ziprin (2001) evaluated the oxidative capabilities of NaCl at various levels on ground beef and chicken. Batches were mixed with 0, 1, 2, 3, 4, or 5%

NaCl based on sample weight. They found that increasing amounts of salt increased oxidative rancidity (Rhee and Ziprin, 2001). Pork fat has a higher number of unsaturated fatty acids, thus having a higher oxidation capability than beef or lamb fat (Romans et al., 2001). Generally, the addition of nitrite in cured sausages reduces the amount of oxidative rancidity, but fresh pork sausages are not cured, and addition of antioxidants are needed.

#### **Addition of Antioxidants**

Antioxidants can delay or prevent oxidative rancidity from occurring. There are several kinds of antioxidants useful to different food systems. Antioxidants occur naturally and can be created synthetically. Antioxidants essentially react at the double bond sites of fatty acids (Romans et al., 2001). They work to terminate the oxidative chain reaction in three main ways: 1) electron donation to peroxy radicals; 2) hydrogen donation to peroxy radicals; or 3) addition of a peroxy radical before it is oxidized (Price and Schweigert, 1978). The more commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate. The Code of Federal Regulations permits the use of BHA or BHT in meat products at a level of 0.01 percent based on fat composition, or a level of 0.02 percent in combination based on fat composition with other antioxidants (9 CFR 424.21). Naturally occurring antioxidants can be found in rosemary, borage, green tea, or pu-erh tea (Martinez et al., 2006). Sebranek et al. (2005) conducted a study on the effectiveness of rosemary extracts compared to BHA/BHT in terms of reduction of oxidation in pork sausage during freezer

storage. Treatments included: 1)control; 2) 200ppm BHT/BHT; 3) rosemary extract at 1500ppm; and 4) rosemary extract at 2500 ppm. Evaluation of storage time on oxidation development was measured by thiobarbituric acid method (TBARS) and sensory panels. CIE  $L^*$ ,  $a^*$ , and  $b^*$  color scores were also recorded over time. The samples were tested every 14 days up to 120 days. In conclusion, Sebranek et al. (2005) determined that treatment with natural antioxidant rosemary extract at 2500 ppm, worked as well or more effectively in fresh-frozen pork sausage than the use of BHA/BHT.

#### 2-Thiobarbbituric Acid Reactive Substances (TBARS)

Presence of aromas and flavors arises from the production of secondary oxidation which can be a mixture of aldehydes, ketones, hydrocarbons, alcohols, etc. (Shahidi and Wanasundara, 2008). Measurement of the amount of secondary oxidation can be carried out by 2-thiobarbituric acid values, oxirane value, *p*-Anisidine value, TOTOX value, carbonyls, hydrocarbons and flourecence (Shahidi and Wanasundara, 2008). The use of 2-thiobarbituric acid values have become the most widely practiced method of predicting oxidative rancidity in meat products. Breakage of hydroperoxides produces the secondary product malondialdehyde (MDA). TBARS produces a value that is measured in macromoles of MDA equivalents per gram of sample (Shahidi and Wanasundara, 2008). Values are obtained by the reaction of MDA with 2-thiobarbituric acid (TBA) to form shades of red color (Fernandez et al., 1997). This hued complex is the result of of two molecules of TBA binding to one molecule of MDA (Shahidi and Wanasundara, 2008). The range of red/pink hues are measured at an absorbance measure at 530- 532 nm

(Shahidi and Wanasundara, 2008; Tarladgis et al., 1960). The intensity of color is directly related to the concentration of the MDA rancidity present in the sample (Fernandez et al., 1997). The absorbance of the TBA-MDA complex is measured to standards that can be produced from 1,1,3,3 tetraethoxypropane (TEP) or 1,1,3,3tetramethoxypropane (TMP) (Fernandez et al., 1997). There are several methods in which TBA values can be obtained including performing testing directly on the sample, its extracts, or distillates (Shahidi and Wanasundara, 2008). Conducting TBARS directly on a sample involves heating the sample with TBA under acidic conditions and extracting the red color through the use of butanol (Fernandez et al., 1997). The extraction method uses trichloroacetic acid to acquire an aqueous acid solution from the meat sample before reacting with TBA. This method is believed to more accurately predict MDA concentration in the meat sample (Fernandez et al., 1997). Distillation is conducted by steaming the MDA out of the product producing a clear solution that is free of interfering solvents (Tarladgis et al., 1960; Shahidi and Wanasundara, 2008). Tarladgis et al. (1960) used the method of distillation on unspecified meat samples in comparison to the Turner et al. (1954) method of extraction. Using the distillation method, Tarladgis et al. (1960) was able to isolate 68% of MDA from 50 ml of known concentration of TEP. Due to this result, these researchers concluded that the distillation method was superior to other TBARS methods based on the theory that distillation did not produce further oxidation during the procedure. Later, a study by Witte et al. (1970) examined a new extraction method and they were able to produce 94% of MDA from a 20 g sample of fresh meat.

They determined that this new extraction method was much more acceptable and easier to produce TBA values than previous distillation methods.

#### Sensory Descriptive Analysis of Fresh Pork Sausage

Sensory descriptive analysis is a method by which the qualitative and quantitative sensory characteristics of a food product are identified and quantified by a group of trained panelists (Meilgaard et al., 2007). Panelists are trained to identify and describe sensory characteristics pertaining to areas of aroma, flavor, appearance, texture, or sound (Meilgaard et al., 2007; Murray et al., 2001). Trained panelists should be able to use the qualitative descriptors to differentiate between samples of similar products by quantifying the attributes (Murray et al., 2001). Use of descriptive analysis can also be used from a quality standpoint to check for addition of ingredients or differences in processing by determining and measuring the sensory attributes of products over time (Murray et al., 2001). Qualitative attributes are put into quantitative forms to determine the degree to which a sensory attribute identified is present (Meilgaard et al., 2007). Quantitative measures are generally expressed using scales such as: Category scales, which limit the amount of words or numbers and mostly ranges from 0-9; line scales, which can be more accurate, but can also make panelists inconsistent due to the length of the 15cm scale; or magnitude estimation, which involves free assignment of the first number, mostly used for single attributes over a large intensity range (Meilgaard et al., 2007).

Sensory descriptive methods have become abundant and a select few are used as standards in the industry. These standards include Flavor Profile Method, Quantitative

Descriptive Analysis (QDA<sup>®</sup>) Method, Spectrum<sup>™</sup> Descriptive Analysis Method, and Free-Choice Profiling. The flavor profile method was developed to be used by a panel of four to six trained panelists to identify a product's aroma and flavor, intensity levels, order of appearance, and aftertaste (Meilgaard et al., 2007). Panelists are selected on their ability to taste, intensity differentiation, olfactory discrimination, and are trained using reference samples and ingredients in the food product (Meilgaard et al., 2007; Murray et al., 2001). QDA<sup>®</sup> was developed to help correct issues from the flavor profile method by integrating statistical analysis (Meilgaard et al., 2007; Murray et al., 2001). Trained panelists use non-technical language to avoid becoming biased, and a broader range of panelists are selected, not just those who are more familiar with the type of product (Murray et al., 2001). ODA<sup>®</sup> generally uses a 15cm line scale, and the results are statistically analyzed and a graphical representation of the data is produced (Meilgaard et al., 2007). Spectrum<sup>TM</sup> uses extensive lists of references, and panelists should have extensive knowledge of the technical principles for each type of reference and attribute discussed or identified (Meilgaard et al., 2007). Free-choice profiling was developed to help development teams with consumer perceptions rather than technical terminology (Murray et al., 2001). Panelists can use however many descriptive terms to identify an attribute, and training is not involved in free-choice profiling (Murray et al., 2001).

Flavor of fresh pork sausage is dependent on the formulation of sage, salt, and pepper along with any other additional spices. A listing of sensory descriptors for pork sausage can be created from a generalization of pork flavors with addition of flavors and textures created from combinations of the added ingredients. General sensory profiles of fresh meats are evaluated using the following descriptors: Aroma – intensity, sweetness, metallic, liver, gamey; color – intensity, hue; flavor – intensity, sweetness, acidic, metallic, bitter, saltiness; texture – coarseness, hardness, tenderness, fattiness, juiciness (Rodbotten et al., 2004). Addition of antimicrobials and antioxidants may also provide a change to sensory descriptors by bringing in more variations of pork flavor. The shelf-life of fresh pork sausage also weighs heavily on the flavors, acceptable or not acceptable, and structural stability of the product. The longer the product is stored, the more offflavors are produced from microbial proliferation and oxidative rancidity. Addition of antimicrobials and antioxidants work to prolong formation of off-flavors and textural problems, but also impart their own sensory profiles. Brewer et al. (1991) evaluated the sensory characteristics of fresh pork sausage formulated with sodium lactate; evaluating the change in pork flavor, saltiness, souring, or any off-flavors. They reported the addition of sodium lactate at higher percentages created a more intense pork and salty flavor, while the higher levels reduced the amount of off-flavor intensity over the course of time. Papadopoulos et al. (1991a) determined the aromatics, tastes, textures, and chemical factors of beef with the addition of sodium lactate. They determined off-notes such as cardboard, painty, and fishy aromatics increased over storage time and were in direct correlation with shelf-life and increased levels of sodium lactate. At higher levels, sodium lactate also produced a mild throat irritation, yet was undetected at lower levels. Additions of organic acids like acetic acids to ground meats produce more off-flavor notes and have negative effects on meat color. Stivarius et al. (2002) reported an increase in off-odor and decreased beef flavor in samples containing acetic acid. They also

reported that ground beef treated with acetic acid had increased discoloration when compared to the control when held under refrigerated retail conditions over time.

Variations on descriptive analysis can also occur from the initial raw materials and methods of production. Flores et al. (1999) determined that post-mortem quality of pork carcasses can affect the final textural and tastes of pork products. DeVol et al. (1988) indicated there is a large variation between the type of pork entering the market and desired palatability. A random sampling of pork loins was taken and evaluated for sensory quality of juiciness, tenderness, amount of connective tissue, and pork flavor intensity. Their findings were comparable to other studies that reported animal-to-animal variations in pork products.

# Relationship of Antimicrobials and Antioxidants to Sensory Descriptors and Consumer Acceptance

Studies on the effects of adding antimicrobial agents and antioxidants to pork sausage correlate with sensory and consumer acceptability. Statistical methods are used to relate sensory to product quality. These statistical methods include: regression analysis (DeVol et al., 1988) and principal components analysis (PCA) (Aaslyng et al., 2007).

## Principal Components Analysis (PCA)

Principal Components Analysis (PCA) is a statistical method used to reduce the wide range of related variables in the data while maintaining the variation present (Jolliffe, 2002). Original data is produced into the "principal components", which are more manageable because they are uncorrelated and ordered so that the first few contain

the most variation that is present from the original (Jolliffe, 2002). PCA biplots are used to represent the first few principal components visually.

#### **Cluster Analysis**

Cluster Analysis works on the same principles of PCA, attributes are grouped based on the degree of similarity between observations (Meilgaard et al., 2007). Cluster analysis follows two types of algorithms; hierarchical and nonhierarchical. In hierarchical, once an observation is clustered it cannot be moved (Meilgaard et al., 2007). Clusters are represented in dendrograms. The dendrograms show the breakdown of cluster groups until single observations are achieved (Meilgaard et al., 2007).

## **Product Development**

Development of a new product is highly dependent on how the producer perceives and meets consumer demands and tastes. Quality function deployment (QFD) is a method of creating new products and relaying consumer needs into measureable technical attributes (Bech et al., 1997). This study expounds on this concept by providing measured values relating to safety of consumption, palatability, and providing a visually appealing food that a consumer may purchase while also providing the producer with an economical product to use in production.

## CHAPTER III

# MATERIALS AND METHODS

# Materials

## Sample

Three sows (3-4 years of age) were purchased (Prestage Farms, West Point, MS) and each one was used per replication for a total of three replications. Each sow was conventionally slaughtered at the Mississippi State University Meats Laboratory. Immediately after exsanguination and proper dressing procedures (skinning), the left side of each carcass was de-boned. Roughly 45kg of each left side was coarse ground (Model 80055 Mixer Grinder, Hollymatic Co., Countryside, IL) with added fresh pork sausage seasoning (Rebel Country Sausage Seasoning, Rebel Butcher Supply Co., Inc, Jackson, MS). A sample from each replication was analyzed to determine fat percentage (LabWave 9000<sup>™</sup> Model FES. CEM Corporation. Matthews, N.C.). Leaf fat was added to increase fat percentage to an average of 23% as needed. Three 9 kg batches were pulled from the coarse ground meat to produce three treatment groups: 2.5% sodium lactate 60% solids (L), 2.5% buffered vinegar pH 6.5-8.0 (V), 2.5% sodium lactate and vinegar 52/ 48% mixture (LV). Two more 9 kg batches were pulled to create a control with 0.02% BHA/BHT (C), and a negative control with no antioxidant or antimicrobial additives (NC). All batches were reground through a 4 mm plate with a four blade knife (80055 Mixer-grinder, Hollymatic Co., Countryside, IL) and stuffed (Risco I-36016 Thiene, Vincenza, Italy) into 7.62 cm diameter plastic tubes (Interstate Packaging, White Bluff, TN), labeled and frozen overnight at -23°C. The frozen logs were sliced to 1.27 cm thickness and placed 6 patties per labeled polystyrofoam tray (White Foam Meat tray – 8  $\frac{1}{2}$ " x 6  $\frac{1}{2}$ " x  $\frac{1}{2}$ ", Instawares, LLC., Kennesaw, GA) and overwrapped (Meat Stretch, LINPAC Filmco, Inc., Aurora, OH). The overwrapped trays with frozen sausage patties were randomly selected, placed and maintained under simulated retail lighting (753 lux) conditions continually until evaluation time, for the respective attribute evaluations. The packages were maintained at a temperature of 1 to 2°C for up to 18 days to simulate temperature and holding times found in refrigerated display cases in tested retail markets.

#### **Retail Display**

A grouping of six units of incandesent lights (Cool White 34 Watt. Sylvania Supersaver Ecologic. Danvers, MA) were suspended from a structure built into the standing cooler in the Ammerman-Hernsberger Food Processing Plant at Mississippi State University. Lengths of the supporting chains were adjusted until an average of 753 lux was achieved at the surface of the sausage products. This lux value was determined since it was an average lux value of retail lighting displays for fresh pork sausage at retail food distributors in Starkville, MS.

#### **Microbial Supplies**

Microbial growth of the samples was measured using Tryptic Soy Agar (Becton Dickson, Sparks, MD) to enumerate general colony growth with no specification on identifying bacteria. Coliform and incidence of *Escherichia coli* was measured using Ecoli/ Coliform Count Plate Petrifilm<sup>™</sup> (3M Petrifilm <sup>™</sup>, St. Paul, MN). A phosphatebuffer solution (0.01 M Phosphate-Buffered Saline pH 7.5, Bacteriological Analytical Manual, U.S. Food & Drug Administration/Center for Food Safety & Applied Nutrition) with an adjusted pH of 7.5 at room temperature was used for serial dilution of the pork sausage samples.

## 2-Thiobarbituric Acid Reactive Substance

Measure of oxidative rancidity was carried out by the procedure of Spainer and Traylor (1991). Absorbance measures were recorded from a UV-VIS Spectrophotometer (UV-1201, Shimadzu Corporation, Australia). Chemicals used for extraction: 2thiobarbituric acid minimum 98%, sodium dodecyl sulfate, propyl gallate, ethylenediaminetetraacetic acid 99.995%, 1,1,3,3 –tetramethoxypropane 99%, 1-butanol ≥99.4% A.C.S reagent, pyridine 99+% A.C.S reagent (Sigma-Aldrich, St. Louis, MO) and glacial acetic acid (Fisher Chemical, Fisher Scientific, Fair Lawn, NJ). Distilled water and 1 M NaOH (Fisher Scientific, Fair Lawn, NJ) were also used to dilute solutions and adjust solution pH.

# **Chroma-Meter**

The Chroma Meter CR-400/410 (Konica Minolta Sensing, Inc. Tokyo, Japan) was used to measure the changes in hue, value, and luminescence of the samples over time.

#### Methods

# **Microbial Analysis**

Aerobic microbial evaluation of sausage samples were conducted on days 0, 7, 14, 16, and 18 of shelf life. Duplicate samples from each treatment, control and negative control representative trays were aseptically removed, weighed, and placed in sterile stomacher bags (BA6141/5TR filter bag, Stomacher ® Lab System, Seward, U.K.). The samples were then diluted with 225 mL of 0.1M (pH 7.5) phosphate-buffered solution to create a 10<sup>0</sup> solution. Each sample was homogenized for 1 minute in a Stomacher ® 400 Circulator (Seward, U.K.) then serially diluted (10<sup>0</sup> to 10<sup>5</sup>) in 9 ml tubes of 0.1 M phosphate- buffered solution to obtain countable plates. Samples were then spread plated in onto pre-poured Tryptic-Soy agar plates (Becton Dickson, Sparks, MD) and incubated at 34°C for 48 hours before counting. On days 0 and 18, samples were plated onto E-coli/ Coliform Count Plate Petrifilm<sup>TM</sup> (3M Petrifilm <sup>TM</sup>, St. Paul, MN), and incubated at 34°C for 24 hours to determine *E. coli* presence or recuperation over time. Total plate counts (TPC) and *E. coli*/ coliforms were expressed as log numbers of colony forming units/mL (CFU).

#### **Instrumental Color Analysis**

Instrumental color determinations were made on the surface of the patties held under retail conditions at 1-2°C. The surface of the patties were evaluated for CIE color  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) using Chroma Meter CR-400/410 (Konica Minolta). Treatments, control, and negative control samples were evaluated on days 0, 4, 7, 11, 14, 16, and 18. A randomly selected package of each sample was chosen at each evaluation day. Three patties from each tray were then selected, and three separate readings on each of the patties, for a total of 9 was recorded. The 9 readings from each treatment, control, and negative control sample were averaged for a final color reading and used for statistical analysis.

#### **Thiobarbituric Acid Reactive Substance Values**

Oxidative rancidity of the sausage patties was determined by the adapted extraction method of Spainer and Traylor (1991). Standard solutions (Table 3.1) were prepared containing 0.05ml sodium dodecyl sulfate (SDS), 5.0ml solution III (0.1 gm propyl gallate and 0.2 gm EDTA), and increasing concentrations of tetramethoxypropane (TMP). Standard solutions were used to compare color pigment increase associated with increased pigment in oxidized meat products. A 5 g sample of each treatment, control, and negative control was added to 65 ml distilled water, 0.01 ml 10% SDS, and 10 ml of solution III. Mixtures were produced in duplicate and homogenized. Standards and homogenates were transferred to test tubes in 1 ml increments and 4 ml of solution I (3.75 gm thiobarbituric acid, 5.06 gm SDS, 119 ml glacial acetic acid; adjusted to pH 3.4) was added to the sample before placing in a 95°C water bath for 1 hr. After cooling, 5 ml of solution II (15:1 solution; n-butanol and pyridine) was added to each sample and standard tubes, prior to centrifugation (Centrific Model 228, Fisher Scientific, Fair Lawn, NJ) at 3000 rpm for 15 min. After centrifuging, the top layer was measured for color absorbance using a UV-VIS Spectrophotometer set at 532 nm, and mg/kg MDA was determined for standards and samples using a standard curve.

## **Sensory Sample Preparation**

Sausage patties were prepared by frying on a griddle top (Griddle 442A, Toastmaster Inc., Booneville, MO). The griddles were preheated to temperature of 176°C for 5mins prior to placement of sausage patties on the griddle. The patties were cooked for 5 min on each side, with an additional 2 minutes on each side until an internal temperature of 71°C was reached measured by a hand held digital thermometer. This sample preparation was chosen to closely resemble pork sausage patty preparation and consumption in typical consumer homes.

#### **Descriptive Sensory Analysis**

Five samples of fresh pork sausage (three treatments, a control, and a negative control) were assessed by an eight member trained panel with experience in excess of 100 hours each in the evaluation of meat products. Panelists participated in six 1-hour training sessions to evaluate pork sausage for specific sensory components of the product within the categories of appearance, aroma, oral texture, basic tastes, flavor and overall quality.

Vol. 0.1 mM TMP	Vol. 10% SDS (mL)	Vol. Sol. III (mL)	Conc. TMP (mM)
(mL)			
0	0.05	5	0
.250	0.05	5	0.5
.625	0.05	5	1.25
1.25	0.05	5	2.5
1.875	0.05	5	3.75
2.5	0.05	5	5
3.75	0.05	5	5.5
5.0	0.05	5	10
10.0	0.05	5	20

Table 3.1 Prepared standard solutions with increasing concentrations of tetramethoxypropane that were used to evaluate the oxidative rancidity of fresh pork sausage samples by measuring absorbance 532 nm using a UV-VIS spectrometer.

The Quantitiative Descriptive Analysis (QDA<sup>®</sup>) method was used throughout all training and sensory sessions (Meilgaard et al., 2007). Previously identified descriptors (Pegg and Shahidi, 2007; Meilgaard et al., 2007; Flores et al., 1999; Rodbotten et al., 2004) and terms deemed useful during training sessions were utilized for sensory evaluation of fresh pork sausage (Table 3.2). Panelists training sessions were held in a group discussion atmosphere to develop final descriptors for consistency in scoring samples. After training, sensory testing was replicated and tasting was conducted on days 0, 7, 14, and 17 of shelf-life. The descriptors were measured using a 15-point intensity line scale; 0=not detected and 15=extremely strong (Figure A2).

Prepared samples were held in whole sausage patty form, wrapped in a packet of Reynold's aluminum foil (Reynolds Wrap, Reynolds Consumer Products Co., Richmond, VA) and placed in chafing dishes to maintain heat until sensory evaluation. Samples were held in chafing dishes for no longer than 30 min. For evaluation, whole sausage patties where quartered, and one quarter of each sample was placed in a 2-oz plastic container with lid (Sweetheart Cup Co., Owning Mills, MD). The cups were coded with three-digit randon numbers that were generated from a random numbers table (Ott and Longnecker, 2001). Each panelist received one cup to evaluate appearance, aroma, texture, flavor, and overall acceptability of each sample. To cleanse their palates between samples, panelists were also provided with water (Mountain Spring water, Blue Ridge, GA), unsalted crackers (Unsalted Tops Saltines, Best Yet, C&S Wholesale Grocers, Inc., Keen, NH), apple juice (Apple Juice from Concentrate, Best Yet, C&S Wholesale Grocers, Inc., Keen, NH), and Styrofoam cups to expectorate samples.

## **Consumer Acceptability**

Three replications of 60 consumer panelists each was conducted to determine consumer acceptability of the control (C) and lactate and vinegar mixture (LV). These two treatments were chosen for the consumer panel based upon the ratings from the descriptive panel and other data. Consumers consisted of faculty, staff and students of Mississippi State University solicited through email notices and by word of mouth. Consumer panels were conducted over one week and after all descriptive sessions were completed. The panelists evaluated the two samples in an eight booth sensory room, where lighting, temperature, and ventilation could be controlled. Panelists received one quarter of each representative sausage patty in a 2-oz plastic lidded container labeled with three-digit random numbers. Sample preparation was conducted using the same

Sensory Descriptor	Definition
Appearance	
Color homogeneity <sup>d</sup>	Presence of color homogeneity of the patty surface
Browning	External browning from heating
Cupping	Amount of concavity in center of patty (lack of flatness)
Aroma	
Cooked complex	Aroma associated with cooked fat and lean with typical sausage spices
Boar taint <sup>a</sup>	Aroma associated with boar meat; hormone-like (skatole)
Rancid <sup>a</sup>	Aroma associated with extremely oxidized fat or oil
Pork <sup>a</sup>	Aroma associated with cooked pork muscle
Oral Texture	
Fimness <sup>d</sup>	Effort required to bite through lean and to convert the sample so that it can be swallowed
Crumbliness <sup>c</sup>	Degree of granularity of muscle fibers
Juiciness <sup>a</sup>	Impression of lubricated food during chewing
Chewy/Springiness <sup>d</sup>	Springs back to original position when compressed/bitten
Basic tastes	
Sour <sup>b</sup>	The taste on the tongue associated with citric acid
Salty <sup>a</sup>	The taste on the tongue associated with sodium ions
Bitter <sup>a</sup>	The taste on the tongue associated with caffeine
Sweetness <sup>b</sup>	The taste on the tongue associated with sucrose
Flavor	
Fat complex <sup>d</sup>	Flavor associated with lipid products such as animal fat and lard
Spice <sup>d</sup>	Flavor associated with pepper, salt, and sage
Rancid <sup>b</sup>	Flavor associated with extremely oxidized fat or oil
Chemical <sup>d</sup>	The taste on the tongue associated with medicine or soap
Pork <sup>b</sup>	Flavor associated with cooked pork muscle meat
Off-flavor <sup>d</sup>	Metallic, old, musty, piggy

 Table 3.2
 Fresh pork sausage sensory descriptors and definitions.

<sup>a</sup>Sensory descriptors and definitions taken from Flores et al. (1999)

<sup>b</sup>Sensory descriptors and definitions taken from Pegg and Shahidi (2007)

<sup>c</sup>Sensory descriptors and definitions taken from Rodbotton et al. (2004)

<sup>d</sup>Sensory descriptors determined during training by descriptive panelists, definitions provided by Civille and Lyon (1996)

procedures that were used for descriptive analysis. Panelists were provided with water, unsalted crackers, and expectorant cups. Each of the two samples were evaluated based on acceptability of flavor, aroma, texture, appearance and overall acceptability. Evaluations were conducted using a 9-point hedonic scale; 1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much and 9-like extremely (Meilgaard et al., 2007)(Figure A2).

#### **Statistical Analysis**

A randomized complete block design with three replications and a factorial structure was utilized to determine differences (P<0.05) in fresh pork sausage patty treatments for microbial growth, changes in color, oxidation and sensory descriptors over time. The Least Significant Difference (LSD) test was used to separate means, when significant differences occurred over time, among treatments.

Sensory descriptors, treatments, and intensity over time relationships were determined by use of Principal Components Analysis (PCA, Statistical Analysis Software, version 9.1 SAS® Institute, Cary, NC). A randomized complete block design with three replications was used to determine the differences (P<0.05) in consumer acceptability between the control and LV samples. Agglomerative hierarchical clustering was used to cluster consumers on the basis of consumer opinion for appearance, aroma, texture, flavor, and overall acceptance (Figure A3). A dendrogram plot was used to determine the number of clusters that should be used to group consumers (Figure A4).

# CHAPTER IV

# **RESULTS AND DISCUSSION**

#### **Microbial Analysis**

Total Plate Counts (TPCs) of all treatments over storage time are shown in Table 4.1. There were a few differences (P<0.05) among treatments with respect to TPCs at all storage times, with means ranging from 2.6 to 7.6 CFU/g (Table 4.1). Initial total plate count (TPC) for fresh pork sausage was  $10^4$  colony forming units (CFU)/g. This was slightly lower than the initial TPC of fresh pork sausage reported by Brewer et al. (1991). Brewer et al. (1991) reported an initial TPC of  $10^6$ , which could be explained by a higher initial microbial load of the pork meat or environment differences between studies. Total plate counts of LV indicated an initial decrease (P<0.05) in microbes at 0-d of 2 logs, as compared to L and C. The negative control and V also showed numerically reduced TPC's compared to the C, but did not differ (P>0.05) statistically from the control. Jensen et al. (2003) hypothesized that due to the weak acid nature of acetic acid, its undissociated properties in fresh meat would act as a more effective antimicrobial than lactic acid. These researchers reported that enhancing pork chops with acetate or a lactate/diacetate mixture is more advantageous than maintaining shelf-life with phosphate and salts alone (Jensen et al., 2003). However, the current study conducted showed that

			Day		
Treatment <sup>1</sup>	0	7	14	16	18
С	4.7 <sup>aA</sup>	$4.6^{abA}$	$7.6^{\mathrm{aB}}$	$7.5^{\mathrm{aB}}$	7.3 <sup>aB</sup>
NC	3.7 <sup>abA</sup>	$4.8^{abA}$	7.3 <sup>aB</sup>	7.3 <sup>aB</sup>	6.8 <sup>aB</sup>
L	5.0 <sup>aA</sup>	5.4 <sup>bAB</sup>	5.4 <sup>bAB</sup>	$6.0^{abAB}$	$6.0^{abB}$
LV	2.6 <sup>bA</sup>	$4.1^{abB}$	4.9 <sup>bBC</sup>	5.4 <sup>bC</sup>	5.6 <sup>bC</sup>
V	3.5 <sup>abA</sup>	3.8 <sup>aA</sup>	$5.7^{bB}$	5.7 <sup>bB</sup>	6.3 <sup>aB</sup>

Table 4.1 Mean TPC (log CFU/g), over time of fresh pork sausage patties

<sup>a, b</sup> Means within each column with different superscripts significantly differ (P<0.05).

<sup>A, B</sup> Means within each row with different superscripts significantly differ (P<0.05).

 $^{1}$  C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

there is no difference (P > 0.05) between sausage treated with V and L except at 7-d (Table 4.1). Differences between studies have occured due to the type of acetic acid used. The vinegar solution used for this study was adjusted by the manufacturer to potentially optimize pH. This adjustment in pH may have led to a decrease in the ability of acetic acid to acidify the cell interior of the bacteria. George et al. (1996) stated that when acetic and lactic acid solutions have an equivalent concentration of undissociated acid, lactic acid is more inhibitive. This may have been the case in the current study. Adjustment of the V pH may have allowed for an equilibrium to be reached between the two products used for L and V treatments. L initially showed a 1 log increase in counts from 0-d, but did not differ (P>0.05) from C. This may be explained by potential contamination of the treatment during processing or with contamination within the other ingredients. However, use of 2% L by Brewer et al. (1991) also showed an increase of TPC at 0-d when compared to treatments with no sodium lactate added. The study conducted by Brewer et al. (1991) is also comparative to the current study in relation to the comparison of a control and 2.5% sodium lactate. Both studies show an increase in

TPC of lactate samples over time until around 10-d. After 10-d, sausage samples treated with 2% (Brewer et al., 1991) and 2.5% (current study) lactate in both studies showed a decrease in counts from controls with no antimicrobials. At the end of shelf-life, in both studies, TPC of lactate treated sausage was lower, but was also no different from control sausages. Total plate counts of L sausage at 16 and 18-d for the current study was recorded at 6.0 CFU/g which is comparable to Brewer et al. (1991) who reported 6.49 CFU/g for sausage treatments with 2% sodium lactate after 18-d of storage. Day 14 TPC of C and NC were higher (P<0.05) than L, V, and LV at 14-d by at least 2 logs with counts of C and NC at 14-d, 7.6 and 7.3 CFU/g, respectively. Fresh pork sausage can be held for up to 10-d at  $4^{\circ}$ C in a normal or modified atmosphere (Cocolin et al., 2004). Spoilage levels are reached at  $10^7$  CFU/g (Arganosa et al., 1987). Controls C and NC reached spoilage between 7 and 14-d, while treatments of L, V, and LV delayed spoilage levels beyond 14-d. The C treatment with the addition of BHA/BHT did not deter bacterial growth. Antioxidants are recorded as generally only delaying oxidation of fat in the product, and not inhibiting microbial growth. Controls C and NC did not differ (P>0.05) at any evaluation time, and TPCs between them did not differ greatly over time (Table 4.1). Day 18 L and LV did not differ (P>0.05) from each other but LV had lower (P<0.05) counts than C, NC, and V. Treatment LV had lower counts (P<0.05) than NC and C for 14, 16, and 18-d evaluations as well. Use of these mixtures have been recorded as lowering counts of psychrotropic microbials such as *Enterobacteriaceae* (Mendonca et al., 1989) and Listeria monocytogenes (Ita and Hutkins, 1991). Effects of LV on shelflife of the sausage are consistent with reports by Adams and Hall (1988) on the

synergistic effects between lactate and acetate. Synergism between lactic acid and acetic acid was determined using a two acid toxicological model by Rubin (1978). The two acids were deemed to be synergistic if the combined inhibition of *Salmonella typhimurium* growth was greater than both acids singularly, and antagonistic if inhibition was less than each acid singularly. A resulting 12% increase in inhibition of the lactic acid/acetic acid mixture was deemed to be synergistic (Rubin, 1978). Adams and Hall (1988) further explained from the previous work from Rubin, that undissociated acid is a toxic factor for microbials. They theorize lactic acid in the acid mixture sets the precedence of a lower pH cellular environment, which allows for greater undissociation of acetic acid.

As expected, TPCs increased (P<0.05) over time for all treatments. However, as in the previously referenced studies, lactate and acetic acid products tended to maintain lower microbial numbers after initial 7-d increases as compared to treatments without inhibitors with LV having the lowest 18-d TPC.

No growth of *E. coli* was indicated throughout the study. Coliform growth occurred only during the third replication and was found only in C, NC, and L sausage patties (Figure A5). Coliform growth may have been due to contamination in this particular replication since no other replications indicated coliform growth and none detected in treatments LVand V initially or at 18-d.

					Day			
Value	Treatment <sup>1</sup>	0	4	7	11	14	16	18
$L^*$	С	$48.6^{\mathrm{aA}}$	$51.0^{\mathrm{aA}}$	$47.9^{\mathrm{aA}}$	$50.0^{\mathrm{aA}}$	$47.2^{aA}$	$48.2^{\mathrm{aA}}$	$48.7^{abA}$
	NC	$47.6^{aA}$	$51.1^{\mathrm{aA}}$	$49.1^{\mathrm{aAB}}$	$48.6^{\mathrm{aAB}}$	$46.9^{\mathrm{aB}}$	$47.6^{\mathrm{aAB}}$	$50.9^{\mathrm{aA}}$
	L	$48.4^{\mathrm{aAB}}$	$50.7^{\mathrm{aB}}$	$47.8^{\mathrm{aAB}}$	$48.1^{\mathrm{aAB}}$	$44.9^{aA}$	$45.8^{\mathrm{aA}}$	$46.9^{\text{bAB}}$
	LV	$50.4^{\mathrm{aA}}$	$48.3^{\mathrm{aAB}}$	$48.5^{\mathrm{aAB}}$	$48.7^{\mathrm{aAB}}$	$46.0^{\mathrm{aB}}$	$47.0^{\mathrm{aAB}}$	$47.4^{abAB}$
	Λ	$49.3^{\mathrm{aABC}}$	$51.0^{\mathrm{aB}}$	$47.9^{aA}$	$52.0^{aC}$	$46.0^{\mathrm{aA}}$	$47.9^{\mathrm{aA}}$	$47.8^{abA}$
$a^*$	С	$15.4^{aA}$	$12.4^{aB}$	$13.6^{\mathrm{aB}}$	$11.6^{\mathrm{aB}}$	$11.0^{\mathrm{aB}}$	$8.5^{\rm abC}$	$7.3^{\rm aC}$
	NC	$15.1^{aA}$	$12.3^{\mathrm{aB}}$	$12.3^{\mathrm{aB}}$	$11.0^{\mathrm{aB}}$	$10.2^{\mathrm{aB}}$	$7.5^{\rm aC}$	$6.9^{\mathrm{aC}}$
	L	$15.7^{\mathrm{aA}}$	$11.9^{\mathrm{aB}}$	$12.6^{\mathrm{aB}}$	$13.3^{\mathrm{aAB}}$	$12.2^{\mathrm{aB}}$	$10.9^{\rm bBC}$	$8.5^{abC}$
	LV	$14.1^{aA}$	$12.9^{\mathrm{aAB}}$	$12.3^{\mathrm{aAB}}$	$13.2^{\mathrm{aAB}}$	$12.2^{\mathrm{aAB}}$	$10.7^{ m bB}$	$10.8^{\mathrm{bB}}$
	Λ	$16.1^{\mathrm{aA}}$	$11.5^{\mathrm{aB}}$	$13.3^{\mathrm{aB}}$	$11.9^{\mathrm{aB}}$	$11.7^{\mathrm{aB}}$	$8.9^{abBC}$	$7.2^{\rm aC}$
$p^*$	С	$12.4^{\mathrm{aA}}$	$10.6^{\mathrm{aA}}$	$11.6^{\mathrm{aA}}$	$10.9^{\mathrm{aA}}$	$11.3^{aA}$	$11.4^{\mathrm{aA}}$	$12.1^{\mathrm{aA}}$
	NC	$12.3^{\mathrm{aA}}$	$10.3^{\mathrm{aA}}$	$11.7^{\mathrm{aA}}$	$11.5^{\mathrm{aA}}$	$11.1^{aA}$	$11.1^{\mathrm{aA}}$	$11.4^{\mathrm{aA}}$
	L	$12.3^{aA}$	$10.3^{\mathrm{aA}}$	$10.8^{\mathrm{aA}}$	$11.2^{\mathrm{aA}}$	$11.2^{\mathrm{aA}}$	$10.9^{\mathrm{aA}}$	$10.9^{\mathrm{aA}}$
	LV	$12.7^{\mathrm{aA}}$	$10.2^{\mathrm{aB}}$	$10.7^{\mathrm{aB}}$	$11.3^{\mathrm{aAB}}$	$10.9^{\mathrm{aAB}}$	$10.4^{\mathrm{aB}}$	$11.1^{\mathrm{aAB}}$
	Λ	$14.2^{aA}$	$10.0^{\mathrm{aB}}$	$11.2^{\mathrm{aB}}$	$10.9^{\mathrm{aB}}$	$11.5^{\mathrm{aB}}$	$11.3^{\mathrm{aB}}$	$11.7^{\mathrm{aB}}$

Table 4.2 CIE L\*a\*b\* color values, over time for fresh pork sausage patties stored up to 18 days at 1-2°C

 $^{A,B,C}$  Means within each row with different superscripts significantly differ (P<0.05). <sup>1</sup> C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

43

#### **Instrumental Color Analysis**

CIE  $L^*$  values ranged from 45.8 to 52.0. There were no differences (P>0.05) between treatments over storage times through 16-d (Table 4.2). This coincides with findings from Jensen et al. (2003) who also reported no differences (P>0.05) between treatments over storage time. Use of acetic acid in previous studies determined that increasing percentages of acetic acid in comminuted fresh meats result in a lighter  $L^*$ color when compared to controls (Stivarius et al., 2002; Arganosa et al., 1987; Kotula and The lappurate, 1994).  $L^*$  values for V were not different (P>0.05) over 0 to 11-d storage time. In addition, V had slightly higher values than C. After 11-d, L\* values for V trended slightly lower (Table 4.2). By 11-d, C sausage samples had surpassed optimal visual shelf-life and were discolored, while V samples still retained some acceptable visual color. Treatment NC at 18-d (50.9) had a lighter color (P < 0.05) than 18-d L (46.9). Use of antioxidants BHA/BHT in previous studies have not influenced a change in  $L^*$  value over time for product (Faustman et al., 2006); however, use of other antioxidants (sodium tripolyphosphate, ascorbic acid, and carnosine) used by Cheng et al. (2007) decreased  $L^*$ values after 7-d refrigerated storage when compared to control treatments. Values for  $L^*$ fluctuated over time and did not have a consistent pattern from 0-d to 18-d, except for C which did not differ (P>0.05) over time (Table 4.2).

CIE  $a^*$  values are positive (redness) or negative (greenness) with 0 indicating grey. Mean values for  $a^*$  in the current study showed a greater amount of difference over time and among treatments than  $L^*$  or  $b^*$  values. There was no difference (P>0.05) in  $a^*$ values among treatments at any of the storage times through 14-d (Table 4.2). Sausage patties at 16-d with LV and L had higher  $a^*$  values (P<0.05) than NC patties after 16-d storage; however, L and NC patties did not differ (P>0.05) at 18-d (Table 4.2). In addition, LV preserved redness through 18-d more than (P<0.05) C, NC, and V. Bradford et al. (1993) reported that use of potassium lactate increased  $a^*$  to 14-d before decreasing in redness. They theorized that redness discoloration may be linked to the ability of oxygen to permeate the film and resulting color change of myoglobin to metmyoglobin. There is little information on the process that allows sodium lactate to maintain higher  $a^*$  values. Kim et al. (2006) theorized that lactate increases the reducing activity of metmyoglobin. These researchers reported that sodium lactate provides color stability through lactic dehydrogenase activity which provides NADH to reduce metmyoglobin to either oxymyoglobin or deoxymyoglobin. Mancini and Ramanathan (2008) used methods from Kim et al. (2006) to measure the effects of sodium lactate on equine myoglobin redox stability. They state that 100 and 200 mM levels of sodium lactate inhibit equine oxymyoglobin oxidation when pH is held between 5.6 and 7.4.

CIE  $b^*$  values are positive (yellowness) and negative (blueness) with 0 indicating gray. There were no differences (P>0.05) among treatments at any storage time for  $b^*$ . Means of  $b^*$  values ranged from 10.0 to 14.2 (Table 4.2). Except for an initial decrease (P<0.05) in  $b^*$  for LV and V from 0-d to 7-d, and then remaining relatively constant, there were no practical differences in  $b^*$  values. Similar studies with the use of acetic acid, sodium lactate, and other antioxidants also indicate no differences in  $b^*$  values (Stivarius et al., 2002; Bradford et al., 1993; Cheng et al., 2007; Lee et al., 2005; Papadopoulos et al., 1991b).

			Day			
Treatment <sup>1</sup>	0	7	14	16	18	
C	$1.8^{\mathrm{aA}}$	$6.9^{\mathrm{aB}}$	$5.6^{\mathrm{aB}}$	$7.6^{\mathrm{aBC}}$	$9.6^{\mathrm{aC}}$	
NC	$3.0^{\mathrm{aA}}$	$8.7^{\mathrm{aB}}$	$6.8^{\mathrm{aB}}$	$11.2^{\mathrm{aBC}}$	$10.7^{\mathrm{aC}}$	
L	$2.8^{\mathrm{aA}}$	$7.3^{\mathrm{aB}}$	$7.4^{\mathrm{aB}}$	$8.1^{\mathrm{aBC}}$	$10.6^{\mathrm{aC}}$	
LV	$2.4^{\mathrm{aA}}$	$7.4^{\mathrm{aB}}$	$8.5^{\mathrm{aB}}$	$7.6^{\mathrm{aBC}}$	$10.0^{\mathrm{aC}}$	
Λ	$3.4^{aA}$	$7.5^{\mathrm{aB}}$	$7.6^{\mathrm{aB}}$	$7.7^{ m aBC}$	$8.5^{\mathrm{aC}}$	
<sup>a</sup> Means within each $^{A, B, C}$ Means within $\epsilon$	column with differen	Means within each column with different superscripts significantly differ ( $P<0.05$ ). <sup>B,C</sup> Means within each row with different superscripts significantly differ ( $P<0.05$ ).	ly differ (P<0.05). tly differ (P<0.05).			
<sup>1</sup> C=control; NC= ne	gative control; L= so	dium lactate; V= acetic a	C=control; NC= negative control; L= sodium lactate; V= acetic acid; LV = sodium lactate/acetic acid mixture.	acetic acid mixture.		

Table 4.3 Thiobarbituric acid reactive substance (TBARS) values over time for fresh pork sausage patties stored up to 18 days at 1-2°C

₹ ₹ 46

#### **Thiobarbituric Active Reactive Substances Values**

There were no differences among treatments on individual evaluation days (P>0.05) for TBARS values (Table 4.3). Bradford et al. (1993) and Brewer et al. (1991) also reported that there were no effects on TBARS with the use of lactate. However; there are studies that report use of sodium lactate decreases TBARS values which indicates decreased oxidation over time when compared to control samples (Maca et al., 1999). Sallum and Samejima (2004) theorize that the ability of sodium lactate to prevent oxidation may be dependent on microbial growth, other nonmeat ingredients and amounts, packaging, and storage conditions. While there were no differences (P>0.05) among treatments at each storage time, TBARS increased (P<0.05) over time, indicating increased oxidation. Levels of oxidation at 0-d were relatively low, and increased (P<0.05) after 7-d of storage prior to stabilization at 16-d. At 18-d, C had a slightly lower value (9.6) compared to NC (10.7). Although not different (P>0.05) at each evaluation time, C maintained lower numerical TBARS values when compared to NC. The addition of BHA/BHT to C is hypothesized as the reason for this trend, as would be expected. This is comparable to previous studies conducted that do not report a difference between a control sausage and sausage with antioxidants, but does show lower oxidative values for the antioxidant product (Cheng et al., 2007; Sebranek et al., 2005). The formulation of fresh pork sausage includes salt, which is a prooxidative ingredient in fresh meat products. Negative control (NC) was only formulated with basic pork sausage ingredients, (salt, sage, and spice) and consistently had higher TBARS values during the study, when compared to antioxidant treatments, especially at 16-d.

#### **Sensory Evaluation**

Descriptive sensory panel evaluations of fresh pork sausage showed minimal differences among samples up to 14-d. Eleven out of the twenty-two attributes showed differences (P<0.05) among treatments (Tables 4.4, 4.5, 4.6). Cooked complex did not differ (P>0.05) among treatments, except for C and V (P<0.05) at 0-d. L (0.3), V (0.4), and LV (0.3) at 17-d had lower (P<0.05) sensory scores for rancid aroma compare to C and NC. Treatments L, V, and LV had higher juiciness values at 17-d; however, only V was higher (P<0.05) in juiciness than C and NC at 17-d (Table 4.4). Choi and Chen (2003) reported use of sodium lactate in sausages made the sausages less springy at 14-d, and related the loss to decreases in water holding capacity and purge over time. While generally no different (P>0.05) than all other treatments, L in the current study had the highest value for firmness at 14-d. This may be due to overcooking and is not considered of great practical significance since there were no other differences in firmness. Perception of saltiness of the V sausage patties was lower (P<0.05) at 0-d when compared to other treatments, but did not differ (P < 0.05) from other treatments on subsequent testing days (Table 4.5). Means of sensory saltiness at 0-d ranged from a low for V of 2.8 to a high of 3.8 for L, but did not differ (P>0.05) in saltiness at 0-d from C (3.0), NC (3.3), or L (3.8). Brewer et al. (1991) and Jensen et al. (2003) theorized that the higher taste perception of salt may be due to ionized sodium lactate which may act as an enhancer. Treatment V also showed the most off flavor numerically at 17-d and was higher (P<0.05) than L or LV. Presence of higher off-flavor values are consistent with

			Ι	Day	
Attribute <sup>1</sup>	Treatment <sup>2</sup>	0	7	14	17
Aroma					
Cooked complex	С	7.3 <sup>aA</sup>	7.2 <sup>aA</sup>	6.6 <sup>aA</sup>	6.4 <sup>aA</sup>
	NC	$6.8^{abA}$	7.1 <sup>aA</sup>	6.9 <sup>aA</sup>	7.3 <sup>aA</sup>
	L	6.8 <sup>abA</sup>	$7.0^{\mathrm{aA}}$	6.9 <sup>aA</sup>	7.3 <sup>aA</sup>
	V	6.3 <sup>bA</sup>	7.4 <sup>aB</sup>	$7.2^{\mathrm{aAB}}$	6.8 <sup>aAB</sup>
	LV	7.1 <sup>abA</sup>	7.2 <sup>aA</sup>	6.8 <sup>aA</sup>	7.0 <sup>aA</sup>
Rancid	С	0.1 <sup>aA</sup>	$0.2^{\mathrm{aA}}$	$0.5^{\mathrm{aB}}$	1.0 <sup>aB</sup>
	NC	$0.1^{aA}$	$0.1^{aA}$	$0.4^{\mathrm{aB}}$	$1.0^{\mathrm{aC}}$
	L	$0.2^{\mathrm{aA}}$	0.3 <sup>aA</sup>	0.3 <sup>aA</sup>	0.3 <sup>bA</sup>
	V	$0.1^{aA}$	$0.1^{aA}$	$0.4^{\mathrm{aB}}$	$0.4^{bB}$
	LV	$0.0^{\mathrm{aA}}$	$0.3^{\mathrm{aBC}}$	$0.6^{\mathrm{aB}}$	0.3 <sup>bAC</sup>
Oral Texture					
Firmness	С	6.1 <sup>aA</sup>	6.4 <sup>aA</sup>	6.4 <sup>aA</sup>	7.0 <sup>aA</sup>
	NC	$6.0^{\mathrm{aA}}$	6.3 <sup>aA</sup>	6.6 <sup>aA</sup>	$7.1^{aA}$
	L	6.9 <sup>aA</sup>	7.2 <sup>aA</sup>	9.1 <sup>bB</sup>	$6.6^{\mathrm{aA}}$
	V	5.5 <sup>aA</sup>	$7.0^{\mathrm{aA}}$	6.4 <sup>aA</sup>	$5.4^{\mathrm{aA}}$
	LV	$7.0^{\mathrm{aA}}$	$7.2^{\mathrm{aA}}$	$6.6^{\mathrm{aA}}$	$7.0^{\mathrm{aA}}$
Juiciness	С	6.6 <sup>aA</sup>	6.0 <sup>aA</sup>	$5.7^{aAB}$	4.7 <sup>aB</sup>
	NC	6.0 <sup>aA</sup>	5.9 <sup>aA</sup>	$5.7^{aAB}$	$4.5^{\mathrm{aB}}$
	L	5.9 <sup>aA</sup>	5.5 <sup>aA</sup>	5.9 <sup>aA</sup>	$5.5^{abA}$
	V	6.8 <sup>aA</sup>	6.1 <sup>aA</sup>	6.6 <sup>aA</sup>	$6.0^{bA}$
	LV	$5.9^{aAB}$	$6.5^{aAB}$	6.7 <sup>aA</sup>	5.5 <sup>abB</sup>

Table 4.4 Significant sensory descriptive attributes for aroma and oral texture, over time, of cooked fresh pork sausage.

<sup>a, b</sup> Means within each column with different superscripts significantly differ (P<0.05). <sup>A, B, C</sup> Means within each row with different superscripts significantly differ (P<0.05).

<sup>1</sup> Anchored 15 cm point scale (0 = absence of attribute; 15 = extremely intense).

 $^{2}$  C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

				Day	
Attribute <sup>1</sup>	Treatment <sup>2</sup>	0	7	14	17
<b>Basic tastes</b>					
Sour	С	$0.5^{\mathrm{aA}}$	$0.7^{\mathrm{aAB}}$	$0.8^{\mathrm{aAB}}$	1.2 <sup>aB</sup>
	NC	$0.5^{\mathrm{aA}}$	$0.7^{\mathrm{aA}}$	$0.8^{\mathrm{aA}}$	$0.7^{abA}$
	L	$0.7^{\mathrm{aA}}$	1.3 <sup>bB</sup>	$0.7^{\mathrm{aA}}$	$1.1^{abA}$
	V	$0.5^{\mathrm{aA}}$	$0.9^{abA}$	$0.8^{\mathrm{aA}}$	0.7 <sup>bA</sup>
	LV	$0.6^{aA}$	$1.0^{abA}$	$0.6^{\mathrm{aA}}$	$0.8^{abA}$
Salty	С	$3.0^{abA}$	3.1 <sup>aA</sup>	3.4 <sup>aA</sup>	2.8 <sup>aA</sup>
	NC	3.3 <sup>abA</sup>	3.8 <sup>aA</sup>	3.6 <sup>aA</sup>	3.1 <sup>aA</sup>
	L	$3.8^{abA}$	$4.0^{\mathrm{aA}}$	$4.2^{\mathrm{aA}}$	3.6 <sup>aA</sup>
	V	2.8 <sup>bA</sup>	3.4 <sup>aA</sup>	3.4 <sup>aA</sup>	3.0 <sup>aA</sup>
	LV	3.8 <sup>aA</sup>	4.1 <sup>aA</sup>	$3.6^{\mathrm{aA}}$	3.7 <sup>aA</sup>
Bitter	С	$0.5^{\mathrm{aA}}$	$0.4^{\mathrm{aA}}$	$0.4^{\mathrm{aA}}$	0.7 <sup>abA</sup>
	NC	0.3 <sup>aA</sup>	$0.5^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	$0.4^{\mathrm{aA}}$
	L	$0.4^{\mathrm{aA}}$	$0.6^{\mathrm{aAB}}$	$0.6^{\mathrm{aAB}}$	$0.8^{bB}$
	V	0.3 <sup>aA</sup>	$0.5^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	$0.5^{abA}$
	LV	$0.4^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	$0.4^{\mathrm{aA}}$	$0.6^{abA}$
Flavor					
Spice	С	$8.5^{\mathrm{aA}}$	7.9 <sup>aA</sup>	$7.7^{\mathrm{aA}}$	5.9 <sup>aB</sup>
-	NC	9.0 <sup>aA</sup>	$8.2^{aA}$	8.4 <sup>aA</sup>	$8.0^{bA}$
	L	8.8 <sup>aA</sup>	$8.6^{aA}$	8.6 <sup>aA</sup>	8.3 <sup>bA</sup>
	V	8.1 <sup>aA</sup>	$7.9^{\mathrm{aA}}$	$7.9^{\mathrm{aA}}$	$6.4^{aB}$
	LV	8.9 <sup>aA</sup>	$8.7^{\mathrm{aA}}$	8.1 <sup>aA</sup>	8.2 <sup>bA</sup>
Rancid	С	$0.2^{\mathrm{aA}}$	$0.5^{aAB}$	$0.8^{\mathrm{aB}}$	$1.7^{\mathrm{aC}}$
	NC	0.3 <sup>aA</sup>	$0.5^{\mathrm{aA}}$	$0.7^{\mathrm{aA}}$	$1.5^{abB}$
	L	$0.1^{\mathrm{aA}}$	0.3 <sup>aA</sup>	$0.4^{\mathrm{aA}}$	$1.0^{bB}$
	V	$0.1^{aA}$	0.3 <sup>aA</sup>	$0.4^{\mathrm{aA}}$	1.1 <sup>bB</sup>
	LV	$0.2^{aA}$	$0.2^{aAB}$	$0.5^{\mathrm{aBC}}$	$0.9^{bC}$
Off flavor	С	$0.4^{\mathrm{aA}}$	0.6 <sup>aA</sup>	$0.9^{\mathrm{aA}}$	$1.4^{abB}$
	NC	$0.6^{aA}$	$0.6^{\mathrm{aA}}$	$0.6^{\mathrm{aA}}$	$1.8^{abB}$
	L	$0.5^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	1.3 <sup>bB</sup>
	V	$0.4^{aA}$	$0.5^{\mathrm{aA}}$	$0.7^{\mathrm{aA}}$	$2.0^{\mathrm{aA}}$
	LV	$0.5^{aA}$	$0.4^{aA}$	$0.8^{\mathrm{aA}}$	1.3 <sup>bA</sup>

Table 4.5 Significant sensory descriptive attributes for basic tastes and flavors, over time, of cooked fresh pork sausage.

<sup>A, B</sup> Means within each column with different superscripts significantly differ (P<0.05). <sup>A, B</sup> Means within each row with different superscripts significantly differ (P<0.05).

<sup>1</sup> Anchored 15 cm point scale (0 = absence of attribute; 15 = extremely intense).

 $^{2}$  C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

Attribute <sup>1</sup> Treatment <sup>2</sup> 0     7     14     17       Overall Acceptability     C $10.5^{aA}$ $9.6^{aAB}$ $8.8^{aBC}$ $8.0^{abC}$ Overall Acceptability     C $10.6^{aA}$ $10.1^{aAB}$ $9.5^{aB}$ $7.7^{bC}$ L $10.6^{aA}$ $9.7^{aAB}$ $9.8^{aBB}$ $8.8^{aB}$ $7.7^{bC}$ V $10.6^{aA}$ $9.7^{aAB}$ $9.8^{aAB}$ $8.8^{aB}$ $8.8^{aB}$ L $10.6^{aA}$ $9.7^{aAB}$ $9.8^{aAB}$ $8.8^{aB}$ V $10.0^{aA}$ $10.0^{aA}$ $9.5^{aA}$ $8.0^{aBA}$ LV $10.1^{aA}$ $9.9^{aAB}$ $9.3^{aAB}$ $9.0^{aBA}$					Day	
$\begin{array}{ccccccc} C & 10.5^{\mathrm{aA}} & 9.6^{\mathrm{aAB}} & 8.8^{\mathrm{aBC}} \\ \mathrm{NC} & \mathrm{NC} & 10.6^{\mathrm{aA}} & 10.1^{\mathrm{aAB}} & 9.5^{\mathrm{aB}} \\ \mathrm{L} & 10.6^{\mathrm{aA}} & 9.7^{\mathrm{aAB}} & 9.8^{\mathrm{aAB}} \\ \mathrm{V} & 10.0^{\mathrm{aA}} & 10.0^{\mathrm{aA}} & 9.8^{\mathrm{aAB}} \\ \mathrm{LV} & 10.1^{\mathrm{aA}} & 9.9^{\mathrm{aAB}} & 9.3^{\mathrm{aAB}} \end{array}$	Attribute <sup>1</sup>	Treatment <sup>2</sup>	0	7	14	17
$\begin{array}{rrrr} 10.6^{\mathrm{aA}} & 10.1^{\mathrm{aAB}} & 9.5^{\mathrm{aB}} \\ 10.6^{\mathrm{aA}} & 9.7^{\mathrm{aAB}} & 9.8^{\mathrm{aAB}} \\ 10.0^{\mathrm{aA}} & 10.0^{\mathrm{aA}} & 9.5^{\mathrm{aA}} \\ 10.1^{\mathrm{aA}} & 9.9^{\mathrm{aAB}} & 9.3^{\mathrm{aAB}} \end{array}$	Overall Acceptability	C	$10.5^{\mathrm{aA}}$		$8.8^{\mathrm{aBC}}$	$8.0^{ m abC}$
$\begin{array}{rrrr} 10.6^{\mathrm{aA}} & 9.7^{\mathrm{aAB}} & 9.8^{\mathrm{aAB}} \\ 10.0^{\mathrm{aA}} & 10.0^{\mathrm{aA}} & 9.5^{\mathrm{aA}} \\ 10.1^{\mathrm{aA}} & 9.9^{\mathrm{aAB}} & 9.3^{\mathrm{aAB}} \end{array}$		NC	$10.6^{\mathrm{aA}}$	$10.1^{\mathrm{aAB}}$	$9.5^{\mathrm{aB}}$	$7.7^{bC}$
$10.0^{\mathrm{aA}}$ $10.0^{\mathrm{aA}}$ $9.5^{\mathrm{aA}}$ $9.3^{\mathrm{aAB}}$ $9.3^{\mathrm{aAB}}$		L	$10.6^{\mathrm{aA}}$	$9.7^{\mathrm{aAB}}$	$9.8^{\mathrm{aAB}}$	$8.8^{\mathrm{aB}}$
$10.1^{aA}$ $9.9^{aAB}$ $9.3^{aAB}$		Λ	$10.0^{\mathrm{aA}}$	$10.0^{\mathrm{aA}}$	$9.5^{\mathrm{aA}}$	$8.0^{abA}$
		LV	$10.1^{aA}$	$9.9^{\mathrm{aAB}}$	$9.3^{\mathrm{aAB}}$	$9.0^{\mathrm{aB}}$
	<sup>1</sup> Anchored 15 cmpoint scale ( $0 = absence of attribute$ ; $15 = extremely intense$ ).	ibute: $15 = extremely intense$ ).				

Table 4.6 Overall acceptability over time of cooked fresh pork sausage.

 $^{2}$  C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

previous work done by Stivarius et al. (2002). At 17-d, sausage patties with added antimicrobials and antioxidants had less (P<0.05) rancidity flavor than C. Off-flavor ranged from 0.4 at 0-d to 2.0 at 18-d which is still relatively low in intensity value. Treatments L and LV had lower (P<0.05) off-flavor scores at 17-d than V. Treatment V had the highest sensory level of off-flavor at 17-d, but did not appear realated to sourness as indicated by the sensory scores for sourness. Papadoupolus et al. (1991b) concluded that use of sodium lactate slowed the decrease of "on-notes" associated with fresh beef during storage. A few other differences (P < 0.05) in sourcess, bitterness and spices are noted in Table 4.4.2 that are of minimal practical significance due to small numerical differences in the intensity values. Overall acceptability (Table 4.6) did not differ (P>0.05) among treatments through 14-d. Treatment LV (9.0) at 17-d had the highest numerical sensory score from the descriptive panel, but did not differ (P>0.05) from L and C. Treatment NC was least (P<0.05) acceptable at 17-d. Although overall acceptability scores trended lower over time, the lactate and acetic acid/lactate combination treatments maintained higher (P<0.05) acceptability scores over time as compared to C and NC. No other differences existed (P>0.05) in acceptability at 17-d.

Four of the sensory descriptive attributes that showed no differences (P>0.05) among treatments by evaluation day are listed in Table 4.7. These attributes are listed as: chewy, sweet, chemical, and pork; and were determined to have neither a negative or positive effect on product acceptability. In the current study, pork flavor showed no significant change (P>0.05) among the treatments or over time, except for C being lower (P<0.05) at 17-d in pork favor than at 0-d. In contrast, Sutton et al. (1997) reported that

				Day	
Attribute <sup>1</sup>	Treatment <sup>2</sup>	0	7	14	17
Oral Texture					
Chewy	С	1.4 <sup>aA</sup>	1.5 <sup>aA</sup>	$1.9^{aAB}$	$2.7^{\mathrm{aB}}$
	NC	$1.0^{\mathrm{aA}}$	$1.7^{aAB}$	$2.1^{aB}$	3.4 <sup>aC</sup>
	L	1.3 <sup>aA</sup>	1.5 <sup>aA</sup>	$2.6^{\mathrm{aB}}$	3.3 <sup>aB</sup>
	V	1.1 <sup>aA</sup>	$1.7^{aA}$	$1.9^{aAB}$	$2.6^{\mathrm{aB}}$
	LV	1.6 <sup>aA</sup>	$1.7^{aAB}$	$2.4^{aAB}$	3.1 <sup>aB</sup>
<b>Basic Tastes</b>					
Sweet	С	1.0 <sup>aA</sup>	1.0 <sup>aA</sup>	$0.9^{\mathrm{aA}}$	1.0 <sup>aA</sup>
	NC	$1.0^{\mathrm{aA}}$	$1.0^{\mathrm{aA}}$	$0.7^{\mathrm{aA}}$	$0.8^{\mathrm{aA}}$
	L	$0.9^{\mathrm{aA}}$	0.9 <sup>aA</sup>	$0.8^{\mathrm{aA}}$	$0.8^{\mathrm{aA}}$
	V	1.1 <sup>aA</sup>	1.0 <sup>aA</sup>	0.9 <sup>aA</sup>	$0.8^{\mathrm{aA}}$
	LV	1.0 <sup>aA</sup>	$0.9^{aA}$	$0.8^{\mathrm{aA}}$	1.1 <sup>aA</sup>
Flavor					
Chemical	С	$0.4^{\mathrm{aA}}$	$0.4^{aA}$	$0.8^{\mathrm{aA}}$	0.9 <sup>aA</sup>
	NC	$0.6^{\mathrm{aA}}$	0.6 <sup>aA</sup>	$0.5^{aA}$	$0.8^{\mathrm{aA}}$
	L	$0.5^{\mathrm{aA}}$	$0.5^{\mathrm{aA}}$	$0.4^{aA}$	$0.7^{\mathrm{aA}}$
	V	0.3 <sup>aA</sup>	$0.4^{\mathrm{aA}}$	$0.5^{aA}$	$0.6^{aA}$
	LV	$0.4^{\mathrm{aA}}$	$0.5^{aAB}$	$0.6^{aAB}$	1.1 <sup>aB</sup>
Pork	С	4.6 <sup>aA</sup>	$5.1^{aAB}$	4.9 <sup>aAB</sup>	4.3 <sup>aB</sup>
	NC	4.6 <sup>aA</sup>	4.6 <sup>aA</sup>	$4.6^{\mathrm{aA}}$	4.4 <sup>aA</sup>
	L	4.9 <sup>aA</sup>	5.0 <sup>aA</sup>	5.0 <sup>aA</sup>	4.6 <sup>aA</sup>
	V	5.1 <sup>aA</sup>	$4.6^{\mathrm{aA}}$	$4.6^{aA}$	4.8 <sup>aA</sup>
	LV	5.0 <sup>aA</sup>	$4.8^{aA}$	4.5 <sup>aA</sup>	4.9 <sup>aA</sup>

Table 4.7 Sensory descriptive attributes of cooked fresh pork sausage showing no significant difference (P>0.05) between treatments over time.

<sup>A, b</sup> Means within each column with different superscripts significantly differ (P<0.05). <sup>A, B</sup> Means within each row with different superscripts significantly differ (P<0.05). <sup>1</sup> Anchored 15 cm point scale (0 = absence of attribute; 15 = extremely intense). <sup>2</sup> C = control; NC = negative control; L = sodium lactate; V = acetic acid; LV = sodium lactate/acetic acid mixture.

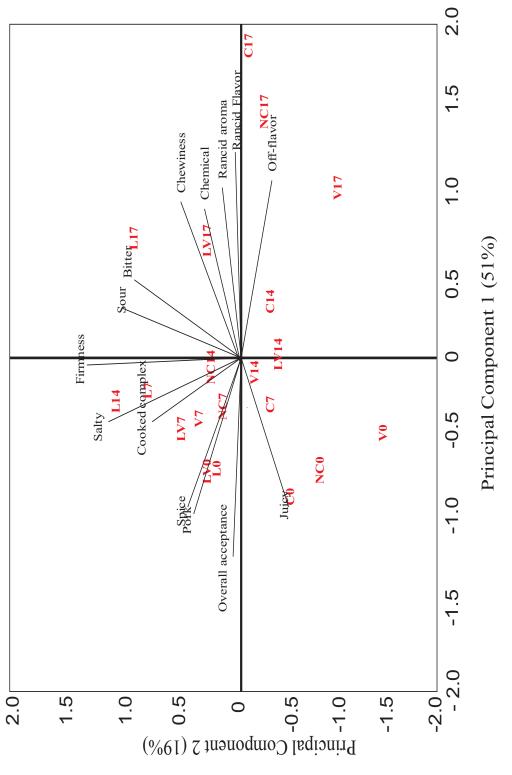


Figure 4.1. Principal components analysis biplot of sensory descriptive analysis data over time and treatments, control, and negative control of fresh pork sausage.

use of sodium lactate increased the intensity of pork flavor over time. Chewiness values ranged from 1.0 to 3.4 and became chewier (P<0.05) over time, but values still indicate that all treatments maintained a relatively soft sensory texture. Chemical flavor did not exceed a mean of 1.1 over time on the 15 point scale, which is still considered very acceptable. Treatment LV was the only treatment where the chemical perception ncreased (P<0.05) after 17-d of storage, in comparison to other treatments and storage times. Other treatments did not differ (P>0.05) over time. This indicates that addition of the antimicrobials and antioxidants used in this study did not create unwanted flavors or objectionable aftertastes. However, the chemical flavor of LV may have resulted from the combination of sodium ions and vinegar components reacting together.

The principal components analysis (PCA) biplot visualizes the variations of descriptive flavor attributes over time and treatments, control, and negative control of fresh pork sausage in the first two principal components (PCs) which show 70% of the variation in the data (Figure 4.1). PC1, explains 51% of the variance. The variance of PC1 relates the attributes more associated with the end of shelf life and off-flavors, basic tastes, aromas, and textures such as: sour, bitter, chewiness, chemical, rancid aroma, rancid flavor, and off flavors. PC2 dimension associates 19% of attribute variation and includes such attributes associated with beginning shelf-life and acceptable flavors, aromas, and textures including: pork, spice, cooked complex, salty, and firmness. This biplot confirms that off-flavors and odors increased as storage time increased and that the lactate and lactate/vinegar mixture had the least amount of objectionable flavor at 17-d.

#### **Consumer Acceptability**

No differences (P>0.05) were found by consumers for appearance, aroma, texture, flavor or overall acceptability (Table 4.8). Consumers were grouped into 5 clusters according to liking and preference of samples (Table 4.9). Cluster 1 panelists made up 13.4% of all panelists and moderately liked LV, but did not like the C sample. Conversely, cluster 5 panelists made up 3.9% of all panelists and liked C and disliked LV. Cluster 3 represents 26.8% of the panelists, and indicates that panelists liked (P<0.05) both C and LV very much. Cluster 4 (22% of panelists) also liked LV very much and slightly liked C. Overall, 96.1% of all panelists liked the LV sample, and 86.6% of panelists liked the C sample. One may conclude from these results that an overwhelming majority of the consumers did not have any dislike to the LV sausage patties and would equally consume sausage with the antimicrobials versus sausage products with only commonly used antioxidants.

	Treat	tment
Attribute <sup>1</sup>	$C^2$	$LV^2$
Appearance	7.4 <sup>a</sup>	7.5 <sup>a</sup>
Aroma	$7.2^{a}$	7.3 <sup>a</sup>
Texture	7.1 <sup>a</sup>	7.3 <sup>a</sup>
Overall Flavor	$7.2^{\mathrm{a}}$	$7.5^{\mathrm{a}}$
Overall Acceptability	$7.2^{\mathrm{a}}$	7.4 <sup>a</sup>

Table 4.8. Consumer panel means for fresh pork sausage C and LV treatments

<sup>a</sup>Means within each row with different superscripts significantly differ (P<0.05).

<sup>1</sup>Hedonic scale was based on a 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). <sup>2</sup>C = control; LV = sodium lactate and vinegar mixture.

Class	Panelist (%)	$C^2$	$LV^2$	
	13.4	4.4	6.8	
2	33.0	7.6	6.7	
~	26.8	8.3	8.3	
4	22.9	6.8	8.3	
S	3.9	7.6	3.9	
Hedonic scale was based C = control; LV = sodiu	<sup>1</sup> Hedonic scale was based on a 9-point scale (1 = dislike extremely, $5 =$ neither like nor dislike, and $9 =$ like extremely) <sup>2</sup> C = control; LV = sodium lactate and vinegar mixture.	either like nor dislike, and 9 = like extr	emely).	

Cluster analysis of consumer panel <sup>1</sup> for fresh pork sausage C and LV treatments.
Table 4.9.

## CHAPTER V

# SUMMARY AND CONCLUSIONS

Addition of sodium lactate, acetic acid, and their mixtures at the rates used in this study were effective at extending shelf-life while maintaining consumer acceptability and maintaining the sensory integrity of fresh pork sausage patties. Treatments L (2.5% sodium lactate), V (2.5% buffered vinegar), and LV (2.5% sodium lactate/acetic acid mixture) consistently out performed typical sausage formulations C (0.02% BHA/BHT), or NC (without antioxidants) in reducing microbial growth and maintaining red color. Also, over the course of the study, LV showed significant improvements compared to other treatments. Treatment LV reduced bacteria counts by 2 log<sup>10</sup> when compared to C samples. Samples from the LV treatment exhibited higher instrumental  $a^*$  (redness) values in comparison to the control after 14-d of storage as well as visually retained acceptable color through 18-d. Analysis of sensory descriptors over time revealed that LV samples had lower intensities for undesirable sensory characteristics such as rancidity and off-flavor as compared to other treatments and controls at the end of the 18-d shelflife. The overall consumer acceptability analysis of C and LV further confirmed the acceptability of using LV at the tested levels, in fresh pork sausages by indicating sensory acceptability scores equal to or exceeding the control scores. Cluster analysis showed that a very high percentage (>85%) of consumers liked both LV and C treated samples. In addition, 96% of panelists liked the LV treatment and 87% of panelists liked the control treatment.

Results obtained from this study may prove to be beneficial in the production of fresh pork sausage patties and other forms of pork sausage. The use of an ingredient that increases shelf-life while maintaining overall consumer acceptability can be very profitable and beneficial to all sections of the food chain; from the producer to the consumer. Addition of sodium lactate, acetic acid, or a mixture of acetic acid and sodium lactate is capable of increasing shelf-life up to an additional week. This is a great improvement, especially when many sliced patties on the market today are only in the case for four to 10 days maximum before off-color and/or oxidation causes them to be removed or sold at a reduced price. Increased shelf-life means less loss to the wholesaler, retailer, processor, and ultimately the consumer. This research indicates that the use of a 2.5% sodium lactate and acetic acid mixture in fresh pork sausage formulations can enhance product quality and potentially increase revenue for producers and all marketing segments, while maintaining consumer appeal and acceptability.

59

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APPENDIX

#### **Informed Consent Form – Pork Sausage patties**

Title of Study: The effects of Sodium Lactate and Acetic Acid derivatives on fresh pork sausage

Study Site: Department of Food Science, Nutrition and Health Promotion, Garrison Sensory Evaluation Laboratory

Name of Researcher(s) & University affiliation: Dr. J. Byron Williams (Extension and Research Professor)

What is the purpose of this research project? To understand the effects Sodium Lactate and Acetic Acid derivatives may have on fresh pork sausage in regards to increasing shelf life, pH and color change, and rancidity.

**How will the research be conducted?** You will be asked to taste <u>8</u> pork sausage samples. You will then be asked to record your responses on the provided score sheets.

Are there any risks or discomforts to me because of my participation? There are no anticipated risks of discomforts. You may discontinue your participation at any point.

**Does participation in this research provide any benefits to others or myself?** Yes, valuable information will be obtained that will help the pork sausage industry understand the alternative use of sodium lactate and acetic acid on product quality.

Will this information be kept confidential? Yes, Only the researcher who designed this study will have access to this information. \**Also, please note that these records will be held by a state entity and therefore are subject to disclosure if required by law.* 

**Who do I contact with research questions?** If you should have any questions about this research project, please feel free to contact Dr. J. Byron Williams at 662-325-8428. For additional information regarding your rights as a research subject, please feel free to contact the MSU Regulatory Compliance Office at 662-325-5220.

#### What do I do if I am injured at a result of this research?

In addition to reporting an injury to Dr. J. Byron Williams at 662-325-8428 and to the Regulatory Compliance Office (662-325-5220), you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Stone Building, Mississippi State, MS 39762, (662) 325-2121.* 

#### What if I do not want to participate?

Please understand that your **participation is voluntary**, your **refusal to participate will involve no penalty or loss** of benefits to which you are otherwise entitled, and you **may discontinue your participation** at any time without penalty or loss of benefits.

You will be given a copy of this form for your records.

Participant Signature

Date

Investigator Signature

Date

Figure A.1. Informed consent form

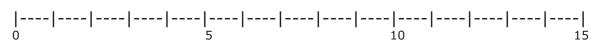
### Sensory Evaluation of Pork Sausage Patties

Date: Day:	Time of		
Panelist Code:	Gender:	F	М

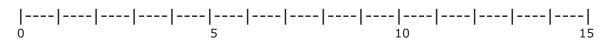
### 0: None 15: High intensity

## **APPEARANCE**

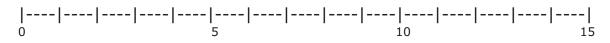
Color homogeneity: Presence of color homogeneity in the patty surface



Browning:



Cupping:



## **AROMA**

Cooked complex: <u>Aroma associated with cooked fat and lean with typical</u> <u>sausage spices</u>

$$\begin{vmatrix} ---- \\ 0 \end{vmatrix}$$
  $\begin{pmatrix} ---- \\ ---- \\ 5 \end{vmatrix}$   $\begin{pmatrix} ---- \\ ---- \\ ---- \\ 10 \end{vmatrix}$   $\begin{pmatrix} ---- \\ ---- \\ ---- \\ 15 \end{vmatrix}$ 

Boar taint: Aroma associated with boar meat; hormone-like (skatole)

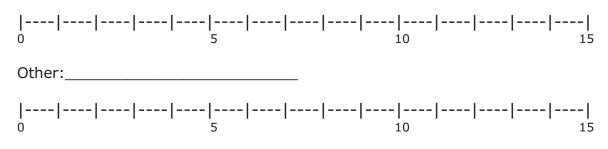
$$\begin{vmatrix} ---- \\ 0 \end{vmatrix}$$
  $\begin{pmatrix} ---- \\ ---- \\ 5 \end{vmatrix}$   $\begin{pmatrix} ---- \\ ---- \\ ---- \\ 10 \end{vmatrix}$   $\begin{pmatrix} ---- \\ ---- \\ ---- \\ 15 \end{vmatrix}$ 

Rancid: Aroma associated with extremely oxidized fat or oil

$$\begin{vmatrix} ---- \\ 0 & 5 & 10 & 15 \end{vmatrix}$$

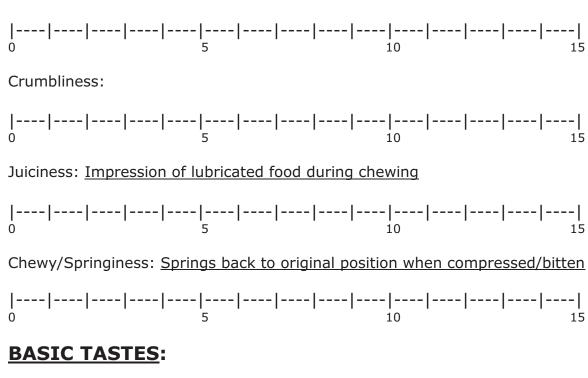
Figure A.2. Score sheet for descriptive sensory analysis

Pork: Aroma associated with cooked pork muscle meat



## ORAL TEXTURE

Firmness: Effort required to bite through lean and to convert the sample so that it can be swallowed



 Sour:
 The taste on the tongue associated with citric acid

----	----

 0
 5
 10

 5
 10
 15

 Salty:
 The taste on the tongue associated with sodium ions

 |----|----|
 5
 10

 0
 5
 10

Figure A.2 continued. Score sheet for descriptive sensory analysis

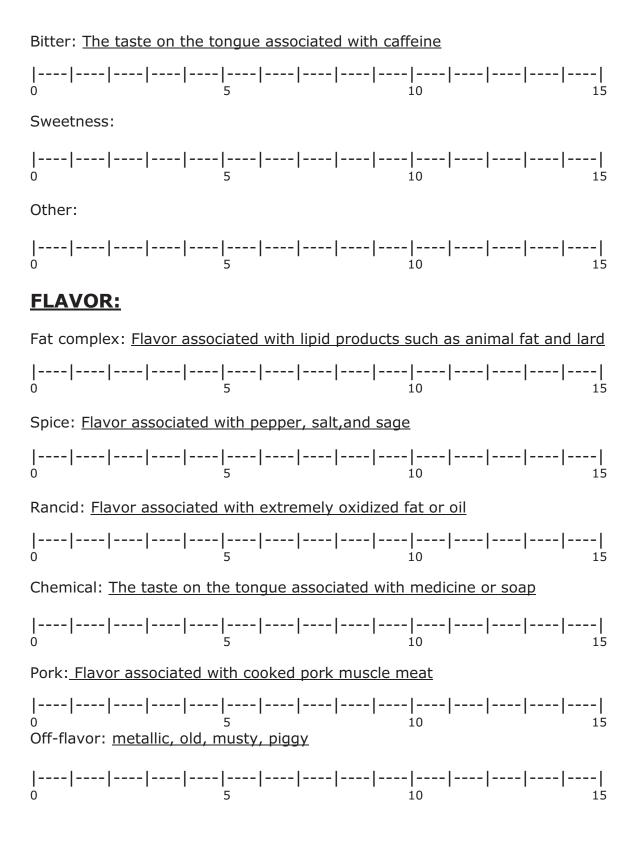


Figure A.2 continued. Score sheet for descriptive sensory analysis

# **OVERALL QUALITY:**

Overall:\_\_\_\_\_

 $\begin{vmatrix} ---- \\ 0 & 5 \end{vmatrix} = \begin{vmatrix} ---- \\ ---- \\ 10 & 15 \end{vmatrix}$ 

Figure A.2 continued. Score sheet for descriptive sensory analysis

### Figure A.3. Score sheet for consumer acceptability tests Samples: Pork Sausage Patties

Date:

Please taste each pork sausage patty sample in the order listed. After chewing if you do not wish to swallow the sample, you may expectorate it in the cup and rinse with the water provided. Rate each sample in each of the five categories listed.

Each column will need one check mark if you choose to evaluate all samples.

515	320	Appearance
		Like extremel
		Like very muc
		Like moderate
		Like slightly
		Neither like no
		Dislike slightl
		Dislike moder
		Dislike very n
		Dislike extrem

ly ch ely or dislike ly rately much Dislike extremely

	_
515 320	Aroma
	Like ext
	Like ver
	Like mo
	Like slig
	Neither
	Dislike
	Dislike
	Dislike
	Dislike

tremely ry much oderately ghtly like nor dislike slightly moderately very much extremely

515	320	Text
		Like
		Neith
		Dislil

ure extremely very much moderately slightly her like nor dislike ke slightly ke moderately ke very much ke extremely

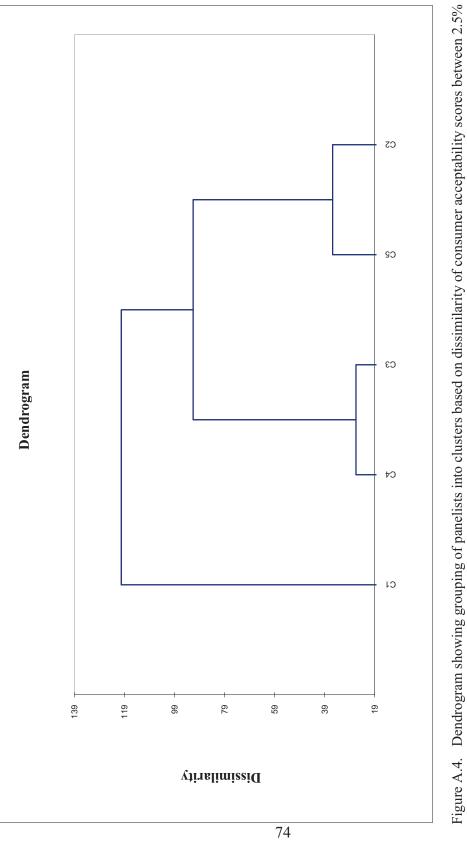
515	320	
		I
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		I
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**Overall Flavor** 

Like extremely Like very much Like moderately Like slightly Neither like nor dislike Dislike slightly Dislike moderately Dislike very much Dislike extremely

515	320	Overall Acceptability
		Like extremely
		Like very much
		Like moderately
		Like slightly
		Neither like nor dislike
		Dislike slightly
		Dislike moderately
		Dislike very much
		Dislike extremely

Thank you for your Participation!





Treatment	Replication	Day	log CFU/g
С	1	1	0.0
С	1	18	0.0
C NC	1	1	0.0
NC	1	18	0.0
L	1	1	0.0
L	1	18	0.0
LV	1	1	0.0
LV	1	18	0.0
V	1	1	0.0
V	1	18	0.0
V C C	2	1	0.0
С	2	18	0.0
NC	2	1	0.0
NC	2	18	0.0
L	2	1	0.0
L	2	18	0.0
LV	2	1	0.0
LV	2	18	0.0
V	2	1	0.0
V	2	18	0.0
V C C	3	1	3.4
С	3	18	6.1
NC	3	1	3.7
NC	3	18	6.3
L	3	1	3.7
L	3	18	7.1
LV	3	1	0.0
LV	3	18	0.0
V	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	1	0.0
V	3	18	0.0

Table A.1. Coliforms log CFU/g for each replication

Replication	Day	log CFU/g
1	0	6.0
1	7	4.1
1	14	7.3
1		7.2
1		7.0
		4.2
2	7	5.4
2	14	7.5
2		8.0
2		7.8
3		4.0
3		4.3
3		8.0
3		7.2
3		7.1
		3.4
		4.7
		7.1
		7.1
		6.0
		3.8
2		5.7
$\frac{1}{2}$		7.6
2		7.7
2		7.0
$\frac{1}{3}$		4.0
3		4.0
3		7.1
3		7.1
3		7.4
		7.0
1		3.8
1		5.6
1		5.7
-		4.9
		3.9
		4.4
$\frac{2}{2}$		5.1
$\frac{2}{2}$		6.8
	1 1	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

Table A.2. Mean TPCs log CFU/g for treatments over time for each replication of fresh pork sausage patties.

Treatment <sup>1</sup>	Replication	Day	log CFU/g
L		18	6.9
L	3	0	4.0
L	2 3 3 3 3 3 3	7	8.0
L	3	14	5.4
L	3	16	5.8
L	3	18	6.1
V	1	0	3.0
V	1	7	4.0
V	1	14	5.9
V	1	16	5.8
V	1	18	6.7
V	2	0	3.7
V	2	7	4.4
V	2	14	5.6
V	2	16	5.5
V	2	18	6.6
V	3	0	3.8
V	3	7	3.1
V	2 2 2 3 3 3 3 3 3 3	14	5.5
V	3	16	5.8
V	3	18	5.4
LV	1	0	1.0
LV	1	7	3.7
LV	1	14	3.8
LV	1	16	4.1
LV	1	18	4.3
LV	2	0	3.7
LV	2	7	3.7
LV	2	14	6.0
LV	2	16	6.0
LV	2	18	6.7
LV	3	0	3.1
LV	3	7	5.0
LV	3	14	4.8
LV	2 2 2 2 3 3 3 3 3 3 3 3 3	16	6.2
LV	3	18	5.9

Table A.2 continued.Mean TPCs log CFU/g for treatments over time for each<br/>replication of fresh pork sausage patties.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
С	1	0	1	53.3	11.6	13.1
С	1	0	2	59.4	9.5	11.2
С	1	0	3	40.2	20.4	12.6
С	1	7	1	51.7	11.8	12.0
С	1	7	2	52.3	12.3	11.0
С	1	7	3	48.4	15.4	12.8
С	1	14	1	46.1	14.0	12.3
С	1	14	2	47.4	11.8	13.4
С	1	14	3	49.2	12.1	12.6
С	1	16	1	50.1	8.4	10.9
С	1	16	2	52.4	7.4	12.5
С	1	16	3	53.2	7.4	13.8
С	1	18	1	50.8	5.5	11.5
С	1	18	2	49.7	7.2	12.8
С	1	18	3	51.7	5.3	12.4
С	2	0	1	44.1	16.6	11.3
С	2	0	2	45.4	17.0	12.9
С	2	0	3	48.1	17.1	14.7
С	2	4	1	53.5	11.3	9.7
С	2	4	2	53.5	6.5	6.8
С	2	4	3	47.8	13.1	9.2
С	2	7	1	42.6	14.3	12.0
С	2	7	2	49.1	11.2	10.6
С	2	7	3	45.0	13.4	10.6
С	2	11	1	45.8	10.0	8.7
С	2	11	2	52.3	8.6	7.7
С	2	11	3	45.7	11.6	9.3
С	2	14	1	50.3	6.7	9.8
С	2	14	2	49.7	7.8	11.4
	2	14	3	44.3	8.3	10.4
С	2	16	1	47.0	5.9	11.6
C C C C C	2	16	2	46.3	7.7	11.5
С	2	16	3	50.1	7.1	10.4
С	2	18	1	45.2	6.5	11.9
С	2	18	2	45.9	8.3	12.3

Table A.3. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
С	2	18	3	47.8	5.6	10.5
С	3	0	1	51.8	13.4	11.5
С	3	0	2	47.8	16.0	10.7
С	3	0	3	47.4	18.5	13.7
С	3	4	1	46.7	18.2	13.9
С	3	4	2	46.4	14.4	11.1
С	3	4	3	49.0	13.8	11.4
С	3	7	1	46.6	12.9	11.6
С	3	7	2	45.6	15.8	11.6
С	3	7	3	50.1	15.2	12.0
С	3	11	1	49.8	14.7	12.5
С	3	11	2	41.3	17.5	13.1
С	3	11	3	57.1	10.3	12.5
С	3	14	1	45.2	13.6	11.2
С	3	14	2	48.9	12.7	10.7
С	3	14	3	44.0	12.1	10.3
С	3	16	1	48.1	11.2	10.9
С	3	16	2	43.4	11.2	10.4
С	3	16	3	43.5	10.3	10.8
С	3	18	1	50.4	10.9	12.5
С	3	18	2	47.7	7.8	12.8
С	3	18	3	49.1	8.8	12.0
NC	1	0	1	50.0	12.5	12.9
NC	1	0	2	45.6	14.3	14.0
NC	1	0	3	42.7	17.3	12.8
NC	1	7	1	47.5	12.5	11.6
NC	1	7	2	50.2	11.4	11.4
NC	1	7	3	54.0	8.7	10.4
NC	1	14	1	50.9	7.3	10.9
NC	1	14	2	49.9	7.0	10.7
NC	1	14	3	47.0	9.3	11.4
NC	1	16	1	54.5	5.5	12.4
NC	1	16	2	45.6	9.3	11.7
NC	1	16	3	52.9	6.8	12.1
NC	1	18	1	54.5	3.9	11.7
NC	1	18	2	57.1	4.3	12.0

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
NC	1	18	3	58.4	4.2	12.0
NC	2	0	1	47.5	15.1	11.4
NC	2	0	2	49.2	16.4	11.9
NC	2	0	3	43.0	14.8	10.7
NC	2	4	1	52.1	10.9	9.1
NC	2	4	2	54.6	9.4	7.6
NC	2	4	3	49.3	11.6	8.2
NC	2	7	1	44.3	15.3	13.5
NC	2	7	2	48.2	11.2	11.5
NC	2	7	3	51.2	11.5	11.8
NC	2	11	1	47.2	9.7	9.1
NC	2	11	2	46.3	9.0	9.0
NC	2	11	3	46.6	8.5	10.7
NC	2	14	1	46.3	8.3	10.8
NC	2	14	2	44.4	9.0	10.7
NC	2	14	3	47.8	9.0	11.4
NC	2	16	1	44.9	6.8	10.3
NC	2	16	2	43.2	8.3	11.4
NC	2	16	3	44.7	7.5	12.3
NC	2	18	1	49.2	5.8	11.3
NC	2	18	2	49.5	5.7	12.2
NC	2	18	3	46.8	9.8	12.4
NC	3	0	1	50.8	14.4	11.9
NC	3	0	2	48.8	14.8	11.9
NC	3	0	3	50.4	16.4	13.5
NC	3	4	1	47.8	14.3	11.2
NC	3	4	2	48.8	15.1	11.8
NC	3	4	3	47.2	15.3	12.2
NC	3	7	1	49.4	12.8	11.4
NC	3	7	2	51.8	12.6	11.4
NC	3	7	3	45.6	14.8	12.0
NC	3	11	1	44.9	15.2	12.3
NC	3	11	2	56.5	12.3	13.0
NC	3	11	3	43.3	13.8	13.3
NC	3	14	1	45.9	15.8	11.9

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
NC	3	14	2	44.4	13.7	10.7
NC	3	14	3	45.6	12.8	11.1
NC	3	16	1	46.8	8.3	10.5
NC	3	16	2	48.3	7.7	10.0
NC	3	16	3	48.0	8.0	9.9
NC	3	18	1	46.4	8.6	10.1
NC	3	18	2	45.5	9.4	9.4
NC	3	18	3	50.8	10.3	11.9
L	1	0	1	47.6	18.1	15.6
L	1	0	2	50.1	14.0	12.5
L	1	0	3	52.1	9.6	10.7
L	1	7	1	52.3	11.4	9.9
L	1	7	2	49.6	11.0	10.3
L	1	7	3	49.9	10.9	10.9
L	1	14	1	45.6	9.6	10.8
L	1	14	2	45.6	11.7	10.7
L	1	14	3	44.5	9.9	11.3
L	1	16	1	47.5	13.0	12.7
L	1	16	2	48.2	10.2	11.3
L	1	16	3	48.4	8.9	10.4
L	1	18	1	54.3	5.3	10.3
L	1	18	2	52.5	4.0	11.4
L	1	18	3	51.4	5.1	11.1
L	2	0	1	45.5	17.9	12.8
L	2	0	2	51.1	17.7	11.4
L	2	0	3	57.1	11.8	10.3
L	2	4	1	48.0	11.5	8.4
L	2	4	2	55.4	9.4	8.1
L	2	4	3	51.3	9.3	6.6
L	2	7	1	43.2	13.5	11.2
L	2	7	2	52.2	12.0	11.1
L	2	7	3	46.2	12.8	11.6
L	2	11	1	51.1	8.3	6.5
L	2	11	2	50.1	10.4	8.6
L	2	11	3	47.2	10.8	9.9

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	2	14	1	41.4	12.8	10.8
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L		14	2	44.3	11.7	10.4
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	2	14	3	41.5	13.4	12.1
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L		16	1	42.6	10.2	10.3
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L		16	2	44.2	11.7	11.0
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	L		16	3	44.6	9.3	9.8
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	L		18	1	43.8	9.1	11.6
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	L		18	2	43.3	9.7	10.9
L30241.918.312.0L30347.216.212.7L34148.213.112.3L34242.917.413.1L34351.613.111.9L37147.714.310.5L37247.414.611.7L37342.012.69.5L311141.317.612.6L311246.615.612.4L314145.313.611.1L314145.313.611.1L314145.313.611.1L314350.612.312.0L316145.711.710.8L316145.711.710.8L316347.610.111.1L318141.212.710.4L318341.111.610.8V10150.120.018.4V10347.919.315.8V10347.919.315.8	L		18	3	47.4	9.1	11.3
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	0	1	42.6	17.9	12.8
L34148.213.112.3L34242.917.413.1L34351.613.111.9L37147.714.310.5L37247.414.611.7L37342.012.69.5L311141.317.612.6L311246.615.612.4L311345.719.915.6L314145.313.611.1L314245.714.912.0L316145.711.710.8L316243.413.410.6L316347.610.111.1L318141.212.710.4L318341.111.610.8V10150.120.018.4	L	3	0	2	41.9	18.3	12.0
L34242.917.413.1L34351.613.111.9L37147.714.310.5L37247.414.611.7L37342.012.69.5L311141.317.612.6L311246.615.612.4L311345.719.915.6L314145.313.611.1L314245.714.912.0L316145.711.710.8L316347.610.111.1L318141.212.710.4L318341.111.610.8V10150.120.018.4V10347.919.315.8V17154.711.411.0	L	3	0	3	47.2	16.2	12.7
L343 $51.6$ $13.1$ $11.9$ L371 $47.7$ $14.3$ $10.5$ L372 $47.4$ $14.6$ $11.7$ L373 $42.0$ $12.6$ $9.5$ L3111 $41.3$ $17.6$ $12.6$ L3112 $46.6$ $15.6$ $12.4$ L3113 $45.7$ $19.9$ $15.6$ L3141 $45.3$ $13.6$ $11.1$ L3142 $45.7$ $14.9$ $12.0$ L3161 $45.7$ $11.7$ $10.8$ L3162 $43.4$ $13.4$ $10.6$ L3163 $47.6$ $10.1$ $11.1$ L3183 $41.1$ $11.6$ $10.8$ V101 $50.1$ $20.0$ $18.4$ V103 $47.9$ $19.3$ $15.8$ V103 $47.9$ $19.3$ $15.8$	L	3	4	1	48.2	13.1	12.3
L37147.714.310.5L37247.414.611.7L37342.012.69.5L311141.317.612.6L311246.615.612.4L311345.719.915.6L314145.313.611.1L314245.714.912.0L316145.711.710.8L316145.711.710.8L316243.413.410.6L316347.610.111.1L318141.212.710.4L318341.111.610.8V10150.120.018.4V10347.919.315.8V10347.919.315.8	L	3	4	2	42.9	17.4	13.1
L37247.414.611.7L37342.012.69.5L311141.317.612.6L311246.615.612.4L311345.719.915.6L314145.313.611.1L314245.714.912.0L314350.612.312.0L316145.711.710.8L316347.610.111.1L316347.610.111.1L316347.610.111.1L316347.610.111.1L318141.212.710.4L318341.111.610.8V10150.120.018.4V10347.919.315.8V17154.711.411.0	L	3	4	3	51.6	13.1	11.9
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	7	1	47.7	14.3	10.5
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	3	7	2	47.4	14.6	11.7
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	7	3	42.0	12.6	9.5
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	11	1	41.3	17.6	12.6
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	11	2	46.6	15.6	12.4
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	11	3	45.7	19.9	15.6
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	14	1	45.3	13.6	11.1
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	L	3	14	2	45.7	14.9	12.0
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	3	14	3	50.6	12.3	12.0
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	3	16	1	45.7	11.7	10.8
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	3	16	2	43.4	13.4	10.6
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	L	3	16	3	47.6	10.1	11.1
L318341.111.610.8V10150.120.018.4V10251.615.014.4V10347.919.315.8V17154.711.411.0	L		18	1	41.2	12.7	10.4
V10150.120.018.4V10251.615.014.4V10347.919.315.8V17154.711.411.0	L	3	18	2	47.1	9.7	10.7
V10251.615.014.4V10347.919.315.8V17154.711.411.0		3		3	41.1	11.6	10.8
V10251.615.014.4V10347.919.315.8V17154.711.411.0	V	1	0	1	50.1	20.0	18.4
V 1 7 1 54.7 11.4 11.0		1	0	2	51.6	15.0	14.4
V 1 7 1 54.7 11.4 11.0		1	0	3			
	V	1	7	1	54.7	11.4	
1   1   1   2   7.5   15.5   12.1	V	1	7	2	49.3	13.5	12.1

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
V	1	7	3	48.9	13.5	11.9
V	1	14	1	45.5	12.5	13.0
V	1	14	2	53.4	9.4	11.7
V	1	14	3	46.1	11.3	10.8
V	1	16	1	52.1	7.8	10.9
V	1	16	2	49.5	11.1	12.0
V	1	16	3	49.1	8.5	12.5
V	1	18	1	50.9	8.3	12.7
V	1	18	2	55.8	4.2	13.0
V	1	18	3	47.1	6.6	11.9
V	2	0	1	54.9	13.2	13.9
V	2	0	2	45.2	16.2	13.8
V	2	0	3	46.7	15.3	12.1
V	2	4	1	49.2	12.3	8.8
V	2	4	2	48.4	11.0	7.7
V	2	4	3	49.1	11.0	7.8
V	2	7	1	47.3	12.5	11.0
V	2	7	2	45.9	12.7	10.0
V	2	7	3	44.1	13.6	10.4
V	2	11	1	48.9	10.9	8.8
V	2	11	2	54.4	9.7	9.8
V	2	11	3	47.4	10.1	7.5
V	2	14	1	45.2	10.2	10.7
V	2	14	2	43.8	10.6	10.3
V	2	14	3	46.1	13.0	12.6
V	2	16	1	45.9	9.1	11.1
V	2	16	2	44.0	9.2	10.9
V	2	16	3	46.1	6.1	11.7
V	2	18	1	44.6	8.3	10.4
V	2	18	2	42.4	7.8	11.3
V	2	18	3	47.9	7.3	10.7
V	3	0	1	50.2	13.6	13.1
V	3	0	2	46.7	17.5	13.6
V	3	0	3	50.6	15.3	12.8
V	3	4	1	49.1	12.6	11.4
<sup>1</sup> C-control NC-	negative control: $I = s$		1			11,1

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
V	3	4	2	50.4	12.1	11.0
V	3	4	3	53.4	12.6	12.0
V	3	7	1	47.2	13.5	11.1
V	3	7	2	46.2	14.5	12.1
V	3	7	3	47.9	14.3	11.3
V	3	11	1	47.9	14.1	12.5
V	3	11	2	51.0	16.2	13.2
V	3	11	3	56.1	13.3	12.3
V	3	14	1	43.3	11.9	11.8
V	3	14	2	47.2	11.7	10.2
V	3	14	3	43.7	14.7	12.1
V	3	16	1	45.9	9.3	8.8
V	3	16	2	47.9	10.4	10.5
V	3	16	3	50.2	8.6	13.1
V	3	18	1	49.5	6.8	12.1
V	3	18	2	44.8	7.0	11.9
V	3	18	3	47.3	8.0	11.2
LV	0	0	1	44.4	14.7	15.7
LV	0	0	2	59.2	11.5	15.4
LV	0	0	3	46.2	15.7	13.2
LV	0	7	1	52.0	10.3	10.9
LV	0	7	2	52.1	10.6	11.2
LV	0	7	3	49.5	10.6	9.8
LV	0	14	1	48.9	11.6	11.3
LV	0	14	2	52.0	9.7	11.4
LV	0	14	3	50.6	10.0	10.9
LV	0	16	1	47.0	11.5	11.8
LV	0	16	2	51.4	11.8	11.7
LV	0	16	3	45.6	11.1	10.7
LV	0	18	1	49.7	8.5	11.0
LV	0	18	2	48.2	9.3	11.3
LV	0	18	3	46.3	10.9	11.3
LV	2	0	1	58.0	11.6	10.3
LV	2	0	2	48.3	14.4	12.3
LV	2	0	3	50.0	14.1	11.6

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
LV	2	4	1	51.0	10.5	6.7
LV	2	4	2	50.9	9.8	7.1
LV	2	4	3	50.1	11.8	9.5
LV	2	7	1	44.6	14.1	10.8
LV	2	7	2	48.8	12.0	11.4
LV	2	7	3	50.6	10.0	8.7
LV	2	11	1	49.9	9.2	7.5
LV	2	11	2	51.0	11.6	10.9
LV	2	11	3	48.9	9.4	7.5
LV	2	14	1	41.0	13.2	10.3
LV	2	14	2	40.9	13.4	10.5
LV	2	14	3	45.5	9.7	9.5
LV	2	16	1	42.5	10.9	10.6
LV	2	16	2	43.4	9.1	9.4
LV	2	16	3	46.2	9.8	9.3
LV	2	18	1	42.4	11.0	11.1
LV	2	18	2	46.9	10.7	11.2
LV	2	18	3	48.9	10.6	10.7
LV	3	0	1	52.3	14.1	12.5
LV	3	0	2	48.3	15.2	11.5
LV	3	0	3	46.8	15.3	12.3
LV	3	4	1	44.7	15.5	12.2
LV	3	4	2	43.2	15.9	11.6
LV	3	4	3	43.2	16.4	12.5
LV	3	7	1	43.9	15.8	11.2
LV	3	7	2	47.4	14.0	11.4
LV	3	7	3	47.4	13.8	11.0
LV	3	11	1	43.8	20.4	14.5
LV	3	11	2	50.3	13.7	12.9
LV	3	11	3	41.7	18.0	13.3
LV	3	14	1	45.4	12.1	9.9
LV	3	14	2	41.1	16.1	12.3
LV	3	14	3	48.6	14.3	12.1
LV	3	16	1	46.0	10.5	9.4
LV	3	16	2	50.1	10.3	10.1

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

LV318150.712.011.LV318247.212.711.	Treatment <sup>1</sup>	Replication	Day	Patty	L*	a*	b*
LV 3 18 2 47.2 12.7 11.	LV	3	16	3	51.1	11.0	10.7
	LV	3	18	1	50.7	12.0	11.2
LV 3 18 3 46.5 11.8 10	LV	3	18	2	47.2	12.7	11.4
	LV	3	18	3	46.5	11.8	10.9

Table A.3. continued. Mean values of  $L^* a^* b^*$  over time for each replication.

Treatment <sup>1</sup>	Replication	Day	TBAR value
С	1	0	0.09
С	1	7	0.68
С	1	14	0.23
С	1	16	0.65
С	1	18	0.52
С	2	0	0.75
C C C	2	7	0.90
С	2	14	0.93
C C C	2	16	1.02
С	2	18	1.76
С	3	0	0.09
С	3	7	1.04
С	3	14	0.76
C C C	3	16	0.76
С	3	18	0.94
NC	1	0	0.08
NC	1	7	0.70
NC	1	14	0.15
NC	1	16	0.76
NC	1	18	0.86
NC	2	0	0.98
NC	2	7	1.32
NC	2	14	1.20
NC	2	16	1.74
NC	2	18	2.15
NC	3	0	0.34
NC	3	7	1.29
NC	3	14	0.92
NC	3	16	0.92
NC	3	18	0.72
L	1	0	0.06
L	1	7	0.76
L	1	14	0.32
L	1	16	0.68
L	1	18	1.36
L	2	0	1.40

Table A.4. Mean TBAR values over time for each replication

 $\frac{L}{^{1}C=\text{control}; \text{ NC}=\text{ negative control}; \text{ L}=\text{ sodium lactate}; \text{ V}=\text{ acetic acid}; \text{ LV}=\text{ sodium lactate/acetic acid mixture}}$ 

Treatment <sup>1</sup>	Replication	Day	TBAR value
L	2	7	1.27
L	2	14	1.26
L	2	16	1.09
L	2	18	1.75
L	3	0	0.06
L	3	7	0.85
L	3	14	0.95
L	3	16	0.95
L	3	18	0.75
V	1	0	0.10
V	1	7	0.67
V	1	14	0.13
V	1	16	1.29
V	1	18	0.76
V	2	0	0.98
V	2	7	1.35
V	2	14	1.26
V	2	16	1.09
V	2	18	1.40
V	3	0	0.14
V	3	7	0.91
V	3	14	1.11
V	3	16	1.11
V	3	18	0.79
LV	1	0	0.08
LV	1	7	0.86
LV	1	14	0.49
LV	1	16	0.76
LV	1	18	0.76
LV	2	0	1.10
LV	2	7	1.10
LV	2	14	1.52
LV	2	16	1.20
LV	2	18	1.97
LV	3	0	0.09
LV	3	7	0.93

Table A.4 continued. Mean TBAR values over time for each replication

 $\frac{LV}{C}$  = control; NC= negative control; L= sodium lactate; V= acetic acid; LV = sodium actate/acetic acid mixture

Treatment <sup>1</sup>	Replication	Day	TBAR value
LV	3	7	0.93
LV	3	14	0.97
LV	3	16	0.97
LV	3	18	0.73

Table A.4. continued. Mean TBAR values over time for each replication

Treatment	Replication	Day	Color	Browning	Cupping	Cooked	Boar	Aroma	Aroma
			Homogeneity			Complex	Taint	Rancid	Pork
	1	0	7.4	5.9	1.2	6.9	0.1	0.1	4.1
	1	7	7.7	6.5	0.8	6.7	0.0	0.3	4.3
	1	14	7.3	7.2	1.3	6.4	0.0	0.7	4.2
NC	1	0	8.4	4.7	1.3	6.7	0.1	0.1	4.3
0	-	7	7.5	6.3	1.5	7.3	0.0	0.1	4.5
0	1	14	7.7	7.1	1.4	7.1	0.1	0.7	4.9
	1	0	7.1	6.9	2.2	6.9	0.1	0.2	4.4
	1	7	7.8	6.5	1.9	7.5	0.1	0.2	4.1
	1	14	7.4	7.7	1.1	7.4	0.0	0.4	5.1
1	1	0	8.4	6.0	0.9	6.5	0.0	0.0	4.3
/	1	7	6.9	5.1	1.0	6.8	0.0	0.2	4.3
1	1	14	7.0	6.3	1.4	6.7	0.1	0.7	4.4
	1	0	8.1	3.4	2.3	6.2	0.1	0.1	4.0
	1	7	7.9	6.6	2.4	8.3	0.0	0.0	4.8
	1	14	8.1	7.5	2.6	6.2	0.0	0.5	4.9
Treatment	Replication	Day	Firmness	Crumbly	Juiciness	Sour	Salty	Bitter	Sweet
	1	0	5.8	6.2	8.0	0.0	0.1	2.9	0.4
	1	7	6.8	5.8	7.2	0.0	0.5	3.8	0.5
	1	14	6.9	5.6	6.9	0.5	1.1	3.2	0.3
7)	-	0	5.7	5.6	8.1	0.0	0.5	3.3	0.3
()	1	7	6.1	6.1	6.9	0.0	0.7	4.3	0.9
0	1	14	7.0	5.2	7.3	0.4	1.2	4.1	0.9
	1	0	6.6	6.3	7.7	0.0	0.4	3.8	0.3
	1	7	7.0	5.7	6.0	0.0	1.6	4.3	0.7
	1	14	7.1	6.4	6.4	0.5	0.5	4.9	0.5
LV	1	0	7.0	5.1	7.8	0.0	0.8	3.9	0.2
7	1	7	6.9	6.1	8.8	0.0	0.8	3.8	0.3
/	1	14	6.8	5.7	8.1	0.2	0.5	3.7	0.2
	1	0	5.3	5.9	8.5	0.0	0.8	2.5	0.4
	1	7	6.9	5.1	7.4	0.4	1.3	4.0	0.6
		14	7.5	5.3	6.1	0.5	1.0	3.6	0.6

Treatment	Replication	Day	Fat	Spice	Flavor	Chemical	Flavor	-ffO	Overall
			Complex		Rancid		Pork	Flavor	
0	1	0	6.8	7.7	0.1	0.3	4.0	0.5	6.6
0	1	7	7.7	7.4	0.6	0.6	5.5	0.5	9.2
7)	1	14	6.5	7.5	0.9	1.1	4.6	0.9	8.4
NC	1	0	6.1	9.3	0.2	0.3	4.2	0.5	10.9
NC	1	7	6.1	7.9	0.3	0.4	4.9	0.3	10.0
AC V	1	14	6.1	8.2	1.1	1.1	4.4	0.9	8.7
L	1	0	6.9	9.2	0.1	0.5	4.2	0.6	10.6
. 1	1	7	7.7	8.4	0.2	0.3	5.2	0.5	9.8
. 1	1	14	6.4	7.7	0.4	0.7	5.0	0.4	9.7
LV	1	0	6.8	9.2	0.0	0.5	4.3	0.4	10.4
LV	1	7	7.4	8.4	0.2	0.6	5.4	0.1	9.6
LV	1	14	7.0	7.7	0.7	0.8	4.5	1.0	9.1
>	1	0	6.6	8.2	0.0	0.3	4.9	0.3	9.4
>	1	7	6.5	8.2	0.2	0.7	5.2	0.3	10.1
>	1	14	6.4	6.9	0.7	0.9	4.2	1.1	8.5

. Means of sensory descriptive attributes for each replication
Table A.5. continued.

Treatment	Replication	Day	Color Hamageneity	Browning	Cupping	Cooked	Boar Taint	Aroma Rancid	Aroma
		•					1 allu	INALIVIU	VIN T
	2	0	7.7	6.0	0.5	7.2	0.0	0.3	4.8
	2	7	7.9	6.4	0.8	7.6	0.0	0.1	4.7
С	2	14	7.9	6.4	0.8	7.6	0.0	0.1	4.7
C	2	0	8.1	6.8	1.0	6.8	0.0	0.1	4.8
U	2	7	7.6	6.9	0.8	7.5	0.0	0.0	4.3
0	2	14	7.6	8.0	1.7	6.2	0.0	0.4	3.9
	2	0	7.8	5.6	1.5	7.0	0.1	0.2	4.7
	2	7	8.3	6.1	0.9	6.3	0.0	0.7	3.9
	2	14	8.9	8.5	0.6	6.7	0.0	0.4	4.4
/	2	0	7.4	5.9	0.4	7.6	0.0	0.1	4.6
>	2	7	7.7	5.6	1.1	7.3	0.0	0.3	4.3
>	2	14	8.6	5.7	1.1	6.4	0.0	0.7	3.9
	2	0	7.0	6.6	1.7	6.3	0.0	0.1	4.0
	2	7	8.0	7.4	0.7	6.2	0.0	0.3	3.9
	2	14	8.3	7.6	1.9	6.4	0.0	0.5	4.4
Treatment	Replication	Day	Firmness	Crumbly	Juiciness	Sour	Salty	Bitter	Sweet
	2	0	6.7	6.7	6.1	1.4	0.8	2.9	0.4
	2	7	7.1	7.2	5.3	2.7	0.9	2.6	0.3
	2	14	7.1	7.2	5.3	2.7	0.9	2.6	0.3
0	2	0	6.9	5.9	5.3	1.4	0.5	3.5	0.4
0	2	7	7.3	6.7	5.5	3.2	1.0	3.4	0.5
0	2	14	6.5	6.6	4.6	3.6	0.8	3.8	0.2
	2	0	7.7	7.1	5.2	1.5	0.8	4.1	0.4
	2	7	7.7	7.0	5.0	2.5	1.0	3.3	0.6
	2	14	7.4	5.7	5.7	4.2	1.2	4.3	0.8
/	2	0	8.1	6.6	5.0	1.9	0.4	3.9	0.5
/	2	7	7.7	6.3	5.8	2.9	1.3	3.1	0.8
/	2	14	6.5	5.1	6.3	3.9	0.9	4.2	0.8
	2	0	5.8	6.1	6.5	1.0	0.4	2.7	0.3
	2	7	7.4	6.9	5.4	2.5	0.7	2.5	0.4
	2	14	56	6.2	6.7	3 1	04	3.6	0.6

92

Treatment	Replication	Day	Fat Complex	Spice	Flavor Rancid	Chemical	Flavor Pork	Off- Flavor	Overall
C	2	0	6.3	9.0	0.3	0.6	4.5	0.7	9.6
C	2	7	6.1	9.3	0.6	0.6	4.6	0.6	9.6
U	2	14	6.1	9.3	0.6	0.6	4.6	0.6	9.6
NC	2	0	6.3	9.1	0.1	1.1	4.5	0.9	9.5
NC	2	7	6.7	9.1	0.5	0.5	4.2	0.9	10.2
NC	2	14	6.0	8.4	0.2	0.4	4.7	0.2	10.1
. 1	2	0	6.5	9.1	0.3	0.9	5.2	0.7	10.2
. 1	2	7	6.3	9.0	0.4	0.5	4.5	0.8	9.3
. 1	2	14	6.6	9.2	0.6	0.1	5.2	0.6	9.8
LV	2	0	6.5	8.8	0.3	0.6	5.1	1.0	9.6
2	2	7	6.2	9.2	0.2	0.5	4.2	0.7	9.9
N	2	14	6.5	8.5	0.5	0.9	3.6	1.3	8.6
~	2	0	6.5	8.1	0.4	0.6	4.6	0.8	9.7
~	2	7	6.2	8.0	0.3	0.3	3.5	0.9	9.1
~	2	14	6.8	7.4	0.5	0.2	4.3	0.9	9.6

Treatment	Replication	Day	Color	Browning	Cupping	Cooked	Boar	Aroma	Aroma
			Homogeneity			Complex	Taint	Rancid	Pork
С	3	0	0.0	7.6	6.6	0.8	7.8	0.0	0.0
С	33	7	7.6	6.5	0.8	7.3	0.0	0.2	4.4
С	33	14	7.7	8.4	0.7	6.9	0.0	0.2	4.5
NC	ŝ	0	8.1	7.0	0.7	7.0	0.0	0.0	4.7
NC	ŝ	7	7.6	5.0	0.5	6.5	0.0	0.2	4.0
NC	33	14	8.9	7.9	1.3	7.2	0.0	0.2	4.8
L	33	0	7.7	6.9	1.6	6.6	0.0	0.1	4.6
L	33	7	7.0	7.0	0.6	7.1	0.0	0.1	5.3
L	33	14	8.1	7.2	2.3	6.5	0.0	0.2	4.3
LV	33	0	7.9	5.9	2.3	7.2	0.0	0.0	4.6
LV	33	7	7.2	7.5	0.6	7.7	0.0	0.5	3.6
LV	33	14	8.6	6.1	1.1	7.3	0.0	0.3	4.5
V	33	0	7.9	5.9	2.3	7.2	0.0	0.0	4.6
Λ	ŝ	7	7.4	7.0	0.6	7.8	0.0	0.0	4.3
Λ	3	14	8.6	7.2	0.8	7.6	0.0	0.2	4.2
Treatment	Replication	Day	Firmness	Crumbly	Juiciness	Sour	Salty	Bitter	Sweet
C	3	0	5.1	5.9	6.9	5.7	2.7	0.6	3.1
C	33	7	5.4	5.9	5.5	2.0	0.7	3.2	0.4
C	33	14	5.9	6.4	5.1	2.7	0.4	3.1	0.3
NC	33	0	5.6	5.4	4.5	1.7	0.5	3.1	0.1
NC	3	7	5.6	5.6	5.5	1.8	0.4	3.6	0.2
NC	3	14	6.3	6.1	5.1	2.3	0.4	2.9	0.4
L	3	0	6.4	6.0	4.7	2.4	0.9	3.5	0.5
L	33	7	6.9	6.1	5.6	1.9	1.3	4.4	0.5
L	3	14	13.0	6.3	5.4	3.0	0.5	3.4	0.5
LV	ŝ	0	5.9	5.8	4.9	2.8	0.7	3.7	0.5
LV	33	7	7.4	6.3	5.3	1.6	0.9	5.6	0.3
LV	33	14	9.9	6.3	5.8	3.0	0.5	3.0	0.3
V	33	0	5.9	5.8	4.9	2.8	0.7	3.7	0.5
Λ	ŝ	7	6.5	6.0	5.6	2.2	0.8	3.7	0.4
Λ	ŝ	14	6.3	6.0	6.9	2.2	1.0	3.0	0.4

Treatment	Replication	Day	Fat	Spice	Flavor	Chemical	Flavor	-ffO	Overall
			Complex		Rancid		Pork	Flavor	
c	3	0	0.8	6.7	8.7	0.1	0.2	5.1	0.1
C	3	7	7.1	7.1	0.2	0.0	5.2	0.6	9.7
C	3	14	7.2	7.1	0.6	0.5	5.5	0.4	9.4
NC	3	0	6.5	8.6	0.7	0.5	5.2	0.2	11.4
NC	3	7	7.4	7.5	0.6	0.9	4.7	0.6	10.0
NC	3	14	6.7	8.5	0.7	0.0	4.7	0.9	9.6
L	3	0	6.7	8.2	0.1	0.2	5.3	0.4	11.1
L	3	7	7.1	8.3	0.3	0.6	5.3	0.3	10.1
L	3	14	6.9	8.8	0.3	0.5	4.8	0.4	9.8
LV	3	0	7.1	8.8	0.2	0.3	5.7	0.2	10.3
LV	3	7	7.0	8.8	0.4	0.7	4.6	0.4	9.4
LV	3	14	7.6	8.1	0.3	0.3	5.3	0.3	10.2
~	3	0	7.1	8.8	0.2	0.3	5.7	0.2	10.3
N	3	7	6.2	7.5	0.4	0.3	5.2	0.3	10.6
~	3	14	6.9	9.4	0.2	0.3	5.4	0.2	10.5