ABSTRACT

MINA KYUNGMIN KIM. Impact of Temperature and pH on the survival of *Listeria monocytogenes* in Souse meat. (Under the guidance of Dr. MaryAnne Drake and Dr. Dana J. Hanson)

Souse meat is an acidic, gelled product made with pork co-products such as ears, hearts, tongues and lean pork trimmings. This fully cooked, ready-to-eat (RTE) product is popular in the southern United States, and represents a significant volume of processed meat sold in this region. There is a "zero-tolerance" policy for *Listeria monocytogenes* in RTE meat products. The growth/survival of *L. monocytogenes* in souse is unknown. The objective of this study was to determine the effectiveness of three different souse formulations in controlling the growth of *L. monocytogenes* at two different refrigerated storage temperatures.

Three souse formulations (pH 4.3, 4.7, and 5.1) were produced in triplicate by a local processing plant. Products were subsequently surface-contaminated with a three-strain cocktail of *L. monocytogenes.* Uninoculated product served as the control. The initial contamination level was 10^5 cfu/cm^2. Products were vacuum-sealed and stored at two different temperatures (5°C and 10°C). Microbial counts were monitored (total aerobic plate count and *L. monocytogenes*) twice weekly through 32 days storage. Data were analyzed by regression analysis and D values were compared using analysis of variance with means separation. Souse meat did not support the growth of *L. monocytogenes*, regardless of product formulation or storage temperature. D values for products with a pH of 4.7 or 5.1 were not different (40days at 5°C, TSAYE; 27 days at pH 4.7, 21 days at pH 5.1 at 5°C, TSAYE) ($p>0.05$). A lower product pH (4.3) decreased *L. monocytogenes* survival (D value

= 9days) compared to higher pH products ($p<0.05$). D values for products stored at 5° C and 10° C did not statistically differ ($p>0.05$). Consumer acceptability of pH 4.3 products was not significantly different from (typical) pH 4.7 product ($p>0.05$).

These results demonstrate that conventionally produced souse meat does not support the growth of *L. monocytogenes* and that inactivation of the organism is favored for products formulated at lower pH ($\leq$4.3).

Impact of Temperature and pH on the survival of *Listeria monocytogenes* in Souse meat

By

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Biography

Mina Kyungmin Kim was born on December 12, 1980 in Evanston, Illinois to her father, Hho-jung Kim and her mother Hye-Gyung Hwang. She spent her first five years at Evanston, Illinois, while her father pursued his Ph.D degree at Northwestern University. After her father finished his study, her family moved to Korea. She lived in Dae-Duk science valley, which has been compared to Silicon Valley, CA in the United States, located in Daejeon city. She graduated Dae-Duk elementary school, Dae-Duk middle school, and Dae-Duk high school. She applied to department of applied biological science, Konkuk University, located in Seoul, Korea, and she accepted. She spent two and a half years in Konkuk University from spring, 1999 to spring 2001. While she was at Konkuk University, she was vice president of first year college student government associates, and freshman representative in the Fermentation club. In the summer of 2001, she decided to study abroad in the United States. She came to the ESL (English as a Second Language) program, called INTERLINK at the University of North Carolina at Greensboro. While she was in the ESL program, she decided to transfer to college in the U.S. She applied to several different universities in the U. S., and she was accepted to North Carolina State University, department of Food Science. She continued her undergraduate study at North Carolina State University from fall, 2002. While she was in NCSU, she was listed in Dean's list in her senior year, and graduated Cum Laude in May 2004. Also she was inducted for Sigma Phi Thau, the Honor Society in Food Science, and has been a member up to now. Upon graduation, she decided to continue her studies at NCSU in Food Science. She started her Master's program in fall, 2004 under the guidance of Dr. MaryAnne Drake and Dr. Dana J. Hanson. During her Master's program, she minored in Food Safety to broaden her knowledge in Food

Microbiology, as well as took extension courses for Food Safety Manager Certification. She was presented part of her M.S. thesis as a poster at the Institute of Food Technologist (IFT) meeting in June 2006 and was awarded 3rd place in the graduate student poster competition in the food microbiology division, and was also awarded the outstanding paper award in KAIFT (Korean American Institute of Food Technologist). While completing her Master's program, Mina was also very active in church activities. She was a piano accompanist in the Duraleigh Presbyterian Church, and also was a leader of a weekly bible study group. After graduation in December, 2006, Mina plans to continue her studies to earn a Ph.D in Food Science.

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Chapter I

Comprehensive Review of Literature

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I. Introduction

1. US and NC Meat Industry

Animal production in the United States has changed over the last 50 years from a system mainly comprised of independent animal producers to one mainly comprised of concentrated animal feeding operations (FAO, 1996). According to the National Agricultural Statistics Service (2003), commercial red meat production for the United States totaled 3.94 billion pounds. Red meat production, especially beef production, is geographically concentrated in midwestern states, including Iowa, Kansas, Nebraska, and Texas (MacDonald et al., 2000). Production from these states accounted for over 51 percent of U.S. commercial red meat production in 2001(NASS, 2002). Red meat production is a very concentrated industry; so most feedlots and slaughter facilities are also concentrated in these areas (FAO, 1996). In commercial processing plants, about 45.7 billion pounds of red meat was produced, and beef accounted for 26.2 billion pounds in 2001 (NASS, 2002). Commercial red meat production in NC increased from 175.1 million pounds in 2002 to 183.3 million pounds in 2003 (NASS, 2003).

The U. S. poultry industry can be classified into three categories: broilers, turkeys, and eggs (FAO, 1996). Among the three segments, broilers dominate about 66 percent of the dollar value of poultry production (Nalivka, 2002). The United States produced more than 8.2 billion chickens, 2.6 billion turkeys and more than 71 billion table eggs in 2000 (FAO, 1996). The poultry industry is vertically integrated. This means that the company or integrator controls all aspects of the process but contracts with individual landowners for growing services (FAO, 1996). The landowners furnish the poultry houses, energy,

and labor, while the companies furnish the animals, a feed, and technical support (FAO, 1996). The basic unit of this arrangement is called "complex" which includes parent flocks, multiplier flocks, hatchery, feed mill, and processing plant (FAO, 1996). Total egg production in NC ranked 11th nationally with production of 2,518 million eggs in 2002, and NC production increased to 2,523 million eggs in 2003 and ranked 10th (NASS, 2004). Broiler production in NC was 735,000,000 chickens annually (NASS, 2004).

The United States is the second largest pork producing country worldwide, after China (FAO, 1996). The pork industry expanded rapidly during the 1990's, so more pork was produced and more hogs slaughtered (FAO, 1996). Pork production totaled 1.55 billion pounds nationally in 2003, which is 6 percent less than the previous year (NASS, 2003). Over the years, the hog industry has changed structurally. First, production and processing have become a large-scale, structured industry (Drabenstott, 1998). This means that fewer farms produced larger numbers of hogs. In 1982, there were approximately 330,000 farms producing hogs and pigs, however over ten years, the number of hog farmers decreased by 42%, while hog production increased 17% (Furuseth, 1997). In addition, the geographic location of pork production is shifting to non-traditional hog states such as Texas, Colorado, Oklahoma and North Carolina.

North Carolina ranked 14th for pork production 30 years ago; now North Carolina ranks second nationally (FAO, 1996). North Carolina has been a traditional tobacco farming state (Furuseth, 1997). However for the past decade, tobacco has played a smaller role in NC life due to its resulting health risk, and at the same time, hog production has increased either via family farming or large-scale structured industry

(Furuseth, 1997). Geographically, lots of animals are found in the traditional tobacco-growing districts of eastern North Carolina (Furuseth, 1997). Along with the growth of large-scale hog farming, casual, family based hog farming is still widespread. About 58% of hog farms in North Carolina have fewer than 25 hogs (Furuseth, 1997).

Throughout recorded history, consumption of meat has indicated a position of social and economic prestige among people and nations (Aberle et al., 2001). As nations become industrialized, and people obtain more economic power, consumption of meat increases. Moreover, there is a relationship between social and economical status and the demand for high quality and greater quantities of meat (Aberle et al., 2001). In addition, the quantity of meat consumption is greatly influenced by social customs, religious beliefs, and personal preference (Aberle et al., 2001).

2. Historical Perspectives

One of the oldest methods of processing meats for preservation is sausage making. In ancient Rome, meats were salted for storage and transportation. Moreover, ancient Egyptians recorded the preservation of meat products by salting and sun drying (Pearson and Gillett, 1996). The word "sausage" is derived from the Latin word "salsus", the Spanish word "salchichón", and the French word "saucisson" (Toldrá, 2002). All three words mean salted or preserved (Johnson et al., 1974). In addition, the Latin word "salumen", which means a group of salted products, became the origin of the Italian word salami. Early sausages were produced by salting and drying, and later, the technique of adding starter cultures was introduced to sausage manufacture.

Early sausage manufacturing was confined to the home or small commercial operations. Therefore each individual manufacturer had their own skills and recipes for making sausages. The individual sausage varieties became distinctive not only because of the uniqueness of the seasoning but also because of the type and coarseness of grind of the meat constituents, the method in which the sausages were stuffed and processed, and such details as the manner in which the string was wound around the casing (Salisbury, 1960). Consumers choose sausage for various reasons; flavor, convenience, variety, economy, and nutritional value (Pearson and Gillett, 1996). Most sausage products are regarded as Ready-To-Eat (RTE) products, which means that they do not require further processing. Therefore, sausages are convenient for consumers since they are easily and quickly prepared (Pearson and Gillett, 1996).

Due to the popular acceptance of certain types of sausage varieties, many sausage-manufacturing operations grew their businesses substantially. As the market grew, the names of certain products were associated with the village or city from which they originated. The versatility within the products, regional preferences for certain types of sausages, and climatic conditions of some geographic areas were responsible for the wide variety of sausage products (Salisbury, 1960). Currently, hundreds of different sausage products are available to consumers, and each sausage type has a distinct flavor, which appeals to certain populations (Pearson and Gillett, 1996). Although most sausages originated from European countries, the demand associated with particular ethnic groups has brought sausage products to the United States (Pearson and Gillett, 1996). Because of the improvement of the distribution system and frequent traveling to different regions, several sausage products have become almost universally acceptable and available (Pearson and Gillett, 1996).

3. Food Safety and HACCP

Hazard Analysis and Critical Control Point (HACCP) is a system that was conceived in the 1960s by the Pillsbury Company and National Aeronautics and Space Administration (NASA), to ensure the safety of foods for astronauts (Motarjemi, 2001). Originally HACCP was developed to ensure the microbiological safety of food during transporting, processing, and preparation (Motarjemi, 2001). Currently HACCP is defined by the CAC (Codex Alimentarius Commission) as a system, which identifies, evaluates, and controls hazards that are significant for food safety. HACCP is a scientific, rational, and systematic approach to the identification, assessment, and control of hazards during production, processing, and manufacturing (Motarjemi, 2001). Unlike the traditional model for food safety assurance that has been used for decades, HACCP does not rely on end product testing to ensure the safety of food batches, but rather on continuous control and monitoring of critical control points along production and processing (FAO, 1996).

The HACCP principles describe a format for identifying and assuring control of factors (hazards) which are reasonably likely to cause a food to be unsafe for consumption, and are based on a common-sense application of technical and scientific principles to the food production process from field to table (Bernard, 1997). HACCP has 7 principles: identifying hazards, determining CCPs (Critical Control Points), establishing critical limits, monitoring, corrective action, verification, and recordkeeping (Aberle et al., 2001).

The first step is conducting a hazard analysis. It is the process of identification and listing of all the food safety hazards that are reasonably likely to occur during the production of a product and of the preventative measures needed to control the hazards (Aberle et al., 2001).

When conducting a hazard analysis, it is important to check all the ingredients and raw materials required for making the product, from receiving to distribution and final preparation for consumption (NACMCF, 1998). The hazard is defined as a biological, chemical, and physical agent that is reasonably likely to cause illness or injury in the absence of adequate control (NACMCF, 1998). If hazard analysis is not done correctly, processing plants cannot effectively control and ensure the safety of product, regardless of how well they follow the instructions provided by HACCP (NACMCF, 1998). The second step is determining the critical control points (CCPs) (NACMCF, 1998). A critical control point is a point at which control can be applied and is essential to prevent or eliminate a food safety hazards or to reduce it to an acceptable level (NACMCF, 1998). Critical control points are located at any step where hazards can be prevented, eliminated, or reduced to acceptable levels, and common examples of CCPs include thermal processing, chilling, testing ingredients for chemical residues, product formulation control and testing for metal contaminants (NACMCF, 1998). Once CCPs are established, critical limits for each identified hazard in CCPs should be established. A critical limit is a maximum/minimum value to which a variable must be controlled at a critical control point to prevent, eliminate, or reduce to an acceptable level a food safety hazard (Aberle et al., 2001). Critical limits are used to distinguish between safe and unsafe operating conditions associated with CCPs (NACMCF, 1998). Monitoring is significant to HACCP plan because 1) it allows tracking the operation, 2) it is used to determine when there is loss of control and a deviation occurs, and 3) it provides written documentation for use in verification. Based on monitoring, corrective action is taken (NACMCF, 1998). The next step is verification of the HACCP plan. This involves a series of steps to determine the validity of HACCP plan and check whether the system is operating

according to the HACCP plan, and if the plan needs to be modified or revalidated to achieve food safety objectives (Aberle et al., 2001; NACMCF, 1998). The last step of HACCP is effective record keeping. The documentation should include a summary of the hazard analysis along with the HACCP plan with listing of HACCP team, assigned responsibilities, description of the foods, its distribution, intended use, and the consumer.

Before developing a HACCP plan, there are several preliminary tasks. First, the process begins with formation of a HACCP team including plant management and personnel, as well as individuals who have expertise in food borne hazards and the particular product and process being used (NACMCF, 1998). Individuals who have specific knowledge and expertise appropriate to the product and process should be included as well as local personnel who are involved in the operation, because they are more familiar with the variability and limitations of the operation (NACMCF, 1998). The second task is developing a flow diagram that describes the food, its distribution, intended use and process (NACMCF, 1998). In this process, it is important to identify a general description of the product including ingredients, processing methods, and the expected consumer, whether it is for the general public or specific populations such as infants, the immunocompromised, or the elderly. The method(s) of distribution should also be identified (frozen, refrigerated, or ambient temperature) (NACMCF, 1998). The purpose of drawing the flow diagram is to provide a clear, simple outline of the steps involved in the process (NACMCF, 1998). A flow diagram describes the stages that are critical for the safety of the product in a written form including the process category, type of product, common name, mode of usage, type of package, shelf-life, labeling instructions, and necessary controls during distribution (Toldrá, 2002). Figure 1 and 2 show the general flow diagrams for dry-cured ham and dry-fermented sausage.

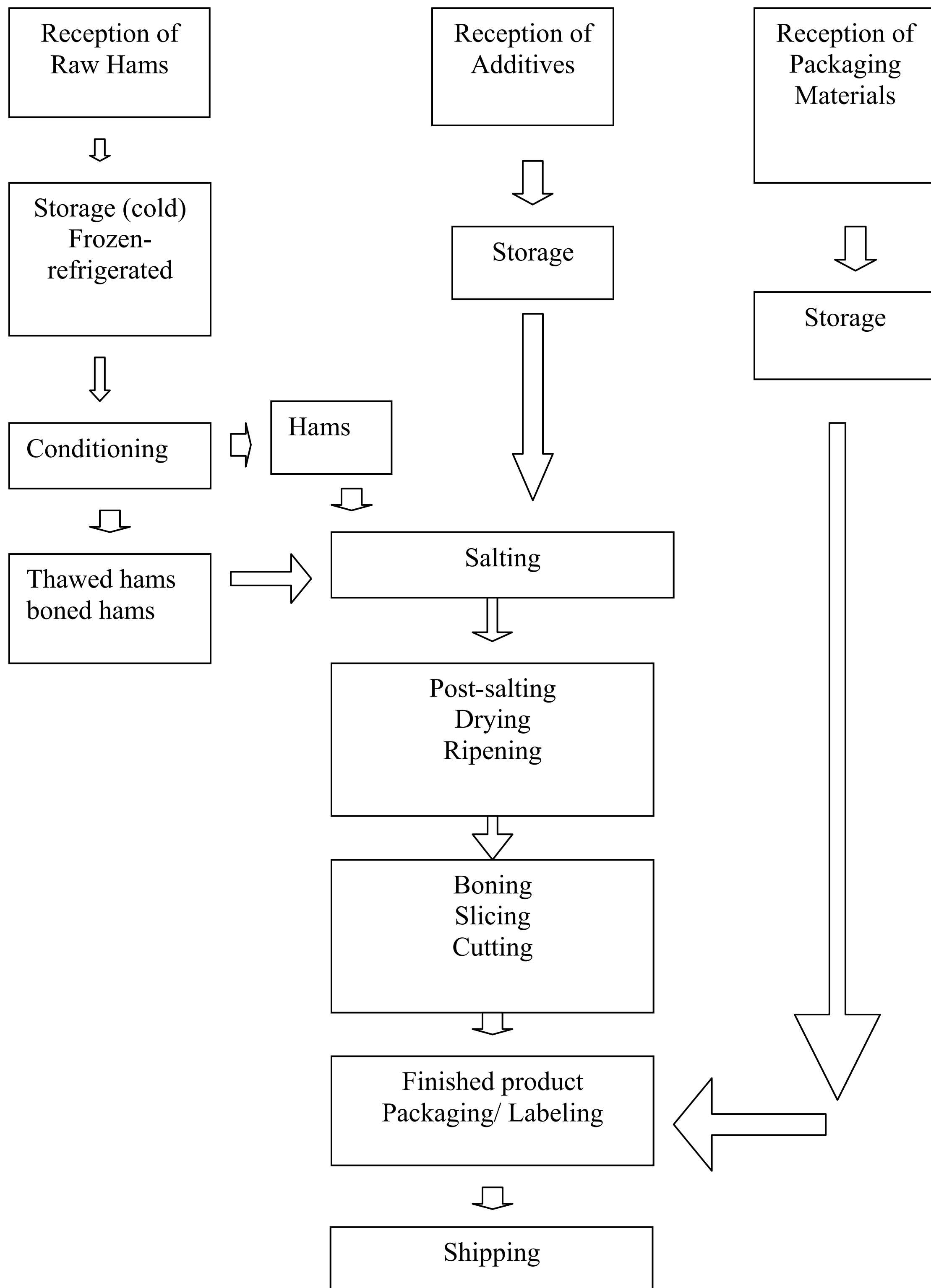

Figure 1. Process diagram for dry-cured ham. (Adopted from Toldrá, 2002)

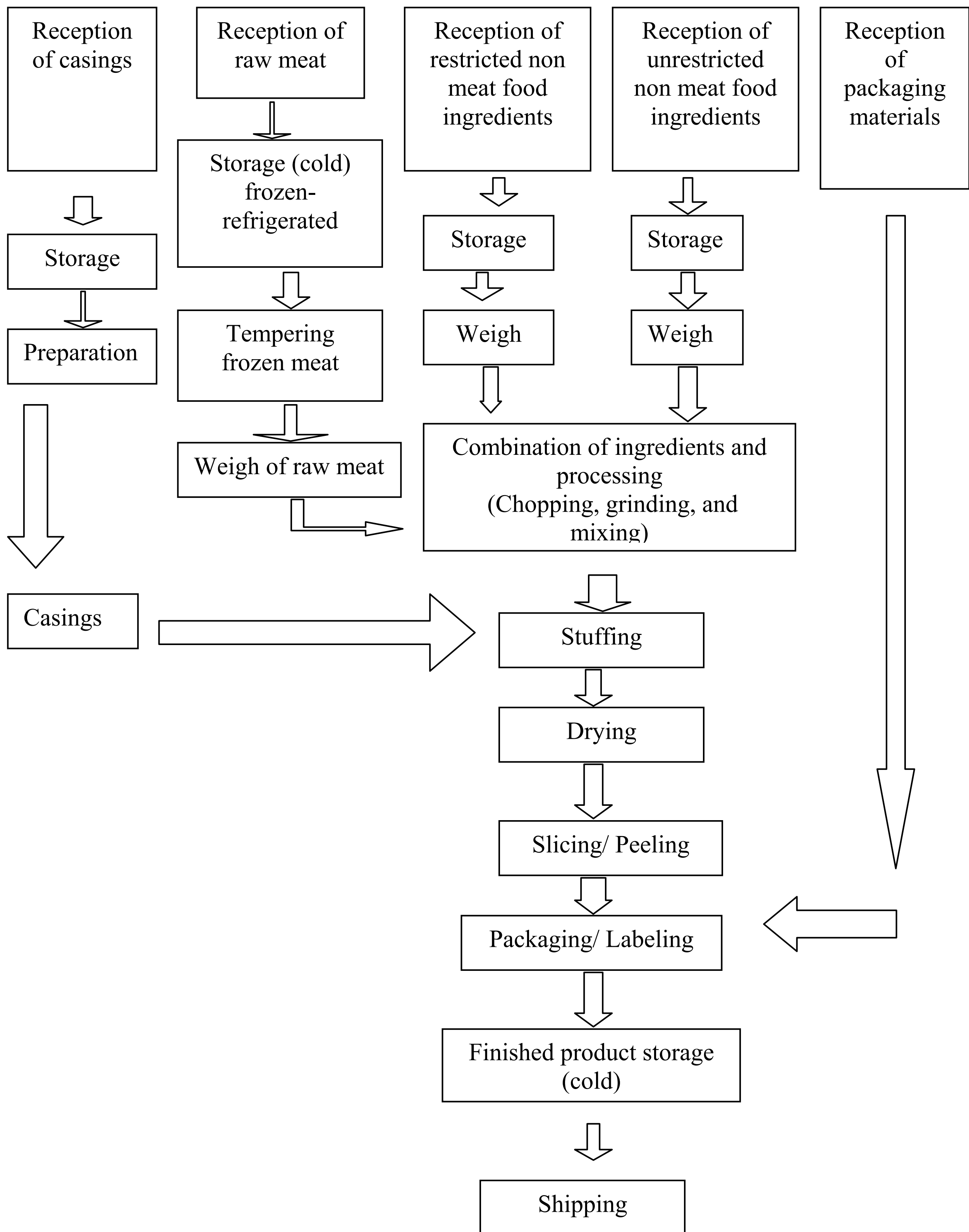

Figure 2. Process diagram for air-dried sausage.
(Adopted from Toldrá, 2002)

Table 1 shows the HACCP plan for air-dried sausage.

Table 1 HACCP for air-dried sausage

Process Step	Potential hazard introduced, controlled or enhanced at this step	Does the potential hazard need to be addressed in the HACCP plan? (Yes/No)	Why? Justification for decision made in previous column	What measures can be applied to prevent, eliminate or reduce the hazard?	CCP (Critical Control Point)
Receiving meat	b-none c-none p-none				No
Meat Cold Storage	b- pathogen c- none p-none	No	Maintain temperature ≤ 41ºC		No
Grinder-Mixer	b- pathogen c- chemicals p- metal	b- yes c- no c- no	b- temperature control necessary to control pathogen growth c- nitrites are used in accordance with Reg. 318.7 (7) (4) letter from supplier p- controlled under SSOP	Maintain raw product temperature at or below 45ºF during grinding	1-b
Natural casings prep.	b- none c- none p- none	No			No
Stuffer	b- none c- none p- none	No			No

Table 1 Continued

Drying room	b- spoilage bacteria c- none p- none	No	Placed in a controlled environment of 55 ºF - 60ºF for 3 days. This is considered a fresh product and will be cooked prior to serving. Spoilage bacteria are self-limiting	Product labeled "uncooked" carries safe handling label and cooking instructions for end user	No
Packaging and labeling	b- none c- none p- none	No			No
Non-meat ingredients receiving	b- none c- none p- foreign materials	No	Effective pest control program. Letter of guaranty from supplier		No
Finished product-cold storage	b- none c- none p- none	No			No

(Toldrá, 2002)

Under the Federal Meat Inspection and Poultry Products Inspection Act, USDA-FSIS (United States Department of Agriculture- Food Safety and Inspection Service) inspects all domestic meat and poultry products to be sold through interstate commerce in the United States (Anonymous, 2003). The Pathogen Reduction Hazard Analysis Critical Control Point (PR/HACCP) final rule was issued by FSIS on July 25, 1996, mandating HACCP implementation as the system of process control in all inspected meat and poultry plants (Toldrá, 2002). The rule changed the regulatory philosophy and roles of both inspector and industry (Anonymous, 2003). In the past, most plants relied on USDA inspectors to identify

plant and process deficiencies before the company took action to correct those (Anonymous, 2003). However, the PR/HACCP rule defined the respective roles, tasks, and responsibilities to both industry and FSIS (Anonymous, 2003). Also, the rule requires all plants to develop and write standard operating procedures for sanitation (SSOPs), to conduct microbial testing for generic *E. coli*, to develop and implement a HACCP system, and to set pathogen reduction performance standards (Toldrá, 2002).

4. Process Validation

Souse is an acidic, gelled pork product, also called headcheese. It is popular in southern areas of the United States, and has a unique "niche" market due to its distinct flavor. The main meat ingredients are based on pork by-products such as ears, skins, hearts, tongues and lean pork trimmings. The non-meat ingredients are vinegar, sugar, salt, and spices. Souse is considered a RTE (Ready-to-eat) product since it is fully cooked. Consumers do not cook the product prior to consumption. During cooking of main meat ingredients, gelatin is produced from pork skin. Gelatin itself has antioxidant properties, and properties to reduce moisture loss, lipid oxidation and color deterioration, and therefore improves the quality of the product (Villegas et al., 1999). *Listeria monocytogenes* is the main concern for souse processing plants as well as other RTE meat products especially during processing and post-process contamination (Barmpalia et al., 2004). Federally inspected establishments are required to comply with one of three alternatives for *L. monocytogenes* control in ready-to-eat meat products (FSIS, 2003). Alternative I requires application of a post lethality treatment to decrease or remove *L. monocytogenes* and an antimicrobial agent or process to limit or restrain growth of this pathogen; for Alternative II, a post lethality treatment or a

growth inhibitor must be employed; and for Alternative III, establishments will control this pathogen with sanitation measures alone (Barmpalia et la., 2004). Souse falls into alternative II, which requires a post lethality treatment or a growth inhibitor (FSIS, 2003). In addition, souse has a pH range of 4.5-5.5, which varies batch to batch. The variation of pH is another safety leak for processors. Figure 3 shows the flow diagram for souse. Table 2 shows the HACCP plan for souse.

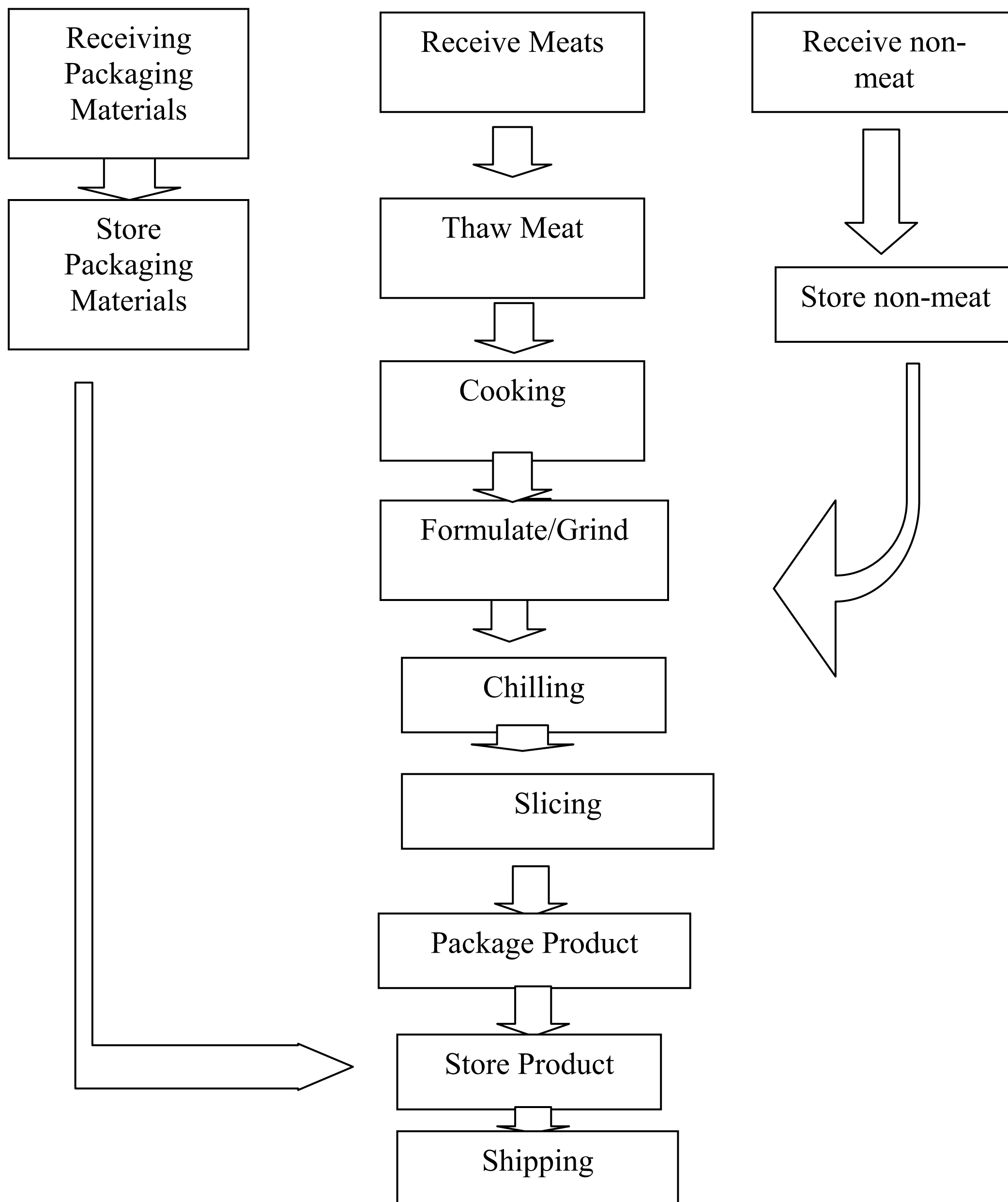

Figure 3. Flow Diagram for Souse. (Personal interview Larry Pierce)

Table 2 HACCP plan for souse

Ingredient process Step	Potential Hazard Introduced, Controlled, or Enhanced at this Step	Is the Potential Food Safety Hazard Significant? Risk: Severity	Justification for Decision	What Control Measures can be applied to Prevent the Significant Hazards?	Is this step a Critical Control Point (CCP)?
Receiving Packaging Materials	B. None C. None P. Foreign Material	No	Letters of guaranty from all suppliers of non-meat ingredients		No
Store Packaging Materials	B. Rodents or Insect damage C. None P. None	No	Control maintained by effective rodent and pest control program		No
Receiving Non-meat ingredients	B. None C. None P. Foreign Materials	No	Letters of guaranty from all suppliers of non-meat products		No
Store None-meat Products	B. None C. None P. Foreign Materials	No	Products received are in sealed packages and kept sealed until used. A continuing letter of guarantee ensures contents of packages are acceptable		No
Receive meats	B. None C. None P. None				No
Thaw Meats	B. Microbial Growth C. None P. None	No	Controlled at a subsequent step (Cooking)		No
Cook	B. Pathogens C. None P. None	Yes	Potential Pathogens present on raw products may survive if not cooked to lethality	Cook product to meet lethality. FSIS Directive 7111.1 AppendixB	Yes (CCP 1B)
Formulate Grind	B. None C. None P. None				No

Table 2 Continued

Chill	B. *Clostridia* C. None P. None	Yes	Improper stabilization could result in pathogen (*clostridia*) growth	Cool products in accordance with FSIS Directive 7111.1 Appendix B	Yes (CCP 2B)
Slicing	B. None C. None P. None				No
Package product	B. *Listeria monocytogenes* C. None P. None	Yes- low	Products have post-lethality exposure	Souse will be covered under SSOP Alternative 3 Plan for Sanitation & Testing of food contact surfaces	No
Store product	B. Microbial growth C. None P. None	Yes- low	Products stored at improper temperature could result in microbial growth	Cooker temperature maintained at <40°F. Temperature maintained on cooker temperature log	No
Ship	B. None C. None P. None				No

(Personal interview Larry Pierce)

The meat processors are responsible for validating safety of their products (HACCP). However, to validate product safety, scientific data is required. The cost of validation is limiting for small processing plants, and most souse making processors are local, family owned, small processing plants.

5. Optimization for safety of RTE meats

The U.S. Department of Agriculture (USDA) regulates all meat products. As mentioned earlier, there are three alternatives to control *L. monocytogenes* directed by USDA-FSIS to reduce, eliminate or suppress/limit the growth of this microorganism (Legan et al., 2004). Due to increased numbers of outbreaks between 1998 and 2000, USDA-FSIS increased their surveillance system for *L. monocytogenes*, and as a result, the numbers of recalls in 2000 increased about 10 fold compared to the number of recalls in 1997 (FSIS, 2003). Since recent technologies such as irradiation are not approved for use to RTE meat products, adding organic acids as antimicrobial compounds to inhibit the growth of *L. monocytogenes* in RTE meat products is commonly applied (Samelis et al., 2001; Zhu et al., 2005). One potential problem for applying antimicrobials to meat products is negative effects on sensory quality (Uhart et al., 2004).

In 2004, Uhart et al. (2004) published research about controlling *L. monocytogenes* in beef frankfurters by using pediocin (6,000 AU), 3% sodium diacetate, and 6% sodium lactate. Application of each antimicrobial alone resulted in a 1-log reduction after 3 weeks storage, and a combination of the three antimicrobials had the greatest effects on inhibiting the growth of *L. monocytogenes* (Uhart et al., 2004). Schlyter and others (1993) found that addition of 2.5% lactate and 0.1% sodium diacetate prevented the growth of *L. monocytogenes* in 42 days of storage at 4˚C in turkey slurries. Yet, 0.1% sodium diacetate itself was not effective against this organism (Schlyter et al., 1993). Organic acids such as lactic acid and acetic acid are the most commonly used antimicrobials in foods (Mbandi and Shelef, 2001). Natural sodium lactate, which is a normal constituent of muscle tissue, has been recognized as an effective food ingredient to control *L. monocytogenes* since the early

1990s (Bacus and Bontenbal, 1991). The combination of 2.5% sodium lactate and 0.2% sodium diacetate can greatly increase the safety of RTE meat products stored either under refrigeration or temperature abuse (Mbandi and Shelef, 2001). Samelis et al. (2001) studied the effectiveness of controlling *L. monocytogenes* on sliced pork bologna by dipping bologna into solutions of 0.5 or 5% lactic acid or acetic acids and 2.5 or 5% sodium acetate, 2.5 or 5% sodium diacetate, 5 or 10% sodium lactate, 5% potassium sorbate or potassium benzoate for 120 days storage at 4°C (Samelis et al., 2001). The results showed that 5% potassium sorbate and 5% lactic acid treatment resulted in significant increases in shelf life from 20 days to 35 days (Samelis et al., 2001). Capita et al. (2001) showed that trisodium phosphate was effective against *L. monocytogenes* in chicken meat, especially after 7 days storage at refrigeration temperature. Glass et al. (2002) found that the addition of combinations of sodium lactate and sodium diacetate in wiener or bratwurst formulations inhibited the growth of *L. monocytogenes* if stored at less than 7°C, along with the curing and smoking process. Geornaras et al. (2005) studied the effectiveness of post process antimicrobial treatments to control *L. monocytogenes* in commercial vacuum-packaged bologna and ham stored at 10˚C for 10 days. Of the 7 treatments, including 2.5% acetic acid, 2.5% lactic acid, 5% potassium benzoate, or 0.5% Nisaplin (commercial form of nisin, concentration of 5,000 IU/ml of nisin), and combinations of Nisaplin and acetic acid, Nisaplin and lactic acid and Nisaplin and potassium benzoate, 6 out of 7 combinations showed listericidal effects except for the products treated with Nisaplin alone (Geornaras et al., 2005). From a sensory perspective, samples treated with 2.5% acetic acid, 2.5 % lactic and or 5% potassium benzoate showed lower values scores with respect to product in appearance, color, odor, texture, and flavor acceptability (Geornaras et al., 2005).

Any RTE products that fall into Alternative III require more intense regulation and verification testing programs, and costs more for processors. Therefore upgrading the Alternative level from Alternative III to either Alternative I or II by adding antimicrobial agents can result in a more cost efficient process.

II. Sausage Products

It is difficult to define sausage in a single definition due to the variety of different types produced. However, sausage can be defined as a comminuted processed meat product made from red meat, poultry or a combination of these with water, binders and seasonings (Essien, 2003). Sausage compositions may vary to a degree; however the government regulatory requirements for moisture, protein, and fat assure a reasonably uniform composition for each product type. Sausage is versatile, so muscle from fish, goats, whales, and donkeys may also be used (Johnson, 1974). Basically, any lean muscle is considered suitable for sausage when blended with the proper type and quantity of fat (Johnson, 1974). The quality of the meat and the additives are carefully checked prior to use. The processing conditions and hygienic handling are highly controlled in order to produce wholesome sausage products (Johnson, 1974). Only the best raw materials should be selected when manufacturing sausages (Barcus, 1984).

1. Raw Materials

a) Meat

Sausages are usually made from lean pork, mixtures of pork and beef, or solely beef. The typical proportions are 50-60% lean pork and 10-20% beef (Toldrá, 2002). Selecting

adequate meat cuts and trimmings is extremely important because it will improve the product's microbiological and chemical qualities as well as extend the shelf life. Therefore, microbiological tests, temperature and pH checks for quality assurance of meat are required.

After slaughter, the pH of meat decreases by conversion of muscle glycogen into lactic acid. As ATP content drops, irreversible links between actin and myosin filaments of muscle are formed, called rigor mortis (Toldrá, 2002). The adequate pH range for meat used for good sausage production is between 5.6 and 6.0 (Roca and Incze, 1990). DFD meat (dark firm and dry) is not permitted due to its higher pH than normal meats (Toldrá, 2002). Their high water binding capacity due to the neutral pH motivates the spoilage of the meat (Toldrá, 2002). On the other hand, PSE meats (pale, soft, and exudative) should be avoided due to serious color problems and protein denaturation (Toldrá, 2002). However, under controlled conditions, PSE muscles can be used for processing without affecting final quality (Toldrá, 2002). For desirable color, meat from older animals, which contains more myoglobin, is preferred.

b) Meat Protein

By body weight basis, protein in meat ranks second only to water in abundance in the animal body, and most of the protein is located in muscle and connective tissues (Aberle et al., 2001). Protein constitutes approximately 15-22% of muscle, and provides many compounds such as peptides and free amino acids during the conversion of muscle to meat (Toldrá, 2002). There are three main groups of proteins in muscle: myofibrillar, sarcoplasmic, and stromal proteins. Myofibrillar proteins are soluble in high ionic

strength buffer (Toldrá, 2002). This protein is the main constituent of the structure of myofibrils, which include myosin and actin (Toldrá, 2002). Titin and nebulin are two large proteins parallel to the long axis of the myofibril, and they contribute the longitudinal continuity and integrity of muscle cells (Robinson et al., 1997). Sarcoplasmic proteins are water-soluble and constitute about 30-35% of the total protein in muscle (Toldrá, 2002). Sarcoplasmic proteins include a variety of metabolic enzymes in the mitochondria, lysosome, microsome, nucleus, or free in the cytosol, and myoglobin (Toldrá, 2002). The most common example of stromal proteins is collagen, which is the basic protein-forming part of the connective tissue that surrounds the fibers and muscles including epimysium, perimysium and endomysium (Toldrá, 2002). Collagen provides strength and supports muscle crosslinks, and thus contributes to toughness of meat (Toldrá, 2002). Also, collagen is used to make gelatin by partial hydrolysis using acid or alkali treatment followed by heat processing in the presence of water (Villegas et al., 1999).

c) Fat

There are several different classes of fat/ lipids in the animal body. Neutral lipids include fatty acids and glycerol and these predominate (Aberle et al., 2001). The function of lipids is to serve as energy sources to contribute to cell membrane structure and function, and involvement in metabolic functions (Aberle et al., 2001). It is recommended to use fresh fats or fats, which were stored for a short period of time (Toldrá, 2002). Reason is that endogenous lipases are active at low temperature, and it may act and generate free fatty acids (lipolysis) during cold storage and even during frozen storage (Toldrá, 2002). The amount of

polyunsaturated fatty acids is important due to the possible development of off-flavor as a result of oxidation (Toldrá, 2002). Yellowish color is developed in fat due to the oxidation of unsaturated free fatty acids. Therefore, the color defects were mostly caused by the defects of raw material containing a high level of unsaturated fatty acids and/or already oxidized fatty acids. The color defects can be cause by long ripening time at a relatively high temperature as well (Toldrá, 2002).

d) Carbohydrates

The major function of added carbohydrates in sausage is to provide a substrate for lactic acid bacteria growth (Pearson and Gillett, 1996). The rate and extent of lactic acid formation, pH drop and evolution of the microflora will depend on the amount and type of carbohydrates added to the mix (Toldrá, 2002). Another function of sugar addition in sausage making is flavor (Pearson and Gillett, 1996). Sugar also softens the product by counteracting the harsh hardening effects of salt by preventing moisture removal (Pearson and Gillett, 1996). Sugar interacts with the amino groups of the proteins and when cooked, forms browning products that enhance the flavor of cured meats. In some instances, the browning reaction may become too pronounced and burned flavors result (Pearson and Gillett, 1996).

Sugar (dextrose or glucose and sucrose) is added to many sausage products at a level of 0.5-1.0%. The purpose is self-limiting because of its contribution to flavor. It is believed by some manufacturers that the use of sugar, particularly dextrose, aids in improving color (Salisbury, 1960). However using excessive amount of sugars during the curing process is not recommended because it lowers the pH and it will affect color

destruction by oxidation of nitric oxide hemoglobin (Brady et al., 1948). Sugar substitutes such as corn syrup, molasses, and other natural sugar containing substances have been used to prevent excessive browning reaction during cooking (Pearson and Gillett, 1996). Sugar should always be stored in a dry atmosphere to promote adherence to the meat when used in dry curing (Moulton, 1935).

e) Water

Moisture, (water) is provided in the form of ice when chopping meats. Adding ice into the grinder is very important in the formulation and preparation of sausages, especially smoked sausages and fresh sausages (Salisbury, 1960). If moisture is not added during the chopping process, the product may become dry and unpalatable (Salisbury, 1960). In addition, product temperature at chopping increases rapidly and addition of ice or water can prevent the temperature from rising too quickly (Essien, 2003). In this regard, chopping temperatures over 16ºC (60ºF) are not permitted if the emulsion is to be held for an extended period of time prior to processing, since these conditions are favorable to bacterial growth (Salisbury, 1960). The addition of excessive amounts of water can decrease the quality of sausage, because unmelted ice can damage fatty tissue which will increase fat losses, and can lead to uneven salt distribution to the final product (Essien, 2003).

f) Chemical additives

Sodium lactate is naturally present as a normal constituent of muscle tissue and has been recognized as an effective and desirable food ingredient to extend product shelf-life, control

pathogens and enhance flavor without affecting other product characteristics (Barcus and Bontenbal, 1991). Preservative characteristics of sodium lactate include feedback inhibition, intracellular acidulation, interference with proton transfer across the cell membrane, and a lowering of the product water activity (Barcus and Bontenbal, 1991). The Food and Drug Administration (FDA) classified natural sodium lactate as GRAS (Generally Recognized as Safe), and the U. S. Department of Agriculture has approved its use in meat and poultry products (Barcus and Bontenbal, 1991). One study showed that the immersion of frankfurters into sodium lactate resulted in listericidal effects (Barmpalia et al., 2004). This study also showed the result that the combination of 1.8% sodium lactate with 0.25% sodium diacetate provided complete inhibition of *L. monocytogenes* growth throughout the 40 day storage period (Barmpalia et al., 2004). Gram positive bacteria have been shown to be more sensitive toward lactate under optimum growth conditions (pH 6.5 at 20°C) than Gram-negative bacteria (Zhu et al., 2005).

Several compounds capable of donating electrons (reductants) are added in meat-curing mixtures to accelerate color development (Toldrá, 2002). For color stabilization and speedy and uniform curing, sodium ascorbate (sodium salt of ascorbic acid- vitamin C) and sodium erythorbate (isoascorbate; isomer of sodium ascorbate) are used (Toldrá, 2002). They facilitate the formation of nitric oxide, exert antioxidant activity, stabilize color and flavor, and inhibit the formation of nitrosamines (Toldrá, 2002).

Some aldehydes, ketones, phenols, and organic acids in wood smoke impart bacteriostatic and bacteriocidal effects to smoked meat products (Aberle et al., 2001). The addition of acids to some meat products, for example, pickled sausage products and pig's feet, has limited bacteriostatic effects (Aberle et al., 2001). One unit of pH decrease

increases preservative effects to meats ten-fold, and acetic acid (vinegar) is the most common acid used for pickled meat processing (Aberle et al., 2001). Alkaline or neutral phosphates have only minimal effects on the curing reaction (Aberle et al., 2001). They increase the water-binding capacity of meat and reduce shrinkage of meat products during subsequent processing (Aberle et al., 2001). Phosphates also retard development of oxidative rancidity and can improve texture (Aberle et al., 2001).

Spices added to meat products can affect sausage quality (Toldrá, 2002). These include seeds (Mustard), leaves (oregano, rosemary), bulbs (garlic, onion) or fruits (pepper, paprika), which can be used in natural form (whole or ground) or as flavoring extracts (essential oils and oleoresins) (Toldrá, 2002). Spices are used for dry fermented sausages for imparting a characteristic and typical flavor and color, and sometimes for antioxidant properties (Toldrá, 2002). When adding spices, care must be taken because of a high risk of microbial contamination (Toldrá, 2002).

g) Salt

Salt (NaCl) is a basic ingredient to all curing mixtures and is the only ingredient absolutely necessary for curing (Toldrá, 2002). Salt is always present in cured meat products and has several roles: bacteriostatic effects, flavor development, and increasing the solubility of myofibrillar protein such as actin and myosin (Toldrá, 2002). Salt can inhibit the growth of undesirable microorganisms by dehydration, altering of osmotic pressure and hence allowing lactic acid bacteria to predominate during the fermentation (Pearson and Gillett, 1996; Barcus, 1984).

Salt influences flavor of the meat since it provides a characteristic salty taste (Toldrá, 2002). Salt also increases the gel strength of sausage batters. However, use of salt alone gives a harsh, dry, salty product that is not very palatable and results in a dark, undesirable colored lean product that is unattractive to consumers (Pearson and Gillett, 1996). As a consequence of undesirable effects of salt on flavor and appearance, salt is generally used in combination with sugar and nitrite and/or nitrate. Only a limited number of products are cured with salt alone. Impurities in salt can affect the solubility of proteins and interfere with the water holding capacity and emulsifying properties of meats (Pearson and Gillett, 1996). Only food-grade salt should be used in curing, since impure salt can cause flavor and color problems (Pearson and Gillett, 1996).

In general, fermented sausages are formulated with 2 to 3% salt, depending on the nature of the product. A salt level of 2% is regarded as a minimum to achieve the desired bind, and no major differences in fermentation rate are generally observed up to 3 % (Salisbury, 1960). At low concentration (less than 2% salt), the growth of undesired microorganisms is favored over that of starter cultures, and at high concentration, more than 5%, the growth of the starter cultures is inhibited (Jensen, 2004). Recently, emphasis has been placed on reducing levels of salt in meat products in view of its relationship to hypertension which is problematic in about 20% of the population (Pearson and Gillett, 1996). Thus processors are attempting to decrease salt in most meat products. Since sodium is the element in salt that causes hypertension, substitution of potassium chloride (KCl) for part of the NaCl has been successfully used for producing sodium-reduced meat products (Pearson and Gillett, 1996).

h) Nitrite/Nitrate

The function of nitrite in meat curing is to stabilize the color of the lean tissues, to contribute to the characteristic flavor of cured meat, to inhibit growth of a number of food poisoning and spoilage microorganisms, and to retard development of rancidity (Pearson and Gillett, 1996). Nitrite reacts with the meat pigment, myoglobin, to form the characteristic cured meat color, nitrosomyglobin, which develops the red cured color (Leistner et al., 2002). Other than the effect on flavor enhancement, the most important reason for its use is its effect on microbial growth (Pearson and Gillett, 1996).

The primary use of nitrites as antimicrobials is to inhibit the growth of *C. botulinum*. Nitrite also inhibits other bacterial spore formers by inhibiting outgrowth of the germinated spore (Duncan and Foster, 1968). Moreover, it also prevents the growth of other spoilage and food borne disease organisms (Pearson and Gillett, 1996). The antimicrobial activity of nitrite is enhanced by low pH under anaerobic conditions and in the presence of salt (Juneja, 2002). Nitrite concentrations of 120 ppm of nitrite added to canned or vacuum-packaged processed meat products can prevent formation of botulinum toxin (Aberle et al., 2001).

Nitrate serves principally as a source of nitrite. Although nitrate was originally approved for color fixation in cured meats, nitrite is more widely used. Nitrate use is limited to only a few products such as country-cured hams and Lebanon bologna and shelf-stable, canned, comminuted products (Pearson and Gillett, 1996). Nitrate reduction to nitrite is a bacterial process and reasonably high levels of nitrate-reducing bacteria are required for significant reduction of nitrate (Salisbury, 1960).

Despite the benefit of nitrite, potential toxicity has been observed. The reaction of nitrous acid to form nitrosamines is well known. Nitrous acid is formed by the breakdown of

nitrite. During the breakdown process, the secondary amines are produced, and these secondary amines are producing nitrosamines (Pearson and Gillett, 1996).

Almost all cured meat have nitrosamines as a result of the interaction of nitrite with secondary amines during curing and cooking (Pearson and Gillett, 1996). Since nitrosamines are carcinogenic compounds, the levels of nitrite use in cured meat have been decreased in recent years (Pearson and Gillett, 1996).

i) Starter cultures

When using starter cultures, proper inoculation and incubation procedures are among the most critical steps for the production of safe, flavorful, uniform, and wholesome fermented sausages (Ricke et at., 2001). Starter cultures have replaced "back-slopping" and natural fermentation methods (Lücke, 1998). Back slopping is using meat from the previous batch to inoculate the following batch (Lücke, 1998). The disadvantage of back slopping is that desired catalase-positive cocci will tend to become "diluted out" and strains with undesirable metabolic properties may be selected (Lücke, 1998). Another method of fermentation is natural fermentation, which relies on indigenous microflora in the meat to serve as the inoculum (Ricke et al., 2001). It has been used before the techniques of adding starter cultures started. Nowadays the majority of fermented sausages produced in Europe are made with starter cultures (Lucke, 1998). Also, the use of commercial starter cultures is the predominant method of inoculation in the United States (Ricke et al., 2001).

Starter cultures are pure microbial cultures that are added to sausage batter either alone or in combination with other pure cultures in order to control the fermentation (Jensen, 2004).

The main purpose of adding starter cultures is to achieve consistency in quality and to ensure stability and safety of the sausage (Jensen, 2004). In the United States, *lactobacillus* or *Pediococcus* are the predominant culture microorganisms, while in Europe, the combination of *Micrococcus* and *Staphylococcus* are most often used (Beuchat, 1976; Nout, 1995). The current commercial starter cultures contain lactic acid bacteria (*Lactobacillus* and *Pediococcus*) to ensure a correct pH drop, and strains of *Kocuria* and *Staphylococcus* are added for additional lipolytic, catalase and nitrate reductase activity (Toldrá, 2002).

Strains used as starter cultures must be generally regarded as safe (GRAS) since they are considered food additives (Toldrá, 2002). There are some requirements that starter cultures must meet for effective use. They must be nonpathogenic, nontoxic or nonallergenic, must have genetic stability, be tolerant of salt and nitrite, be able to grow at manufacturing pH and temperatures, contribute to safety, flavor or nutrition, have non-decarboxylase activity, and be resistant to phage infection (Toldrá, 2002). The accomplishment of these requirements contributes to the safety of sausage and extends its shelf life, which contributes to a substantial reduction in processing time and a sausage with standardized quality. The presence of 10^6 -10^7 rather than 10^2 active lactic acid bacteria per gram of fresh sausage batter leads to a more predictable and more rapid pH decrease and to earlier development of firmness, and may improve product safety (Lücke, 1998).

Yeast and mold strains are available as starter cultures for air-dried (not or only lightly smoked) sausages where these organisms readily colonize the surface (Lücke, 1998). The application of yeasts or molds is restricted to the external surface (Toldrá, 2002). The presence of molds on the external surface of the sausages contributes to the characteristic appearance and quality that is desirable in some Mediterranean areas (Toldrá, 2002).

Debaryomyces hansenii and *Candida famata* appear to be the only yeast species in meat starter cultures because they grow at low water activity and have also been observed to affect color and flavor when added to the sausage mixture (Toldrá, 2002). Molds contribute to the characteristic aroma, flavor and appearance of air-dried sausages (Lücke et al., 1990). However, colonization of the sausage surface by the wrong mold leads to unsatisfactory product quality and increases the risk of mycotoxin formation (Lücke et al., 1990). *Pennicillium nalgiovense* and *P. chrysogenum* are the most common fungi available as starter cultures (Toldrá, 2002). The sausages should be dipped into a suspension of conidia of an appropriate starter mold before ripening (Lücke et al., 1990).

j) Casings

Casings are used to protect sausage contents, to reduce the loss of moisture during storage, and to hinder the penetration of microbes into the product (Danilov, 1969). Stuffing into casings is done manually or with an automatic sausage filler of various constructions. The filler is packed closely with the stuffing and the casings are pulled onto the nozzle of the filler (Danilov, 1969).

Casings are classified into two groups based on their origin: natural and synthetic. Natural casings are originated from animal intestine, and are irregular and lacking in uniformity but give a handmade appearance (Toldrá, 2002). Casings of animal origin are stored in dry salt and should be extensively washed with disinfectant, due to the potential for fecal contamination (Toldrá, 2002). Animal casings used in sausage production include beef small intestines (round), large intestines including colon and rectum or middles, and caecum (blind end); hog small intestines, middles (chitterlings), and rectum (bung); sheep thin

intestines and blind end (caecum) (Danilov, 1969). Natural casings are more expensive than artificial casings (Potter and Hotchikiss, 1998). Synthetic casings are made of materials permeable to evaporation, gas, and smoking. Artificial casings are extruded tubes of regenerated collagen, cellulosic materials, or plastic films (Potter and Hotchikiss, 1998). Synthetic casings have uniformity and a regular pore size so that processors can control drying.

2. Classification

Sausage classifications vary substantially, and there are a variety of sausage classifications available based on the characteristics of products, type of meat ingredients and processing methods used in their manufacture (Johnson et al., 1974; Aberle et al., 2001). Classification is based on degree of chopping; amount of cooking; degree of smoking; amount of water added; amount of curing; amount of fermentation; and amount of moisture in the final product (Toldrá, 2002).

Based on the degree of chopping, the most useful classification separates “coarse ground” and “emulsified” products, according to the Encyclopedia of Food Technology (Johnson and Peterson, 1974). Grinding the meat items and mingling the fats and lean particles in a uniform mix us used to prepare coarse ground products. On the other hand, emulsified products are prepared by mixing, chopping, and emulsifying ground meats with ice, salt, spices, and curing salts to produce an emulsion (Johnson and Peterson, 1974). Most emulsified sausages are cooked or smoked, and examples are bologna, knockwurst, bratwurst, frankfurters and liver sausages (Essien, 2003).

Sausage products can also be divided into uncured, fresh product and cured product depending on the amount of curing. The term “curing” or “cured” meat is used for a variety of meat products, but the meaning varies by country of origin and the kind of product. In general, the term “curing” means the use of curing salt such as sodium chloride and nitrate/ nitrite, which generate the color or texture profiles in the product (Toldrá, 2002). Basically, cured- meat products can be arranged into two main groups based on their respective processes: dry cured and wet or pickle cured (Toldrá, 2002). Dry curing is rubbing the dry cure such as salt alone or nitrite/nitrate on the surface of the meat or mixing it into the mincer. On the other hand, wet curing consists of pickle injection of the cure into the piece by pumping, or the entire piece being soaked in the curing brine.

The USDA Meat Inspection system classifiew sausage into 6 categories, which covers most of the sausage products: fresh sausage, uncooked, smoked sausage, cooked smoked, cooked, dry and semi-dry sausage, and cooked meat specialties (Toldrá, 2002). Table 3 shows sausage classification in the United States.

Table 3 Classification of Sausage produced in the United States

Classification	Characteristics	Examples
Fresh sausage	Fresh meats (chiefly pork); uncured, comminuted, seasoned, and usually stuffed into casings; must be cooked fully before serving	Fresh pork sausage Bratwurst Bockwurst Breakfast sausage
Uncooked, smoked sausage	Fresh meats, cured or uncured, stuffed, smoked, but not cooked; must be fully cooked before serving	Smoked, country-style pork sausage Mettwurst Kielbasa

Table 3 Continued

Cooked, smoked sausage	Cured or uncured meats; comminuted, seasoned, stuffed into casings, cooked and sometimes smoked; usually served cold	Frankfurters Braunschweiger Bologna
Cooked sausage	Cooked, not smoked	Liver sausage
Dry and semi-dry sausage	Cured meats; air dried, may be smoked before drying; served cold	Genoa salami Pepperoni Summer sausage
Cooked meat specialties	Specially prepared meat products;	Luncheon meats, loaves, sandwich spreads, jellied products, souse

(Adopted from Barcus, 1984; Ziegler, 1962)

a) Fresh sausage

Fresh sausages are made of fresh, uncured meat, generally cuts of fresh pork and sometimes beef. The taste, texture, tenderness and color of the products are directly related to the ratio of fat to lean (Toldrá, 2002). The term pork sausage implies ground and seasoned fresh pork product (Ziegler, 1962). Several kinds of fresh pork sausages have their own distinct texture, seasoning, and meat contents, and therefore have a different taste. Usually, pork loins, hams, and shoulders are commonly used for fresh sausage manufacturing (Toldrá, 2002). The country style pork sausage is a coarse, ground meat product, and either stuffed into different sizes of hog casings or unstuffed (Ziegler, 1962). Breakfast style pork sausage is finely ground and seasoned with sage, pepper, and salt (Ziegler, 1962). A lean sausage has 20-25% fat content with a mild seasoning of sage (Ziegler, 1962). It is the most popular but also the most expensive. In this category, bratwurst, fresh thuringer, and bockwurst are included (Ziegler, 1962).

b) Smoked sausage

The smoking process is divided into two classes; natural and liquid (Essien, 2003). It gives desired flavor, color, antimicrobial, antioxidant, and preservative effects to sausage products (Essien, 2003). Natural smoking is using wood such as beech, oak, hickory, juniper, etc., and liquid smoke contains hundreds of compounds, most notably phenols, carbonyls, and acids (Essien 2003; Hansen, 2000). The most popular smoker is the air-conditioned chamber with hot and cold water showering facilities (Ziegler, 1962). The first stage of smoking is drying and warming the product up to 130°F-140°F for 15-20 minutes (Ziegler, 1962). In the second stage, smoking is applied at a temperature of 145-155°F to avoid excessive drying and tough shell (Ziegler, 1962). The last stage is showering with hot water in the smoker or putting the product into a conventional type water cooker until the internal temperature reaches 155°F (Ziegler, 1962). The product is then showered using cold water for 3 to 5 minutes, hung at room temperature to dry, and placed in a cooler with a temperature of 45-55°F (Ziegler, 1962).

There are two types of sausages depending on the degree of cooking prior to smoking: uncooked and smoked sausage, and cooked and smoked sausage. Currently USDA does not allow manufacturing uncooked and smoked sausage. In the US, cooked and smoked sausage is regarded as a fully cooked product, and thus regarded as RTE (Ready-to-Eat) foods. Therefore, it is not necessary to cook it prior to consumption, however many people do reheat the product prior to consumption.

c) Cooked sausage

Manufacturers produce product that has already been cooked, sliced or diced, and the primary reason for cooking is for safety and convenience (Eissen, 2002). Various cooking

methods are used in cooked sausage manufacture such as oven cooking, steaming, smoking, drying or a combination, and deep fat frying (Eissen, 2002). The most important thing during the cooking process is monitoring the time and temperature to achieve consistent quality (Eissen, 2002). At an external temperature of 160°C, sausage should be cooked to a core temperature of between 85°C and 95°C in six minutes (Eissen, 2002). To avoid *Trichinella*, the minimum internal temperature of 58.3°C is required in the US (Vernam, 1995). Microbial spoilage related to cooked sausage is affected by initial microbial load, level of preservatives, and temperature of cooking and storage. Post- processing contamination is the primary concern for processors (Eissen, 2002).

Liver sausage falls into this category. The main ingredients for liver sausage are pork livers, hearts, tongues, brains, sweetbreads, kidneys pork shoulders and wheat flour (Ziegler, 1962). If liver sausage is stuffed into hog casings, the cooking should be done after stuffing (Ziegler, 1962). Then it should be cooled by dipping into the cold water, and hung in a cool place to dry (Ziegler, 1962).

d) Semi-dry and Dry fermented sausage

Semi-dry sausages are generally of higher moisture content than dry ones, but they are also stable at ambient temperature because of a combination of lowered pH (<5.3) and lowered water activity, a_w (<0.95) (Jensen, 2004). Good Manufacturing Practices (GMPs) published by the American Meat Institute Foundation (AMIF) in 1997, have defined semi-dry sausage as chopped or ground meat products that, as a result of bacterial action or direct addition of organic acids, reach a pH of 5.3 or less and are then dried to remove 15% of the moisture resulting in a moisture/protein ratio complying with federal meat and poultry

inspection requirements (AMIF, 1997). On the other hand, dry sausages are ripened and dried for longer times, resulting in lower a_w values (usually <0.89), which make them stable even if the pH is not lowered (Jensen, 2004). In the other definition, dry sausages are chopped or ground meat products that as a result of bacterial action or direct addition of organic acids, reach a pH of 5.3 or less and are then dried to remove 25 to 50% of the moisture (AMIF, 1997).

The stability of semi-dry or dry sausages is enhanced by heat treatment to inactivate most undesired microorganisms except for resistant spores. The length of time required for making sausage varies depending on the kind of product, the desired final quality, and its diameter. In most cases, the total processing time takes less than a week for a rapid process, for a regular process it takes about 3 weeks; and slow process requires around 90 to 120 days (Toldrá, 2002).

e) Specialties

Specialty sausages are made with edible hog by-products such as hearts, tongues, shoulders, and any pig trimmings available containing fat. Souse, headcheese, scrapple are the typical type of specialty sausage, and all of them are essentially the same product with different names, all made by cooking edible trimmings for a long time, adding spices and vinegar, and cutting. Souse is what it is called in North Carolina. The products under specialties are the products, which do not fit the other classifications.

III. Microbiological hazards associated with sausage

1. *Listeria monocytogenes*

Listeria monocytogenes is an agent of foodborne disease that has been emerged over the past two decades, and especially after the 1981 outbreak of listeriosis in Nova Scotia, Canada attributable to contaminated coleslaw (Schlech et al., 1983). Between 1930 and 1950, a few human listeriosis cases were reported; however, hundreds of human listeriosis cases are now reported every year (Rocourt et al., 1992). About 35-50% of sporadic human listeriosis infections and all major outbreaks in Europe and North America in the 1980s were associated with *L. monocytogenes* serotype 4b (Swaminathan, 2001). In contrast, isolates recovered from foods in many countries belong to serotypes 1/2a and 1/2c (Swaminathan, 2001). Healthy adults are not affected, but *L. monocytogenes* can cause a food borne infection when ingested by susceptible individuals including young children, elderly, immuno-compromised individuals or pregnant women (Aberle et al., 2001). Women can be infected with *L. monocytogenes* at any time during pregnancy, but most cases of listeriosis are reported in the third trimester (Slutsker and Schuchat, 1999). Usually three to seven days after the onset of symptoms, a woman may abort the fetus or have premature delivery (Gellin and Broome, 1989). In the first trimester, listeriosis may result in spontaneous abortion. Listeriosis is rarely life threatening to the mother and is not known to cause increased risk in subsequent pregnancies (Skidmore, 1981; Farber and Peterkin, 1991). Actual numbers of susceptible individuals are difficult to determine because these individuals belong to diverse groups including the elderly, cancer and transplant patients, and persons with immunosuppressive diseases such as AIDS (Morris and Potter, 1997). Symptoms of mild listeriosis include influenza-like symptoms such as diarrhea and fever; in severe cases, abortion and meningitis may occur (Aberle et al., 2001).

L. monocytogenes can survive in natural decaying vegetation and can grow in soil and water (Swaminathan, 2001). Contamination can occur in food processing plants, by worker's shoes, clothing, and on transport equipment, through animals which excrete the bacterium or have contaminated hides or surfaces, raw plant tissue, raw food of animal origin and possibly healthy human carriers (Swaminathan, 2001). Once it gets into the food processing environemnt, *L. monocytogenes* attaches to various kinds of surfaces including stainless steel, glass, and rubber, and biofilms have been reported in the meat and dairy processing environment (Jeong et al, 1994). This microorganism can even survive on the fingers after hand washing and in aerosols (Swaminathan, 2001).

L. monocytogenes can grow in low water activity, low pH, low temperature, and high salt (Aberle et al., 2001). Optimum water activity for *L. monocytogenes* is a_w> 0.97, and for most strains the minimum a_w for growth is 0.93 (Lou and Yousef, 1999). This microorganism can survive for long periods at water activity at or below 0.83 (Shahamat et al., 1980). The pH for growth of *L. monocytogenes* ranges between 5.6 and 9.6, although recent study has shown that *L. monocytogenes* can initiate growth at an initial pH of 4.4 (Lou et al., 1999). Below pH 4.3, the organism can survive, but not multiply (Swaminathan, 2001). The presence of organic acids in tryptic soy broth inhibits the growth of *L. monocytogenes*, and this inhibition increases as the incubation temperature decreases (Ahamad et al., 1989). One unit of pH decrease will result in a 10-fold increase of the preservative effects to meat products (Aberle et al., 2001). For pickling meat products, vinegar (acetic acid) is the most commonly used acid. *L. monocytogenes* can initiate growth in the temperature range of 0 to 45 ºC (Swaminathan, 2001). At lower temperature, the growth rate is much slower, but the organism still grows at refrigeration temperatures (Swaminathan, 2001). Temperatures

below 0ºC preserve or moderately inactivate the bacterium and *L. monocytogenes* can be inactivated when exposed to temperatures over 50ºC (Swaminathan, 2001). Survival and injury during frozen storage is dependent on the rate of freezing (Swaminathan, 2001).

2. *Staphylococcus aureus*

Staphylococcus aureus is responsible for food poisoning worldwide, and the source of food poisoning are staphylococcus enterotoxins (SEs) (Jablonski and Bohach, 2001). Unlike many other forms of food borne gastroenteritis food borne illnesses, which are caused by ingestion of live microorganisms, Staphylococcal food poisoning is due to toxins called entertoxins (SEs) produced by the microorganism in contaminated food (Jablonski and Bohach, 2001). This form of food poisoning is called intoxication. Most *S. aureus* strains reside in the external regions of the human body or animals. Humans are a main reservoir of this microorganism, and are usually responsible for human disease associated with *S. aureus* (Jablonski and Bohach, 2001). *S. aureus* is present in the nasal passages and throats and on the hair and skin of 50 percent of healthy normal individuals, and exists in air, dust, sewage, water, milk, food or food equipment, and environmental surfaces (AMIF, 1997). Today, the main cause of SFP is humans who contaminate food during preparation (Jablonski and Bohach, 2001). A survey of more than 700 outbreaks related to *S. aureus* shows the most common conditions for *S. aureus* contamination was inadequate refrigeration; preparation of foods far in advance; poor personal hygiene; inadequate cooking or heating of food; and prolonged use of warming plates when serving foods, since it promotes S. aureus growth and SE production (Bryan, 1976). One of the difficulties in controlling SFP is the large number of human and animal reservoirs (Jablonski and Bohach, 2001). This microorganism has an

ability to persist in sites like mucosal surfaces, due to its intracellular survival. It can be internalized by many different cell types, including bovine mammary epithelial cells (Bayles et al., 1998; Wesson et al., 1998).

The highest risk for SFP outbreaks occur in late summer when temperatures are warm and food is more likely to be stored improperly (Jablonski and Bohach, 2001). The second highest risk season is between November and December, due to leftover holiday food (Jablonski and Bohach, 2001). SFP is usually a self-limiting illness presenting with emesis following a short incubation period (Jablonski and Bohach, 2001). The maximum incubation time is 6 to 10 hours, with mean incubation period of 4.4 hours, although the incubation periods can be as short as 1 hour (Lovejoy, 1991). The common symptoms are nausea, abdominal cramps, diarrhea (either watery or possibly bloody), headaches, muscular cramping, prostration, general weakness, dizziness, chills and perspiration (Jablonski and Bohach, 2001). Symptoms last for 1 to 88 hours, with a mean of 26.3 hours (Jablonski and Bohach, 2001). Death due to SFP is not common, but the fatality rate ranges from 0.03% for the general public to 4.4% for more susceptible populations including children and the elderly (Holmberg and Blake, 1984). The effective dose of SE is achieved when populations of *S. aureus* are greater than 10^5 cells per gram of contaminated food (Anonymous, 1992). Approximately 1 ng of SE per gram of contaminated food is sufficient to cause symptoms associated with SFP (Jablonski and Bohach, 2001). Although levels of 1 to 5 µg of ingested toxin are usually associated with outbreaks, the actual levels of detectable SE to cause illnesses were less than that (Gilbert and Wieneke, 1973).

If sausages are fermented at no more than 25 ºC for 2-3 days, and the initial count of *S. aureus* is below 10^4/g, the risk of enterotoxin formation is low (Holley et al., 1988a), even if

the sausages are not smoked (Metazopoulos et al., 1981a, b). The presence of salt and nitrite does not affect the growth of *S. aureus* as this bacterium is a poor competitor but hardy survivor under anaerobic conditions, at low pH values and low temperatures (Lücke, 1998). It may grow at high fermentation temperatures during the lag phase of the development of lactic acid bacteria and may reach high numbers in the outer layers of the sausage (Barber and Deibel, 1972). The initial pH value and the initial activity of the lactic acid bacteria are critical for control of *S. aureus* (Niskanen and Nurmi 1976; Metaxopoulos et al., 1981a,b). *S. aureus* is an osmotolerant bacterium (Jablonski and Bohach, 2001). However, the production of toxin (SEs) is reduced under osmotic stress (Jablonski and Bohach, 2001; Troller, 1972). Outside the body, *S. aureus* is one of the most resistant non-sporeforming human pathogens and can survive for long periods of time in a dry environment (Jablonski and Bohach, 2001). It can survive at a_w less than 0.86 (Jablonski and Bohach, 2001).

IV. Methods to control or minimize risk

Hurdle Technology

The microbial safety and stability, as well as the sensory and nutritional quality of most foods, is based on an application of combined preservative factors, called hurdles (Leistner, 2000). The most important hurdles used in food preservation are temperature, water activity, pH, redox potential, preservatives, and competitive microorganisms such as lactic acid bacteria (Leistner, 2000). Some hurdles influence the safety and quality of foods due to their antimicrobial properties and their flavor improvement on the products (Leistner, 2000). The same hurdle can have a positive and negative effect on foods depending on its intensity (Leistner, 2000). Therefore, hurdles in foods should be kept in

the optimal range, and will improve the microbial stability and safety but also the sensory and nutritive quality as well as the economic aspects of a food (Leistner, 1994a, 1994b, and 2002). The basic concept of food preservation by hurdle technology is putting microorganisms in a hostile environment in order to inhibit their growth or shorten their survival or cause their death (Leistner, 2000).

As the important manifestation of the hurdle concept is the interference by the food with the homeostasis of microorganisms (Gould, 1988). Homeostasis is the strong tendency of organisms to maintain a stable and balanced internal environment (Leistner, 2002). When cells channel the energy needed for biosynthesis into maintenance of homeostasis, their growth is inhibited (Montville and Matthews, 2001). Microorganisms in stable hurdle-technology foods may strain every possible repair mechanismto maintain homeostasis and overcome the hostile environment; by doing this they become metabolically exhausted, and this situation is called "auto sterilization" (Leistner, 1995). The repair of a disturbed homeostasis demands much energy, and thus the restriction of the energy supply inhibits repair mechanisms in microbial cells and leads to a synergistic effect of preservative factors (Leistner, 2000). In addition, it is more effective to use different hurdles in small amounts in a food instead of one preservative in larger amounts, because different factors affect different target sites in the bacterial cells, and thus they can act synergistically (Leistner, 1994a).

a) Aw

The availability of water is measured by the water activity (aw) of a food, and it is defined as the ratio of the vapor pressure of water in a food (Farkas, 2001). Water

activity (a_w) is a major factor in the preservation of foods to the vapor pressure of pure water (Scott, 1957). Food preservation by dehydration is achieved by inhibiting growth of microorganisms by removing the available water required for the growth (Farkas, 2001). The optimum water activity for *L. monocytogenes* is $a_w > 0.97$, and most strains of this pathogen can grow at a_w values as low as 0.90 (Lou and Youself, 1999). In the vital range of growth, decreasing a_w increases the lag phase of growth and decreases the growth rate. Food borne microorganisms are grouped according to their minimal a_w requirements (Table 4).

Table 4 Minimal a_w levels required for growth of foodborne microorganisms at 25°C

Group of microorganisms	Minimal a_w required
Most bacteria	0.91-0.88
Most Yeasts	0.88
Regular molds	0.80
Halophilic bacteria	0.75
Xerotolerant molds	0.71
Xerophilic molds and osmophilic yeasts	0.62-0.60

(Farkas, 2001)

Differences of minimum a_w values for growth depend on the difference in osmoregulatory capacities (Gould, 1973). Mechanisms of tolerance to low a_w are different in bacteria and fungi, however the most important thing is that the cell osmoregulation mechanism operates to maintain homeostasis with respect to water content (Gould, 1973). Jones (1995) showed that safety of low fat meat products decreases by removal of fat from a food formulation, which results in increased moisture content (Jones, 1995).

b) RH

Modified atmosphere packaging (MAP) is applied to control atmosphere inside the package during the storage time. In 1996, Beumer et al. studied MAP on sliced, cooked, and vacuum or modified atmosphere packed meat product, mainly to observe the growth of *L. monocytogenes* on this product (Beumer et al., 1996). They found that normal background microflora, mostly lactic acid bacteria in this case, did not affect the growth of *L. monocytogenes* in 30% CO_2, 70% N_2 and vacuum packed product (Beumer et al., 1996).

c) pH

Most microorganisms grow best at pH values around 7.0, whereas few grow below pH 4.0. Bacteria tend to be more fastidious in their relationships to pH than molds and yeasts, with pathogenic bacteria being the most fastidious (Jay, 2000). The pH is the most important factor influencing the effectiveness of many food antimicrobials including organic acids, nitrite, and sulfites. Antimicrobials effects of weak acids are most effective in their undissociated or protonated form because they are able to penetrate the cytoplasmic membrane of a microorganism more effectively (Jay, 2000). In addition, their action results from the undissociated molecules rather than the anion, and they are more undissociated at lower pH (Leistner, 1997). The lower the pH of food, the greater the proportion of acid in the protonated form and the greater the antimicrobial activity. The pKa of a weak acid indicates how much of the compound will be in the most active form in a given food application (Davidson, 2002). Undissociated organic acids are lipophilic, so be able to enter into the cell easily (Ray, 2004). Once the undissociated acid enter the cell, it dissociates to generate H+ in cytoplasm (Ray, 2004).

This mechanism causes a reduction in internal pH, which eventually destroys the proton gradient across the cell membrane and dissipates the proton motive force and the ability of the cell to generate energy (Ray, 2004).

d) Bacteriocin

Many microorganisms produce bacteriocins, which can be defined as proteins or protein complexes with bacteriocidal activity directed against species that are usually closely related to the producer bacterium (Chikindas and Montville, 2002, Klaenhammer, 1988). Bacteriocins are different from antimicrobial substance in their mechanism of synthesis, structure, and function (Chikindas and Montville, 2002). The use of bacteriocin-producing starter cultures in fermentation does not require special consideration if the starter culture is GRAS (generally regarded as safe) (Muriana, 1996). Many species of LAB have GRAS status and many studies have been conducted on bacteriocins produced by LAB. However, inside the food matrix, bacteriocins are absorbed to food macromolecule, since bacteriocins are amphiphilic peptides (Zhu et al., 2005).

e) Thermal processing

Designing heat treatments to achieve special lethality for certain food borne pathogens is regarded as a critical control point in food processing, and is fundamental to assure the shelf-life and microbiological safety of processed foods (Juneja, 2002). When designing thermal processes, the most important factor to consider is the target organisms' heat resistance (Juneja, 2002). Overestimating leads to decreases in quality

of the product by changing organoleptic attributes and nutritional qualities, also underestimating increases the chances for survival of target microorganisms (Jujena, 2002). Using relatively mild heat treatment is widely accepted since it is effective in destroy non-spore forming pathogenic microorganisms, and reducing level of naturally present spoilage microorganisms (Juneja, 2002). It is using very high temperatures for a short time to kill viable microorganisms that are significant for public health standpoint and also are capable of growing during normal storage temperature (Juneja, 2002).

f) Temperature

Preservation effects due to cold storage (refrigeration) occur because of decreased rates of chemical reactions and hence decrease in microbial growth (Farkas, 2001). While pure water freezes about 0°C, most foods freeze below 0°C even below -2°C (Farkas, 2001). The proper temperature for using refrigeration is below the minimal growth temperature of most foodborne microorganisms (Farkas, 2001). However, establishing minimum temperature may be difficult due to the resulting changes in a_w and solute concentration that occur after freezing (Gill, 2002).

g) Additions of antimicrobials to formulation

Lactic acid is one of the most widely used antimicrobials in food. Food grade acid should be of GRAS status, and is produced by controlled fermentation of refined carbohydrate sources (Shelef, 1994). Visser et al. (1988) conducted experiments with veal tongues supplemented with 2% lactic acid and stored under vacuum-packaged

conditions. They observed 3 log reductions in bacterial counts (Visser et al., 1988). In addition to antimicrobial properties, lactates are used as humectants and flavor enhancers, and contribute to increase cooking yields and water holding capacity for meat and poultry products (Reid, 1969).

The food industry prefers using naturally present antimicrobials to artificial antimicrobials. In 1994, Beuchat et al. researched the lethality of carrot juice to *L. monocytogenes*. The lethal effects on *L. monocytogenes* were shown over a 48-hour time period within a pH range of 5.0 to 6.4 with various concentrations of carrot juice from 1% to 100%; 10% showed the greatest effect, while 0.1% showed the least effect (Beuchat et al., 1994).

Summary

Souse is an acidified, gelled pork product and is classified as a specialty sausage product. Due to its ubiquitous nature and ability to grow at refrigeration temperature, as well as increased numbers of outbreaks related to RTE (Ready-to-Eat) meat products, *Listeria monocytogenes* has posed a safety concern for RTE meat processors. Since recent technologies, such as irradiation, is not permitted to RTE meat products, organic acid was used widely for controlling this pathogen. There have been a number of testing the effectiveness of various combinations of organic acids on RTE meat products. Yet, there has been no published study evaluating the effect on souse meat, particularly with respect to the growth of *L. monocytogenes*.

The objective of this study is to determine effectiveness of three different souse formulations in controlling the growth of *L. monocytogenes* at two different refrigerated storage temperatures

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Chapter 2 Manuscript I

Impact of Storage Temperature and Product pH on the Survival of *Listeria monocytogenes* in Souse Meat

Impact of Temperature and pH

on the Survival of *Listeria monocytogenes*

in Souse Meat

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Abstract

Souse meat is an acidic, gelled pork product that is considered a fully cooked, ready-to-eat (RTE) food. There is a "zero-tolerance" policy for *Listeria monocytogenes* in RTE meat products. The growth/survival of *L. monocytogenes* in souse is largely unknown. In this study, the effectiveness of three different souse formulations in controlling the growth *of L. monocytogenes* at two different refrigerated storage temperatures was evaluated. Three souse formulations (pH 4.3, 4.7, and 5.1) were produced in triplicate and surface-contaminated with a three-strain cocktail of *L. monocytogenes* at an initial contamination level of 10^5 cfu/cm^2. Products were vacuum-sealed and stored at two different temperatures (5°C and 10°C). Uninoculated product was prepared as the control. Microbial counts were monitored twice weekly through 32 days storage on selective and nonselective media. Souse meat did not support the growth of *L. monocytogenes*, regardless of product formulation or storage temperature. D values for products with a pH of 4.7 or 5.1 were not statistically different (D value = 40 days) ($p>0.05$). A lower product pH (4.3) decreased *L. monocytogenes* survival (D value = 9 days) compared to higher pH products ($p<0.05$). D values for products stored at 5° C and 10° C did not differ significantly ($p>0.05$). Consumer acceptability of pH 4.3 products was not different from (typical) pH 4.7 product ($p>0.05$). These results demonstrate that conventionally produced souse meat does not support the growth of *L. monocytogenes* and that inactivation of the organism is favored for products formulated at lower pH ($\leq$4.3).

Keywords: Challenge study, Souse, *Listeria monocytogenes*, pH and Temperature

Introduction

Souse is a value-added gelled pork product made with pork co-products such as ears, heart, tongues, and skins. Vinegar is used as a natural acidulant to achieve a final pH of 4.5 to 5.5. Souse is also called “headcheese” in different U.S. regions, especially in the northern part of the United States. Souse represents a significant volume of processed meat sold in the United States and displays a distinct flavor. Like sliced luncheon meat, hot dogs, poultry rolls, and meat and poultry salads, souse is considered a Ready-to-Eat (RTE) product. As with other RTE meat products, *L. monocytogenes* is a major food safety concern usually present as a consequence of post-processing environmental contamination (Barmpalia et al., 2004). Indeed, *L. monocytogenes* is zero-tolerance in RTE products.

L. monocytogenes is a Gram positive, non-spore forming, mobile, facultative, rod-shaped bacterium (Farber and Peterkin, 1991). Gellin and Broome (1989) reported that this pathogen was readily isolated from the natural environment including soil, water, and vegetation (Gellin and Broome, 1989). In France, Thévenot et al. (2005) investigated the prevalence of *L. monocytogenes* in 13 dried sausage-processing plants including the surfaces of equipments floors, walls, and steaming and drying rooms were investigated. Fifteen percent of these surfaces were positive for *L. monocytogenes* before operation, and 47.3% were positive during the working day (Thévenot et al., 2005). Yűcel et al. (2005) investigated a variety of meats for *L. monocytogenes* contamination. Contaminated product included: minced meat (2 out of 42), chicken meat (3 out of 26), and beef (1 out of 19) (Yűcel et al., 2005). In addition, many studies have reported that *L. monocytogenes* can grow readily on processed meat at refrigerated temperature (Grau and Vanderlinde, 1990; Samelis et al., 2002; Glass and Doyle, 1989; Glass et al., 2002; Geornaras et al., 2005).

Due to its ubiquitous nature and ability to grow at refrigeration temperature, this pathogen is a significant health hazard (Zhu et al., 2005). Healthy adults are not usually affected, but the organism can cause severe illnesses if ingested by the susceptible population, which includes young children, the elderly, immuno-compromised individuals, or pregnant women (Aberle et al., 2001). It has been estimated that *L. monocytogenes* causes severe disease in about 2,500 persons annually in the United States, with a 20 to 25% mortality rate (Mead et al., 1999). Three serotypes (1/2a, 1/2b and 4b) are associated with most sporadic cases of listeriosis in the United States. Serotype 4b is the cause of almost all outbreaks of listeriosis in Europe (Kathariou, 2002). In November 1998, the public health department of Tennessee, New York, Connecticut, and Ohio reported significant increases of listeriosis cases to the Center for Disease Control (CDC), and subsequently, USDA-FSIS increased their surveillance for *L. monocytogenes* (Graves et al., 2005).

Various researchers have investigated the prevalence of *L. monocytogenes* in RTE meats. Wang and Muriana (1994) tested 93 randomly selected retail frankfurter samples for the presence of *L. monocytogenes*, and 7 out of 93 samples (7.4%) were positive for this pathogen. Samelis and Metaxopoulos (1999) investigated the incidence and principal sources of *Listeria* spp. and *L. monocytogenes* contamination in processed meat and processing plants. Among incoming raw materials, turkey necks were most heavily contaminated with *L. monocytogenes* (30.1%). Among equipment, the cutter and the cutting machine showed the highest prevalence of contamination (Samelis and Metaxopoulos, 1999). Soriano et al. (2001) also investigated the presence of *Listeria* species in raw and RTE foods. Among 103 samples collected from meat (pork, beef, and chicken), fish (salmon, hake, and sole), vegetables (lettuce, and spinach) and Spanish potato omelets, *L. monocytogenes* was

found only on 3 samples (raw lettuce, RTE lettuce, and raw pork products), which constitutes a 2.9% rate of detection.

Currently, RTE meat processors are required to follow CFR 9 Part 430 with a "zero-tolerance" policy for *L. monocytogenes* in their products (FSIS, 2003). In CFR 9 Part 430, any situation involving recontamination of *L. monocytogenes* after post-lethality treatments in RTE meats can be covered by one of three alternatives (FSIS, 2003). Under Alternative I, a facility controls *L. monocytogenes* by using a post lethality treatment and applying an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes* (FSIS, 2003). The use of a post lethality treatment reflects the elimination of any possible presence of the pathogen in the product which can be explained as any hazard reasonably likely to occur from a HACCP point of view (FSIS 2003). The use of an antimicrobial agent or growth inhibitor (growth suppressing/ limiting process) is an effective measure for limiting the growth of *L. monocytogenes* if present (FSIS, 2003). In alternative II, facilities can choose either a post lethality treatment or a growth inhibitor for controlling *L. monocytogenes*; and for alternative III, facilities choose to control this pathogen with sanitation measures alone (FSIS, 2003; Barmpalia et al., 2004).

Recent technologies such as irradiation are not approved for RTE meats. Instead, the addition of organic acids as antimicrobial compounds is commonly applied to inhibit the growth of *L. monocytogenes* in RTE meats (Mbandi and Shelef, 2001; Samelis et al., 2001; Zhu et al., 2005). Thus, many researchers have investigated the effectiveness of organic acids as antimicrobial agents in the formulation of RTE meat products. Uhart et al. (2004) evaluated the control of *L. monocytogenes* in beef frankfurters using pediocin (6,000 AU), 3% sodium diacetate, and 6% sodium lactate during 3 weeks storage at 4°C. The application

of each antimicrobial alone resulted in a 1-log reduction and a combination of the three antimicrobials had the greatest effect on inhibiting the growth of *L. monocytogenes,* with a 1.5-2-log reduction (Uhart et al., 2004). Schlyter et al. (1993) found that the addition of 2.5% lactate and 0.1% sodium diacetate in combination prevented the growth of *L. monocytogenes* across 42 days of storage at 4˚C in turkey slurries (Schlyter et al., 1993). Mbandi and Shelef (2001) found that the combination of 2.5% sodium lactate and 0.2% sodium diacetate greatly increased the safety of RTE meat products stored under refrigeration or temperature abuse situations.

Samelis et al. (2001) investigated the effectiveness of using aqueous dipping solutions of organic acids (2.5% or 5% lactic or acetic acid) or salts (2.5% or 5% sodium acetate or sodium diacetate, 5 or 10% sodium lactate, 5% potassium sorbate or potassium benzoate) for controlling *L. monocytogenes* on sliced pork bologna during 120 days storage time at 4°C. Dipping pork bologna slices into 5% potassium sorbate and 5% lactic acid resulted in a significant increase in shelf life from 20 days to 35 days (Samelis et al., 2001). Capita et al. (2001) showed that trisodium phosphate was effective against *L. monocytogenes* in chicken meat, especially after 7 days storage at refrigeration temperature. Glass et al. (2002) found that the addition of combinations of sodium lactate and sodium diacetate in wiener or bratwurst formulations inhibited the growth of *L. monocytogenes* if stored at less than 7°C. Geornaras et al. (2005) investigated the effectiveness of post processing antimicrobial treatments to control *L. monocytogenes* in commercial vacuum-packaged bologna and ham stored at 10˚C for 10 days. Of the 7 treatments, including 2.5% acetic acid, 2.5% lactic acid, 5% potassium benzoate, or 0.5% Nisaplin (a commercial form of nisin,

concentration of 5,000 IU/ml of nisin), and combinations of Nisaplin with each acid, 6 of the 7 treatments showed listericidal effects.

Few studies have been conducted with souse meat. Ingham et al. (2004b) validated headcheese formulation (similar to a souse meat formulation) with respect to its ability to control *L. monocytogenes* growth and survival. *L. monocytogenes* levels in artificially contaminated headcheese decreased during 31 days of storage at 5°C and after 31days, no survivors were detected. The investigates concluded that the product formulation of headcheese (avg. pH 4.3) can be considered an effective antimicrobial agent, and headcheese can fall under Alternative 2 of the USDA regulations (Ingham et al., 2004b). This is likely to be a consequence of vinegar (acetic acid), which can be regarded as a growth inhibitor or antimicrobial agent in the product formulation.

Meat processors are responsible for validating the safety of their products as part of a HACCP program by providing scientific data. However, the cost of validation is limiting for small manufacturers. Most souse processors are local, family-owned, small processing facilities. The objective of this study was to determine the effectiveness of three different souse formulations in controlling the growth of *L. monocytogenes* at two different refrigerated storage temperatures. Consumer acceptance of effective formulations was also evaluated.

Materials and Methods

Culture preparation

Three strains of *L. monocytogenes* were used (Scott A, 1/2a and 1/2b) in this study. *L. monocytogenes* Scott A was obtained from USDA-ARS (United States Department of

Agriculture- Agricultural Research Service, North Carolina State University, Raleigh, NC). Strains 1/2a and 1/2b were obtained from Dr. Sophia Kathariou (Department of Food Science, North Carolina State University, Raleigh, NC). Stock cultures were stored at -80°C in 20 % (w/v) glycerol (Sigma chemicals Co., St. Louis, MO). Strains were activated by transferring a loopful of stock culture into 9 ml of Tryptic Soy Broth (TSBYE;Weber Scientific, Santa Maria, CA) supplemented with 0.6 % yeast extract (Fisher Scientific, Fair Lawn, NJ) and incubated at 37 °C for 20 h (VWR Scientific product, Bridgeport, NJ). This process was repeated twice. Growth curves were conducted for each strain, and the stationary phase of each strain was reached after 20 h at 37 °C in TSBYE. Stationary phase cells were used as inoculum in this study.

Cocktail Preparation

Overnight (20 h) cultures of each strain (200 mL) were grown in TSBYE. Each strain was aliquoted into 8-25 ml sterile centrifuge tubes (VWR Scientific products, Bridgeport, NJ) and centrifuged at 6708-x g for 5 minutes at 20 °C (Spectrofuge Corp., Durham, NC) to pellet the cells. Twenty-five ml of 0.1% sterile peptone water (PW; Fisher Scientific, Fair Lawn, NJ) was added to four of the tubes which were vortexed for 3 min to re-suspend the cells. The re-suspended cells were poured into the remaining four tubes and vortexed for 3 min. This process was repeated for each strain. Plate counts (pour plates on TSBYE) for each strain at this point were ca. 10^{10} cfu/ml. One hundred ml of each strain were then mixed with 200 ml PW to yield a total of 500 mL of cocktail for challenge studies. The cocktail had a plate count of ca. 10^{9} cfu/ ml.

Souse preparation

Souse meat was produced by a state-inspected, commercial processing plant (Pikeville, NC). The processing plant had their own slaughtering facilities and supplied their own meats and ingredients. Storage of raw and cooked meats was physically separated to reduce the possibility of cross-contamination. The primary raw materials for souse meat for this manufacturer included pork ear, lean pork (90% lean, lean pork), and pork hearts. Non-meat ingredients included vinegar, sugar, salt, and a spice mixture containing salt, sugar, black pepper, red pepper, and sage. The detailed formulation can be found in Table 1a. The formulation and cooking time used by this manufacturer typically were not consistent because of ingredient and process variability. However, in this study, special efforts were taken to maintain a consistent time and temperature for all souse replications.

Raw meats were thawed for 4 h at room temperature prior to cooking. Souse was prepared in a custom-designed, steam-jacketed kettle. After thawing, pork ears were cooked for 1 h at 100°C to soften the cartilage. Lean pork and pork hearts were added to the cooker, and cooking continued for 2 h at 100°C. In commercial product formulation cooking, time varies because of the high buffering capacity of meat. Yet in this study, cooking time was kept consistent to reduce the variations on the product formulation throughout the experimental replications. Following cooking, water was drained 1(29 lbs) and the cooked meat mixture was transferred to the grinder for further processing. Spice mix was added and the mixture was ground for approximately 10 minutes in room temperature.

The entire batch of ground and spiced meat was divided into three equal weights by pouring into stainless steel pans (12″x 20″x 2 1/2″). The purpose of dividing into three subgroups was to apply different amounts of vinegar to create the three experimental pH treatments

evaluated in the study. Apple cider vinegar was purchased from a local grocery store (White house National fruit product Co. Inc., Winchester, VA). The control souse was treated with the same amount of vinegar as commercial product manufactured at this facility, which was 7.8% (v/v) representing a product pH of ca. 4.7. For the high pH souse (pH 5.1), 50% of the normal vinegar volume was added, which was approximately 4.07% (v/v). For the low pH souse (pH 4.3), 150% of the amount of vinegar added to the control souse was applied with 11.29% (v/v). Upon addition of vinegar to each batch, the pH was taken for verification, and vinegar volume adjusted to reach target pH. After addition of vinegar and thorough mixing, the souse batches were transferred to a chilling room (65 % RH, 4°C) overnight. The next day, the chilled souse was transported under refrigerated conditions to North Carolina State University, Department of Food Science for microbial analysis and *L. monocytogenes* challenge. This entire procedure was replicated three times. For each experimental replication, samples of souse meat were sent to a commercial laboratory for ash, moisture, fat, and protein analysis using standard AOAC methods. Moisture was analyzed by forced draft oven, fat was analyzed by Soxhlet extraction with ether, protein by the Kjeldahl method, and ash content was determined by muffle furnace combustion.

Sample Inoculation

Trays of souse meat were cut with stainless steel knives into consistent sized squares to obtain a uniform surface area (appx. 85 cm^2). Approximately 100 sample squares were produced from each replication of each pH formulation. Surface area of squares was calculated and recorded for each experimental replication. Souse meat squares were surface-contaminated (to simulate post process contamination) with *L. monocytogenes* by immersion into the cocktail solution for 2 minutes. After immersion, souse squares were placed on

elevated glass rods to expose all surfaces and dried under aseptic conditions for 1 minute at room temperature. The surface contamination procedure was selected based on preliminary experiments that suggested no differences among immersion and drying times of 1 to 5 minutes. After drying, samples were transferred to commercial vacuum package bags (Food Saver; San Francisco, CA) and vacuum-packaged (Food Saver; San Francisco, CA). Prior to this study, plate counts were conducted on bags and no colonies were found <10 cfu/cm^2. Control samples were prepared in the same manner except that sterile PW was used as the immersion solution. Both control and contaminated samples were assigned to two different storage temperatures (5°C and 10°C) for 32 days.

Microbiological analysis

Products stored at each temperature were tested after 0, 4, 7, 11, 14, 18, 21, 25, 28, and 32 days for background microflora and *L. monocytogenes*. TSAYE and Oxford media (Weber Scientific, Santa Maria, CA) with Modified Oxford antimicrobic supplement (BD Difco, Sparks, MD) (MOX) were used for the microbiological analyses. MOX agar was prepared by the aseptic addition of modified oxford antimicrobic supplement (rehydrated with 10ml sterile distilled water) to sterile oxford medium base as recommended by the manufacturer. MOX was used for selective isolation and identification of *L. monocytogenes* from processed meat and poultry products (Lee and McClain, 1989), while TSAYE was used as a nonselective medium to give an overall picture of microbiological load.

For microbiological analysis, a vacuum–sealed square was removed from storage and the bag was cut open with a sterile knife; 50ml of 0.1% PW was directly added to the vacuum bag using aseptic procedures. The bag was shaken 30 times to rinse off surface bacteria.

Preliminary experiments were conducted to compare surface washing and stomaching (data not shown). No differences were observed between the two procedures ($p>0.05$). Appropriate dilutions were made and pour or spread plating was conducted. For TSAYE, samples were pour-plated and for Oxford media, samples were spread-plated. Plating was done in triplicate for each media.

Sensory analysis

Following microbial analysis, typical pH souse (4.7) and the low pH souse formulation (pH 4.3) were selected for consumer acceptance testing. Products for consumer testing were manufactured at the same facility and transported to NCSU next day. Souse samples for sensory analysis were temporarily stored at 5°C food-grade refrigerator for three days. Sensory testing was conducted in compliance with NCSU human subjects regulations. Souse meat was cut into 2.4 x 2.2 x 4.9 cm pieces (avg. 24.7g), placed into 2oz (59ml) lidded plastic portion cups (Sweetheart Company, Bolingbrook, Il), coded with random three-digit numbers and then stored at 5°C (less than 24 h) until testing.

In order to recruit souse meat consumers, consumer testing was conducted at a local retail outlet (Raleigh, NC). Seventy-five souse consumers voluntarily participated and received a $5 store coupon for participation. Consumers first answered a brief questionnaire containing thirteen demographic questions. Consumers were then presented with the two-souse samples presented in a randomized balanced order of presentation. Consumers evaluated souse meat for overall acceptability, appearance, flavor, and texture using a 9-point hedonic scale anchored on the left with dislike extremely and on the right with like extremely. A forced choice preference question was asked after both products were evaluated.

Statistical analysis

D-values were calculated as the time in days, required for one log reduction of the *L. monocytogenes* population in each treatment. D-values were calculated by regression analysis (PROC REG procedure in SAS) (Statistical Analysis Software, Version 9.1, SAS Institute, Cary, NC). Analysis of variance of D-values was done (PROC GLM procedure in SAS) with least square means used to determine significant differences at a α-level of 0.05. Consumer data were also analyzed by analysis of variance with means separation to determine if differences existed in acceptance between treatments (SAS, v 9.1, Cary, NC).

Results

The average pH for the three different souse formulations is presented in Table 2. Table 3 shows the proximate analysis results. Both pH and composition were within the targeted or expected range. *L. monocytogenes* was not detected in control samples throughout 32 days of storage. Microbial counts on control samples by TSAYE were inconsistent and were generally less than 10 CFU/cm^2. Tables 4a-d and Figs. 2a-d show the survival of *L. monocytogenes* throughout 32 days storage at the two different refrigeration temperatures. At 5°C, *L. monocytogenes* on pH 4.3 souse plated in TSAYE showed 3-log reductions, from 5.88 to 2.26, whereas pH 4.7 and pH 5.1 formulations showed *L. monocytogenes* reductions of only 1-log. Similar patterns were observed when samples were plated on MOX. Four-log reductions were observed at pH 4.3, whereas only 2-log and 1-log reductions were observed on pH 4.7 and 5.1 formulations.

Table 4c shows the survival of *L. monocytogenes* throughout 32 days at 10°C recovered on TSAYE. The highest reduction of *L. monocytogenes* was observed (2.5-log reduction) on the low pH (pH 4.3) formulation. The normal or typical formulation (pH 4.7) showed a 1.27-log reduction of this pathogen, and a 0.92-log reduction was observed on the highest pH formulation (pH 5.1). Table 4d shows the survival of *L. monocytogenes* throughout 32 days at 10°C storage using MOX. As with recovery by TSAYE, similar patterns were observed with 3.47-log reductions on pH 4.3 product, which was the highest log reduction of *L. monocytogenes*.

Table 5 shows overall D-value comparisons of *L. monocytogenes* survival. Individual graphical comparisons of *L. monocytogenes* D-values in souse meat stored at 5°C and 10°C on TSAYE and MOX agars, respectively, are shown in Figs 3a-3d and Figs 4a-4d. D-values for *L. monocytogenes* on souse with pH 4.7 and pH 5.1 were not different at 5°C ($p>0.05$) in both TSAYE and MOX. D-values of *L. monocytogenes* were lower (more rapid destruction) in low pH products (pH 4.3) compared to higher pH products ($p<0.05$).

Consumer acceptance testing using non-inoculated samples was conducted at a local pork product store (Raleigh, NC). Souse meat with conventional pH (4.7) were chosen for consumer testing since the pH 4.3 souse was more bacteriocidal to *L. monocytogenes* and we wanted to determine if this change in formulation impacted consumer acceptance. Among those that participated, 58% were female, 42% male, > 60% were between 36 and 65 y, and the majority of them were responsible for shopping for their household (97%) (Table 7). There were no statistically significant differences in liking for the four attributes tested (appearance, flavor, texture, and overall acceptability) between the two souse meats ($p>0.05$). When asked to indicate preference, 60% of participants preferred the lower pH formulation.

Discussion

Souse meat is classified as a specialty meat product. This product is formulated to contain an organic acid (acetic acid) as an antimicrobial agent. The objective of our study was to determine the effectiveness of product pH, achieved by adding different amounts of acetic acid to in terms of controlling/inhibiting the growth of *L. monocytogenes*. For control samples which were not initially contaminated with *L. monocytogenes*, total microbial numbers were low and in most cases, the numbers were less than 10 CFU/cm^2. Since souse meat is a fully cooked RTE product, background microflora should not be present unless cross contamination or post-process contamination has occurred. In this challenge study, stationary-phase cells were chosen because comparatively speaking, these cells are most resistant (Davis et al., 1996). The intracellular pH of *L. monocytogenes* is around 8.0, and this intracellular pH is maintained by the cell in order to conduct normal physiological processes (Shabala et al., 2002). Homeostasis is the strong tendency of organisms to maintain their internal environment (Leistner, 2002). The maintenance of homeostasis requires an intact cell membrane and the expenditure of energy. Therefore, any treatments that disrupt membranes or interfere with the generation of cellular energy will hinder or abolish homeostasis and will lead to cell stress and/or death (Mackey, 2000). In this study, the low pH of souse meat (pH 4.3, 4.7 and 5.1) provides an acidic external environment, which requires *L. monocytogenes* to work and expend energy to maintain homeostasis. As a result, growth of *L. monocytogenes* on souse meat was not observed regardless of product pH or storage temperature.

Several challenge studies have been conducted testing the survival of *L. monocytogenes* on food commodities. Hwang (2005) studied the effectiveness of pH and storage temperature on the behavior of *L. monocytogenes* in ham and potato salad. Three different pHs (pH 3.8, 4.2, and 4.6) of mayonnaise were prepared, and inoculated with 8 different strains of *L. monocytogenes.* These different mayonnaise formulations were used to prepare ham and potato salad, which were stored at 4, 8, and 12°C. The pathogen grew on all three pH values with no significant differences in pathogen growth data (Hwang, 2005). In terms of storage temperature, *L. monocytogenes* was able to grow in ham salad, and the growth rate was increased as the storage temperature increased. However, this pathogen was inactivated in potato salad (Hwang, 2005). Beumer et al. (1996) investigated the ability of sliced, cooked meat products such as luncheon meat, ham and chicken breasts to support the growth of *L. monocytogenes*. *L. monocytogenes* grew on artificially contaminated meat samples, regardless of storage temperature (7 °C and 30 °C), packaging type (vacuum packaging, modified atmosphere (30% CO_2/ 70% N_2), or the presence of competitive microflora (mostly lactic acid bacteria). McKellar et al. (1994) also tested Canadian retail wieners produced in six representative processing plants for survival of *L. monocytogenes*. Among 61 samples tested, 40 samples (65.6%) supported the growth of this pathogen when stored under vacuum at 5°C up to 29 days (McKellar et al., 1994).

In the present study, growth of *L. monocytogenes* was inhibited in souse meat regardless of product pH and the storage temperature across 32 days storage. One of the reasons for the lack of growth can be assumed to be the low product pH, which makes cells focus on maintaining homeostasis instead of using energy for replication. In addition, control of this organism was favored for products formulated at pH $\leq$4.3 (Table 5). This pH

formulation represents a product pH below what is typically used for commercial product. Decreasing product pH resulted in faster inactivation of the organism. When the pH in a food is reduced below the lower limit for cell growth, *L. monocytogenes* lose viability as well as stop growing (Ray, 2004). The inactivation of *L. monocytogenes* can be explained by the pKa of the acetic acid used in the souse. Normally, weak acids that have higher dissociation constants (pK) showed more effectiveness toward pathogen inhibition (Ray, 2004). Undissociated organic acids are lipophilic, so they can enter into the cell. Once the undissociated acid enters the cell, it dissociates to generate H+ in the cytoplasm (Ray, 2004). This mechanism causes a reduction in internal pH, which eventually destroys the proton gradient across the cell membrane and dissipates the proton motive force and the ability of the cell to generate energy (Ray, 2004).

In this study, acetic acid was added to the formulation as natural ingredient, which can play a role as an acidulant or antimicrobial for controlling pathogens. Acetic acid is regarded as a weak acid with a pK value of 4.8. The target pH for this study was 4.3, 4.7, and 5.1. Inhibition of *L. monocytogenes* was highest on the low pH formulation (pH 4.3), and this can be explained by the amount of undissociated acid present. At pH 4.3, the pH is less than the pKa of acetic acid (4.8), and a larger proportion of the acid is undissociated compared to higher pH values. According to the International Commission on Microbiological Specifications for Foods (1980), at pH 4, acetic acid is 84.5% in the undissociated form, and at pH 5 only, 34.9% is undissociated (ICMSF, 1980).

Our results are in agreement with previous studies. Scott et al. (2005) concluded that *L. monocytogenes* could not grow at pH < 4.39 when other factors were optimum. Buncic et al. (1999) challenged on smoked and dried sausage produced no starter culture with *L.*

monocytogenes for 35 days (Buncic et al., 1999). In this case, both the pH of sausage (4.8) and the smoking and drying process inhibited the growth of *L. monocytogenes* (Buncic et al., 1999). Ingham et al. (2004a) also reported that summer sausages at pH 4.7-4.9 were able to completely inhibit the growth of *L. monocytogenes* when held at either 5 or 21°C for 11 weeks (Ingham et al., 2004a).

There were no significant differences in survival of *L. monocytogenes* when comparing the two refrigerated storage temperatures (5°C and 10°C) ($p>0.05$). Several studies have shown survival of *L. monocytogenes* at low temperature and even below freezing (0°C). *L. monocytogenes* can survive for several weeks or months in many chilled and frozen foods, however viable cell numbers decrease over time (Lou and Youself, 1999). *L. monocytogenes* was found to grow in vacuum-packed sliced roast beef even below 0°C (Hudson et al., 1994). However the growth at low temperature (-1.5°C) was slow with a lag time of 174 days and a generation time of 100 h (Hudson et al., 1994). In our study, the storage time was 32 days, which represents a normal shelf life for souse, and the results showed no support for growth of *L. monocytogenes* under the conditions employed in this study.

Palumbo and Williams (1994) investigated control of *L. monocytogenes* on the surface of frankfurters by acid treatments. No difference in inhibition of *L. monocytogenes* was observed between acid treated and non-acid treated samples when stored at 12°C. They concluded that inhibition of *L. monocytogenes* by acid treatments was lost when the storage temperature was abused. In our study, storage temperature at 5 versus 10 °C did not significantly affect inhibition of *L. monocytogenes* ($p>0.05$), although D values for the highest pH/temperature combination (pH 5.1, 10 °C) were higher than for other pH/temperature combinations as evaluated by TSAYE (Table 5).

Among challenge studies conducted with *L. monocytogenes* on RTE meats, limited publications have conducted sensory analysis. Bampalia et al. (2004) did sensory testing with 25 untrained panelists on pork frankfurters with or without antimicrobials added for the formulation and with or without dipping in a 2.5% solution of acetic acid or lactic acid. Negative sensory effects on the flavor acceptability were not detected (Bampalia et al., 2004). With respect for our study, consumer testing by the consumer intercept method was performed with 75 souse-consuming volunteers. No differences in acceptance of souse meat formulations pH were detected in flavor, texture, appearance, or overall acceptance.

Conclusions

Conventionally produced souse meat does not support the growth of *L. monocytogenes* regardless of product formulation. The inactivation of this organism was favored in products formulated at lower pH. The acceptability of souse meat was not impacted by decreased pH. Decreasing the pH of souse meat during formulation may be beneficial to the souse producer to further ensure safety without loss of sensory quality.

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Tables and Figures

Table 1a. Souse Formula for initial batch

	Souse Formula	
Ingredients	volume (lbs)	volume (%)
Ear	25	11.0
Skin	40	17.5
Heart	10	4.4
Water	153	67.1
Total	228*	100

* 129 lbs of water was taken out after cooking of initial batch

Table 1b. Souse formula after division

	Meat	Vinegar	Total
pH 4.3	33	4.2 (11.3*)	37.2
pH 4.7	33	2.8 (7.8*)	35.9
pH 5.1	33	1.4 (4.1*)	34.4

(*) indicates the percentage by vol./vol.

Table 2. Average pH values of experimental replications

Target pH	4.3	4.7	5.1
Average pH	4.41	4.8	5.14
STDEV	0.06	0.07	0.02

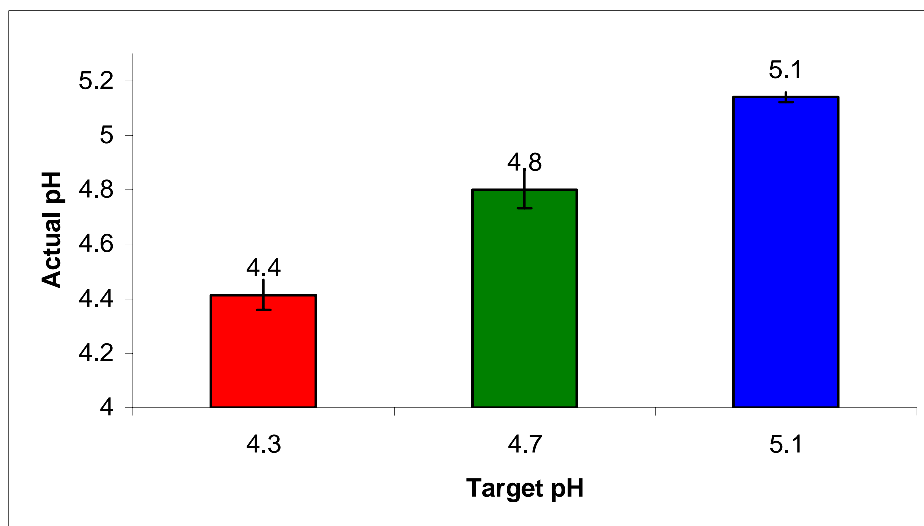


Figure 1. Average pH values throughout the replications. The data are means. Error bars indicate standard deviations for experiments performed in triplicate.

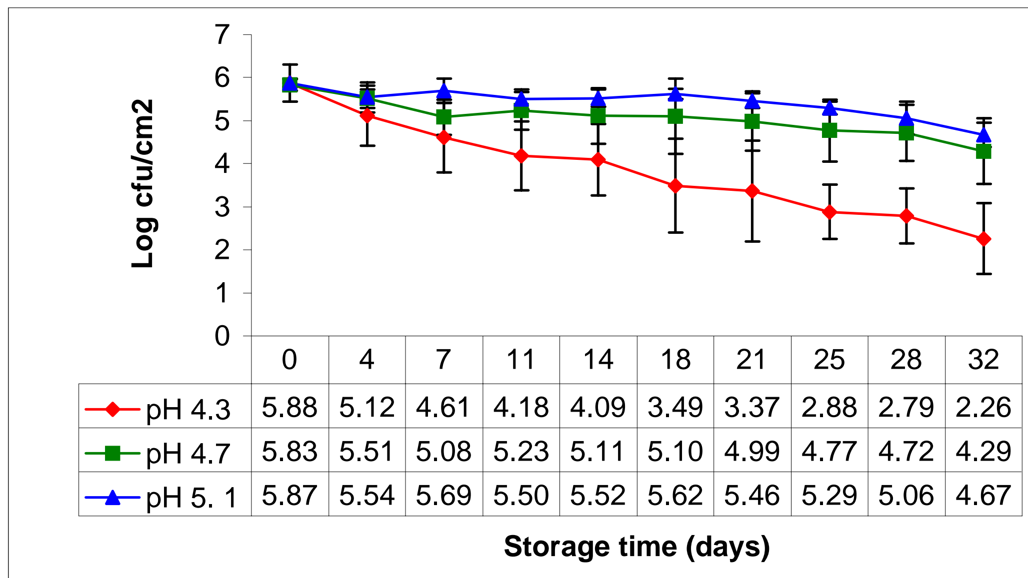
Table 3. Proximate Analysis of Different pH Souse Meat Formulations

	pH 4.3	pH 4.7	pH 5.1
Moisture	74.1^{a} (1.89)	72.1^{a} (2.18)	72.7^{a} (2.71)
Fat	9.9^{a} (3.57)	8^{a} (2.29)	9.3^{a} (2.85)
Ash	1.8^{a}(0.16)	1.8^{a} (0.17)	1.7^{a} (0.36)
Protein	13.1^{a} (1.99)	15.7^{a} (1.72)	14.6^{a} (1.23)

Numbers in parentheses are the standard deviations.

Table 4a. Survival of *L. monocytogenes* throughout 32days at 5°C storage temperature using TSAYE

	Average CFU/cm2			Average (Log CFU/cm2)			Log (standard deviation)		
Time	**pH 4.3**	**PH 4.7**	**pH 5.1**	**pH 4.3**	**pH 4.7**	**pH 5. 1**	**pH 4.3**	**pH 4.7**	**pH 5.1**
0	7.67E+05	7.00E+05	1.11E+06	5.88	5.83	5.87	0.09	0.13	0.43
4	2.66E+05	3.58E+05	4.52E+05	5.12	5.51	5.54	0.70	0.22	0.35
7	9.59E+04	1.56E+05	5.93E+05	4.61	5.08	5.69	0.81	0.41	0.28
11	5.16E+04	2.22E+05	3.57E+05	4.18	5.23	5.50	0.80	0.44	0.23
14	3.24E+04	2.32E+05	3.60E+05	4.09	5.11	5.52	0.83	0.64	0.20
18	2.50E+04	3.02E+05	4.27E+05	3.49	5.10	5.62	1.09	0.88	0.12
21	2.57E+04	2.07E+05	3.05E+05	3.37	4.99	5.46	1.17	0.69	0.17
25	1.82E+03	1.26E+05	2.07E+05	2.88	4.77	5.29	0.63	0.72	0.15
28	1.43E+03	1.03E+05	1.53E+05	2.79	4.72	5.06	0.64	0.65	0.38
32	7.14E+02	3.89E+04	5.57E+04	2.26	4.29	4.67	0.82	0.76	0.28

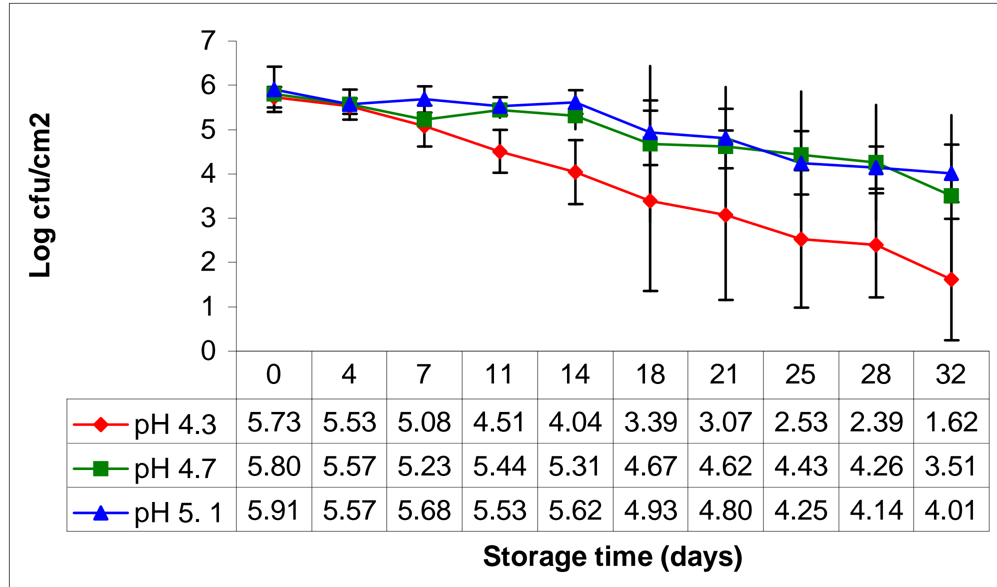


	0	4	7	11	14	18	21	25	28	32
pH 4.3	5.88	5.12	4.61	4.18	4.09	3.49	3.37	2.88	2.79	2.26
pH 4.7	5.83	5.51	5.08	5.23	5.11	5.10	4.99	4.77	4.72	4.29
pH 5. 1	5.87	5.54	5.69	5.50	5.52	5.62	5.46	5.29	5.06	4.67

Figure 2a. Survival of *L. monocytogenes* throughout 32 days at 5°C storage temperature using TSAYE. The data are means. Error bars indicate standard deviations for experiments performed in triplicate

Table 4b . Survival of *L. monocytogenes* throughout 32 days at 5°C storage temperature using MOX

	Average (CFU/cm2)			Average (Log CFU/cm2)			Log (standard deviation)		
Time	**pH 4.3**	**pH 4.7**	**pH 5.1**	**pH 4.3**	**pH 4.7**	**pH 5. 1**	**pH 4.3**	**pH 4.7**	**pH 5.1**
0	5.97E+05	6.67E+05	1.56E+06	5.73	5.80	5.91	0.23	0.17	0.51
4	3.60E+05	4.34E+05	4.75E+05	5.53	5.57	5.57	0.17	0.25	0.34
7	1.67E+05	1.96E+05	5.86E+05	5.08	5.23	5.68	0.46	0.24	0.29
11	4.77E+04	2.90E+05	3.73E+05	4.51	5.44	5.53	0.48	0.17	0.20
14	2.54E+04	2.57E+05	4.77E+05	4.04	5.31	5.62	0.72	0.30	0.27
18	5.08E+04	2.68E+05	1.72E+05	3.39	4.67	4.93	2.04	1.76	0.73
21	4.52E+04	1.67E+05	1.18E+05	3.07	4.62	4.80	1.91	1.34	0.67
25	5.50E+03	1.16E+05	5.37E+04	2.53	4.43	4.25	1.55	1.43	0.71
28	1.07E+03	7.02E+04	2.34E+04	2.39	4.26	4.14	1.18	1.30	0.48
32	2.79E+02	2.16E+04	2.12E+04	1.62	3.51	4.01	1.37	1.82	0.65

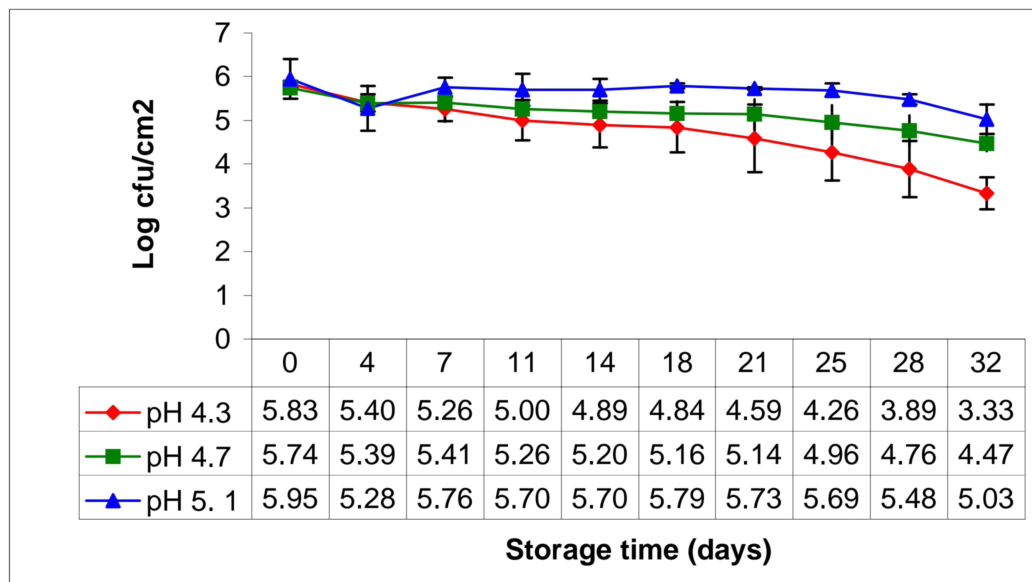


	0	4	7	11	14	18	21	25	28	32
pH 4.3	5.73	5.53	5.08	4.51	4.04	3.39	3.07	2.53	2.39	1.62
pH 4.7	5.80	5.57	5.23	5.44	5.31	4.67	4.62	4.43	4.26	3.51
pH 5. 1	5.91	5.57	5.68	5.53	5.62	4.93	4.80	4.25	4.14	4.01

Figure 2b. Survival of *L. monocytogenes* throughout 32 days at 5°C storage temperature using MOX. The data are means. Error bars indicate standard deviations for experiments performed in triplicate.

Table 4c. Survival of *L. monocytogenes* throughout 32days at 10°C storage temperature using TSAYE

	Average CFU/cm2			Average (Log CFU/cm2)			Log (standard deviation)		
Time	**pH 4.3**	**pH 4.7**	**pH 5.1**	**pH 4.3**	**pH 4.7**	**pH 5. 1**	**pH 4.3**	**pH 4.7**	**pH 5.1**
0	6.79E+05	6.28E+05	1.29E+06	5.83	5.74	5.95	0.08	0.25	0.45
4	2.73E+05	2.56E+05	2.62E+05	5.40	5.39	5.28	0.20	0.15	0.51
7	2.10E+05	3.54E+05	6.29E+05	5.26	5.41	5.76	0.28	0.44	0.21
11	1.43E+05	2.18E+05	6.09E+05	5.00	5.26	5.70	0.46	0.29	0.37
14	1.22E+05	1.74E+05	5.64E+05	4.89	5.20	5.70	0.51	0.22	0.25
18	1.16E+05	1.68E+05	6.28E+05	4.84	5.16	5.79	0.58	0.27	0.06
21	8.55E+04	1.72E+05	5.34E+05	4.59	5.14	5.73	0.77	0.34	0.03
25	3.22E+04	1.19E+05	5.18E+05	4.26	4.96	5.69	0.63	0.39	0.15
28	1.91E+04	7.58E+04	3.12E+05	3.89	4.76	5.48	0.64	0.35	0.11
32	2.87E+03	3.16E+04	1.39E+05	3.33	4.47	5.03	0.36	0.18	0.34

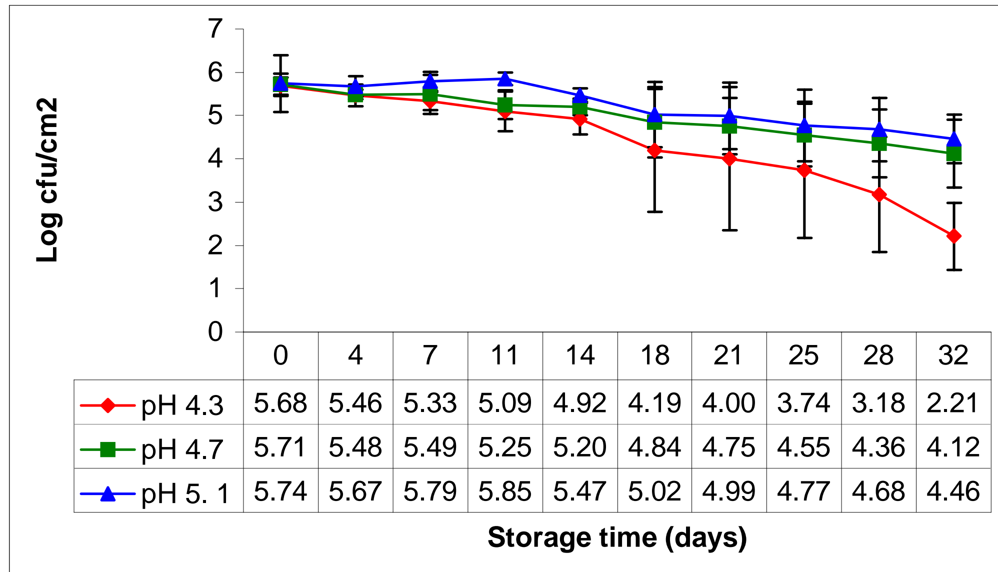


	0	4	7	11	14	18	21	25	28	32
pH 4.3	5.83	5.40	5.26	5.00	4.89	4.84	4.59	4.26	3.89	3.33
pH 4.7	5.74	5.39	5.41	5.26	5.20	5.16	5.14	4.96	4.76	4.47
pH 5. 1	5.95	5.28	5.76	5.70	5.70	5.79	5.73	5.69	5.48	5.03

Figure 2c. Survival of *L. monocytogenes* throughout 32days at 10°C storage temperature using TSAYE. The data are means. Error bars indicate standard deviations for experiments performed in triplicate.

Table 4d. Survival of *L. monocytogenes* throughout 32days at 10°C storage temperature using MOX

	Average CFU/cm2			Average Log (CFU/cm2)			Log (standard deviation)		
Time	**pH 4.3**	**pH 4.7**	**pH 5.1**	**pH 4.3**	**pH 4.7**	**pH 5. 1**	**pH 4.3**	**pH 4.7**	**pH 5.1**
0	5.16E+05	5.84E+05	1.45E+06	5.68	5.71	5.74	0.20	0.26	0.66
4	3.27E+05	3.10E+05	5.47E+05	5.46	5.48	5.67	0.25	0.11	0.24
7	2.37E+05	4.32E+05	6.77E+05	5.33	5.49	5.79	0.21	0.45	0.22
11	1.74E+05	2.23E+05	7.51E+05	5.09	5.25	5.85	0.46	0.33	0.15
14	1.04E+05	1.71E+05	3.08E+05	4.92	5.20	5.47	0.36	0.19	0.15
18	8.52E+04	1.44E+05	2.18E+05	4.19	4.84	5.02	1.42	0.81	0.75
21	8.00E+04	1.01E+05	2.09E+05	4.00	4.75	4.99	1.65	0.65	0.77
25	3.73E+04	7.76E+04	1.63E+05	3.74	4.55	4.77	1.57	0.72	0.83
28	1.39E+04	5.42E+04	9.58E+04	3.18	4.36	4.68	1.33	0.78	0.73
32	3.60E+02	2.51E+04	4.60E+04	2.21	4.12	4.46	0.78	0.78	0.56



	0	4	7	11	14	18	21	25	28	32
pH 4.3	5.68	5.46	5.33	5.09	4.92	4.19	4.00	3.74	3.18	2.21
pH 4.7	5.71	5.48	5.49	5.25	5.20	4.84	4.75	4.55	4.36	4.12
pH 5. 1	5.74	5.67	5.79	5.85	5.47	5.02	4.99	4.77	4.68	4.46

Figure 2d. Survival of *L. monocytogenes* throughout 32days at 10°C storage temperature using MOX. The data are means. Error bars indicate standard deviations for experiments performed in triplicate.

Table 5. Overall D-Values for *L. monocytogenes* survival on souse meat at different pH formulations and different cold storage temperatures across 32 days.

	5C		10C	
	TSAYE	MOX	TSAYE	MOX
pH	Average	Average	Average	Average
pH 4.3	9.9[c]	8.6[b]	15.5[c]	11.5[b]
pH 4.7	40.5[b]	27.2[a]	34.2[b]	26.8[a]
pH 5.1	39.0[b]	21.5[a]	91.4[a]	36.4[a]

Means within each media type followed by different letters are different ($p<0.05$)

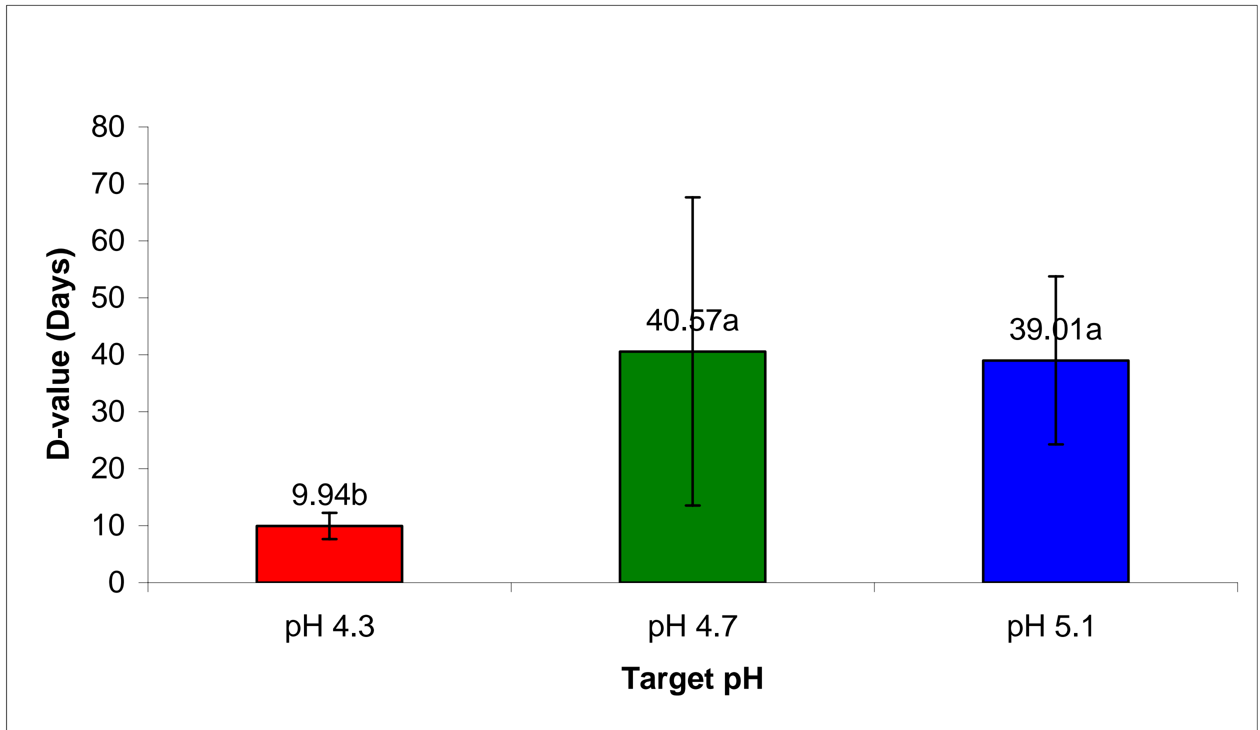


Figure 3a. D-value comparison based on cell recoveries using TSAYE, 5°C storage temperature. The data are means. Error bars indicate standard deviations for experiments performed in triplicate. Letters above bars indicate significant differences ($p<0.05$).

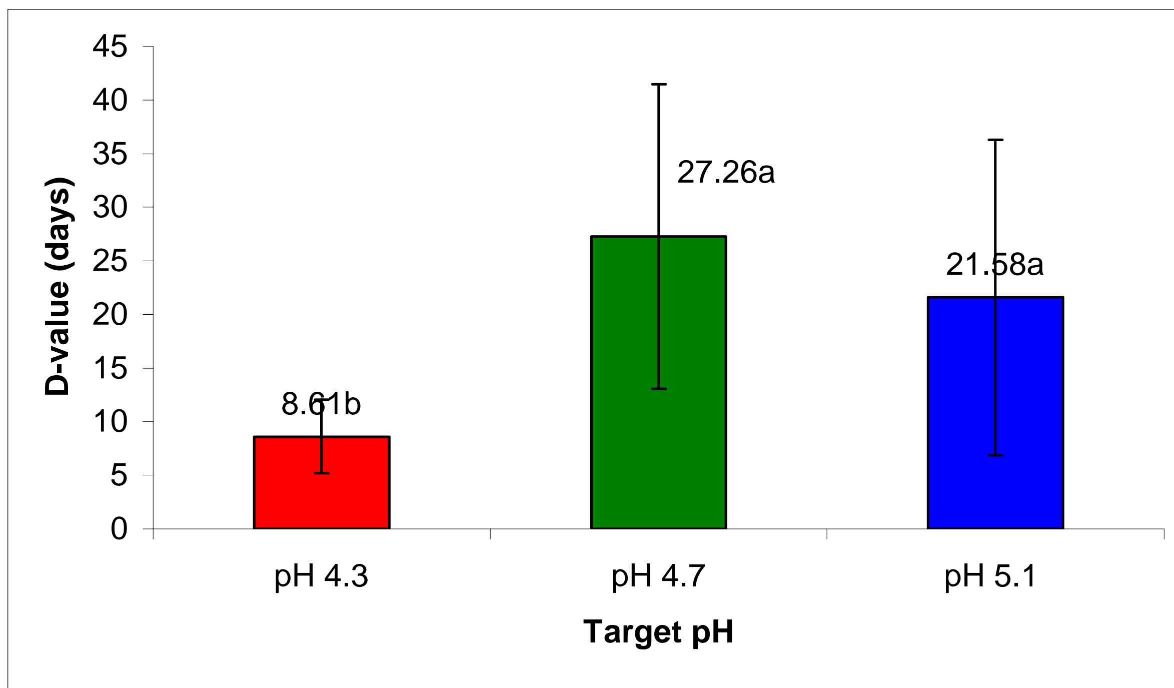


Figure 3b. D-value comparison based on cell recoveries using MOX, 5°C storage temperature. The data are means. Error bars indicate standard deviations for experiments performed in triplicate. Letters above bars indicate significant differences ($p<0.05$).

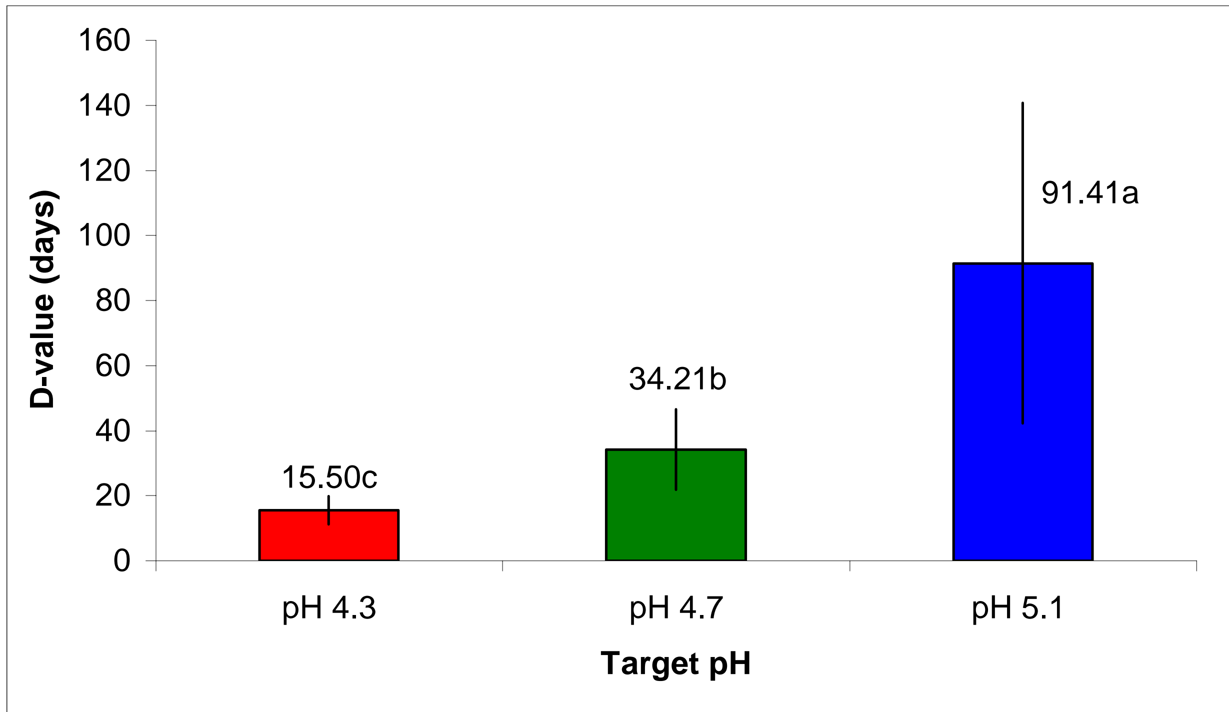


Figure 3c. D-value comparison based on cell recoveries using TSAYE, 10°C storage temperature. The data are means. Error bars indicate standard deviations for experiments performed in triplicate. Letters above bars indicate significant differences ($p<0.05$).

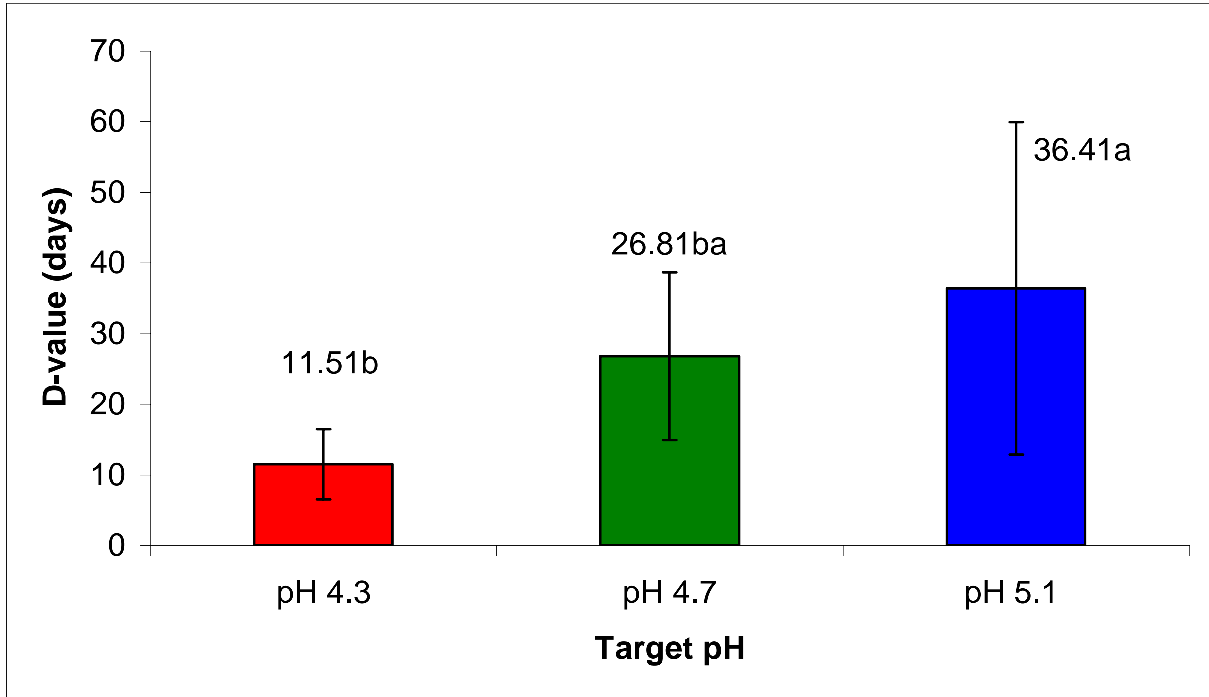


Figure 3d. D-value comparison based on cell recoveries using MOX, 10°C storage temperature. The data are means. Error bars indicate standard deviations for experiments performed in triplicate. Letters above bars indicate significant differences ($p<0.05$).

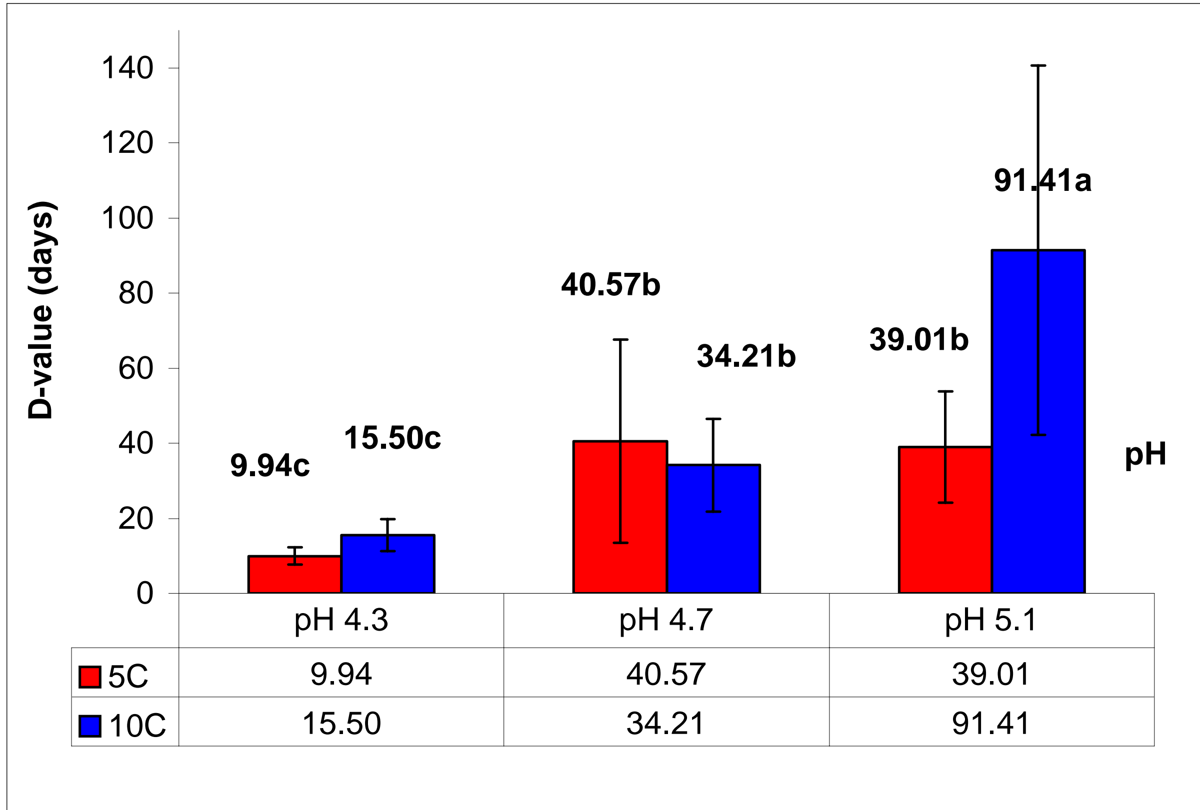


Figure 4a. Comprehensive D-value comparison based on cell recoveries using TSAYE. The data are means. Error bars indicate standard deviations for experiments performed in triplicates. Means followed by different letters are different ($p<0.05$). Letters above bars indicate significant differences ($p<0.05$).

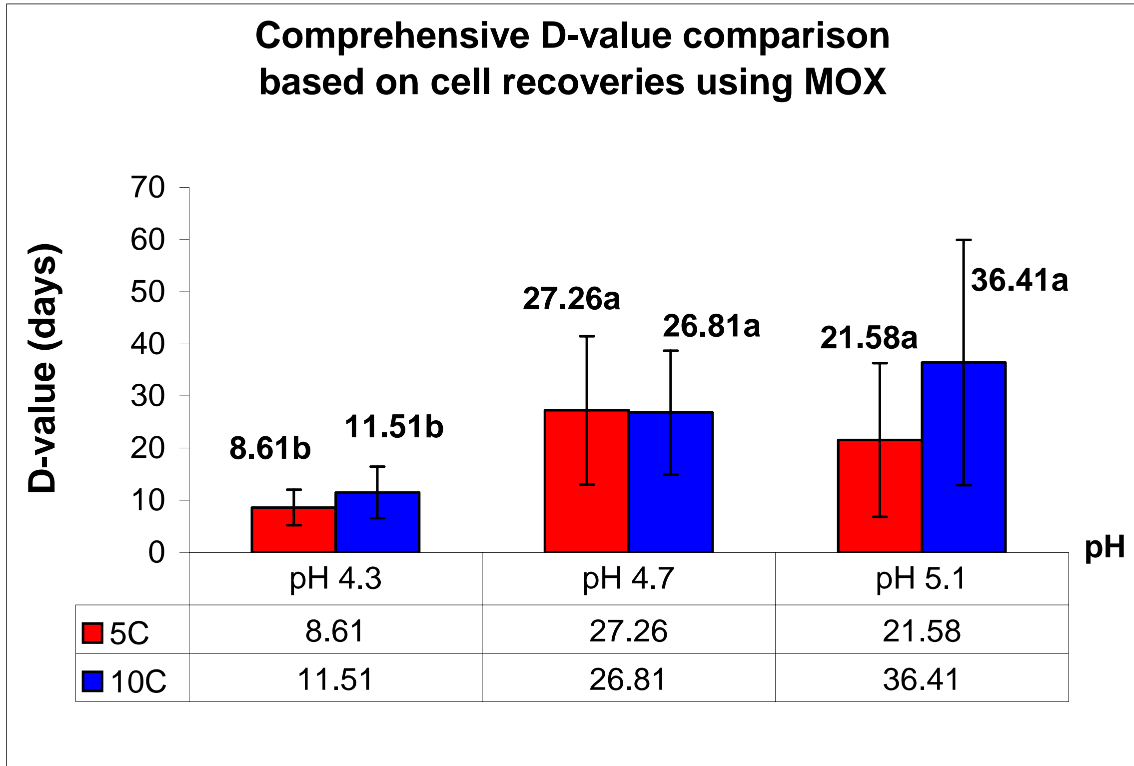


Figure 4b Comprehensive D-value comparison based on cell recoveries using MOX

The data are means.
Error bars indicate standard deviations for experiments performed in triplicate.
Letters above bars indicate significant differences ($p<0.05$).

Table 6a. Percent injured cells comparing TSAYE and MOX at 5°C

5°C	pH 4.3	pH 4.7	pH 5.1
TSAYE	3.87	5.06	5.45
MOX	3.8	4.89	5.1
Injured Cell	0.07	0.18	0.35
% Injury	**2.78**	**3.88**	**6.2**

Table 6b. Percent injured cells comparing TSAYE and MOX at 10°C

10C	pH 4.3	pH 4.7	pH 5.1
TSAYE	4.73	5.15	5.6
MOX	4.39	4.98	5.26
Injured Cell	0.34	0.17	0.35
% Injury	7.76	3.27	6.13

Table 7. Results from Consumer Acceptance Testing

Demographic Information	
	Results (n=74)
Gender (% males/ females)	38/ 58% (not answer 4%)
Age group (%)	< 18yrs 0% 19-25 yrs 5.4% 26-35 yrs 8.1% 36-45 yrs 17.6% 46-55 yrs 32.4% 56-65 yrs 17.6% >65 yrs 18.9%
Shop for household (% yes/no)	97 / 3 %
Meat consumption	Never 0% Less than once a month 1.4% 2-4 times a month 12.2% More than once a week 36.5% More than once a day 50%
Factors influencing the choice of pork products [*]	Price 67.6% Flavor 66.2% Health/Nutrition 39.2% Brand 27.0% Texture 25.7% Availability 24.3% Processing method 21.6% Produced without additives, colors and flavor 18.9% Country or Region of origin 16.2% Packaging 16.2%
Pork Products Consumption [*]	Bacon 85.1% Boneless pork loin 58.1% Breakfast sausage 75.7% Dry-cured (country) ham 59.5% Ham (deli) 54.1% Ham (smoked) 48.6% Pork chops 81.1% Pork ribs (bone-in) 64.9% Pork tenderloin 66.2% Pork sausage 82.4% Specialty products 29.7%

Table 7. Continued

<table>
<tr><td>Pork By-products Consumption *</td><td colspan="2">Back fat (fresh) 47.3%
Chitterlings 37.8%
Ears 12.2%
Feet (fresh) 39.2%
Feet (pickled) 17.6%
Heart 4.1%
Kidney 4.1%
Liver 37.8%
Stomach 6.8%
Tail 13.5%
Tongue 8.1%
Other 10.8%</td></tr>
<tr><td>Specialty Pork Products Consumption *</td><td colspan="2">Head Cheese 18.9%
Souse 59.5%
Loaves 10.8%
Liver Pudding 50.0%
Scrapple 10.8%
Jellied Products 1.4%</td></tr>
<tr><td>Frequency of Purchasing Specialty Pork Products and/or Pork By-products</td><td colspan="2">Never 12.2%
Less than once a month 25.7%
2-4 times a month 37.8%
More than once a week 21.6%
More than once a day 1.4%</td></tr>
<tr><td>Frequency of purchasing Souse Meat</td><td colspan="2">Never 29.7%
Less than once a month 44.6%
2-4 times a month 18.9%
More than once a week 4.1%
More than once a day 1.4%</td></tr>
<tr><td colspan="3">Consumer Acceptance Testing **</td></tr>
<tr><td>Attributes</td><td>Sample 1 (pH 4.3)</td><td>Sample 2 (pH 4.7)</td></tr>
<tr><td>Appearance</td><td>6.7[a]</td><td>6.6[a]</td></tr>
<tr><td>Flavor</td><td>6.6[a]</td><td>6.3[a]</td></tr>
<tr><td>Texture</td><td>6.4[a]</td><td>6.4[a]</td></tr>
<tr><td>Overall Acceptability</td><td>6.6[a]</td><td>6.4[a]</td></tr>
<tr><td colspan="3">Consumer preference Testing</td></tr>
<tr><td>Consumer preference</td><td>59%</td><td>41%</td></tr>
</table>

*Consumers were allowed to choose more than one category, so category percentages do not add up to 100
** Consumer acceptability or perceived intensity scored on a 9-point hedonic scale, where 1= dislike extremely, and 9= like extremely
Means in a row followed by different letters are different ($p<0.05$)