Final Report (June 2005 to October 2006)
Jerky Validation for Small and Very Small Meat and Poultry Businesses

EXECUTIVE SUMMARY

Thermal Process for Jerky Provides Proper Lethality for Controlling Pathogens

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USDA/FSIS issued a Compliance Guideline that provides jerky processing parameters for controlling pathogens. The Guideline was aimed at providing additional processing information for small and very small processors of jerky. The objectives of this study were: Phase 1) to develop a survey tool and collect information relative to manufacturing equipment, processing techniques, ingredients and end item characteristics typical of jerky products produced by small and very small manufacturers primarily in Kansas, Nebraska, Missouri, and Wisconsin and Phase 2) to determine the effects of typical thermal processing temperatures and times on reducing *E. coli* O157:H7 and *Salmonella* in chopped and formed beef jerky.

Phase 1: Development of a four-page survey tool was a coordinated effort between Kansas State University and the University of Wisconsin, with input from the University of Nebraska. It contained questions directed at detailing equipment, ingredients and processing techniques used in jerky manufacture to gain very specific information providing up to 168 data points.

Of the 78 plants contacted, 37 contributed surveys for a 47% response rate. Of these, 33 plants contributed 61 product samples. Analytical results of product samples revealed a mean a_w of 0.74 (SD 0.10); mean NaCl of 6.85% (SD 2.5%); and mean pH of 5.85 (SD 0.33). Some key results garnered from the survey include thermal processing extremes and nearly a complete lack of relative humidity instrumentation. These ranged from processing jerky at 51.6°C for 45 min followed by 3°C increases every 45 minutes to reach an oven temperature of 62.8C maximum, to processing jerky at 93.3°C for 6-7 h. Perhaps the single most important finding is the depth of variability within this industry. The survey tool provided the means to quantify some of the extremes in this variability and in that way will serve as a pivotal source of information for developing challenge studies.

Phase 2: A thermal process was validated to determine its ability to control pathogens during the production of chopped and formed all-beef jerky. A mixture of *E. coli* O157:H7 or *Salmonella* spp. was added to raw batter (ca. 7.0 $\log_{10}$ CFU/g). Jerky strips were placed on screens and put into a smokehouse. Jerky strips were sampled throughout processing and reductions of *E. coli* O157:H7 or *Salmonella* populations were determined. Greater than or equal to 5.0 $\log_{10}$ CFU/g reductions were seen for both pathogenic populations after 45 min of processing at dry bulb temperatures of 55.6°C and relative humidity (R.H.) at <10%, followed by 45 min with dry bulb temperatures at 77.8°C and R.H. at <10%. However, product was dried further at 77.8°C for an additional 3.25 h to achieve moisture-to-protein ratios of approximately 0.75:1 and water activity levels of $\leq$ 0.70 characteristic of shelf stable jerky. At the end of the thermal process, approximately 7.2 $\log_{10}$ CFU/g reductions of both *E. coli* O157:H7 and *Salmonella* spp. were observed, thus meeting USDA/FSIS validation guidelines for being

deemed safe and shelf stable. A reviewed short paper of this study is available on the web at at www.asi.ksu.edu/cattlemensday. This

APPLICATION TO SMALL PROCESSORS

The information from the survey indicated that small processors of jerky have varied processing parameters/methods and need to have more control of their processes. It also determined that there are limited means to incorporate humidity into their smokehouses. In addition, although processors stated various product parameters (salt, pH and water activity levels) these levels were inconsistent with our testing results. The results indicate a need to education small processors about jerky processing parameters and the need to have routine analysis of product completed.

From the preliminary studies it was determined that even at higher water activity levels for kippered beef effective log reductions were achieved for controlling *E. coli* O157:H7 and *Salmonella* spp. More research is needed to further substantiate the data. In the validation study, it was also determined that at low (<10%) relative humidity levels greater than 5 log reductions of *E. coli* O157:H7 populations and greater than 6.5 log reductions of *Salmonella* spp. were observed when chopped and formed jerky was dried to <0.70 water activity level and a 0.75:1 moisture-to-protein ratio was achieved. This is helpful information to small processors that may not be able to achieve high relative humidity levels in their smokehouses.

Although we tested a less expensive water activity meter (Pawkit, Decagon, Pullman, WA) that was approximately $400, we did not get consistent results compared to a standard water activity meter (Model CX2, Aqualab, Pullman, WA). Decagon is coming out with a new hand held water activity meter and has recognized that differences have been observed. This new model may be more adequate in analyzing water activity levels and should be tested. Some suggestions would be to have small processors send samples to universities labs for testing periodically to determine that low water activity levels and 0:75:1 moisture-to-protein ratios are being met for their jerky products.

OTHER ACTIVITIES

The Survey Phase of the study will be developed into a manuscript and submitted to either Meat Science or Journal of Muscle Foods.

The M.S. thesis chapter on the Validation Phase of M.N. Roberts thesis will be developed into a manuscript and submitted to the Journal of Food Protection. In addition, funds have been secured to conduct additional validation research on chopped and formed jerky.

STATUS OF ANY NEW PUBLICATIONS OR TRAINING MATERIALS BEING DELIVERED OR FINALIZED

1. A poster presentation was given at 51st Intl. Cong. Meat Sci. Tech. in Baltimore, MD in August of 2005. The reference and text and poster presentation follows.

2. In addition, the M.S. graduate (Lonneker) conducting the survey phase also completed a M.S. report focused on processing parameters for controlling *Salmonella* species and *Escherichia coli* O157:H7 in jerky products. The report has been completed and an abstract is provided. Principle investigators Boyle and Getty served as committee members with Dr. Melvin Hunt serving as the major advisor.

3. The preliminary studies and the validation part of the jerky project are the main focus of a M.S. thesis by Michelle N. Roberts. She will be defending her thesis in December 2006. The chapter on the preliminary studies is included.

4. The validation study of the project was reported in a progress report form at the Annual Food Safety Consortium Meeting in Fayetteville, AK in October 2006. The reference and text follows.

5. The validation study is also being reported in a Cattleman's Day Report published by Kansas State University in March 2007. A submitted report is included.

6. The validation study was also the main chapter in Michelle N. Roberts' M.S. thesis. Both a copy of the thesis abstract and the chapter on validation is included.

7. References for Michelle N. Roberts' M.S. thesis is included to support the Chapter on Preliminary Studies and the Chapter on Validation Study.

1. Lonnecker, S.M., Boyle, E.A.E., Getty, K.J.K., Buege, D.R., Ingham, S.C., and Searls, G.A. 20005. Determination of baseline product characteristics and production methods in small and very small jerky plants. 51st Intl. Cong. Meat Sci. Tech. Baltimore, MD (see paper below). The poster copy is also attached.

DETERMINATION OF BASELINE PRODUCT CHARACTERISTICS AND PRODUCTION METHODS IN SMALL AND VERY SMALL JERKY PLANTS

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Key Words: Jerky, water activity, salt concentration, jerky production

Introduction

Jerky products have long been a popular meat based food in the United States. For much of its history, these products have enjoyed production with minimal supervision or intervention by regulatory entities. However, in the light of a jerky related Salmonellosis outbreak during October 2003, new scrutiny has been turned to jerky production nationwide. The USDA FSIS (2004) response to this outbreak was the issuance of the "Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants," which provide thermal processing guidelines, humidity requirements and end product recommendations that may be applied to any jerky production scenario. The USDA did not stop with simply issuing these new guidelines. In Spring 2004, USDA solicited proposals to conduct research focused on providing small and very small manufacturing plants a means for validating their HACCP plans, specifically by achieving a 6.5 log reduction in pathogenic microorganisms. In order to develop validation studies, there was a need to determine the current state of the jerky industry in these facilities.

Objectives

The objective was to develop a survey tool and collect information relative to manufacturing equipment, processing techniques, ingredients and end item characteristics typical of jerky products. The targeted segment of the jerky industry were small and very small manufacturers primarily in Kansas, Nebraska, Missouri, and Wisconsin. This information will be used as a baseline for the design of lethality challenge studies in whole muscle and ground and formed jerky.

Methodology

Development of a four page survey tool was a coordinated effort between Kansas State University and the University of Wisconsin, with input from the University of Nebraska. It contained questions directed at detailing equipment, ingredients and processing techniques used in jerky manufacture to gain very specific information providing up to 168 data points. Surveys were mailed to 78 small and very small manufacturers of jerky that were identified through state meat processing associations, state and federally inspected plant lists and internet resources such as state or county health department listings. The packet mailed to processors included a cover letter, the four page survey, and a request to provide four samples of each jerky product they manufactured for complimentary NaCl, pH and water activity (a_w) analysis. Approximately one month after surveys were mailed, a follow-up telephone call was made to processors who had not

yet returned the completed survey. In some cases, another copy of the survey was sent to processors if the first copy had been misplaced.

Survey data were compiled and means generated for quantitative data. Qualitative data were compared to determine if consensus existed among industry practices. Jerky samples that were returned with surveys were stored in original packaging at ambient temperature for up to two weeks before analysis. All lab analyses were done by KSU or UW, with one exception. NaCl analyses for jerky samples received by UW were done at KSU or a commercial lab. All samples were pulverized in a blender (Waring Blender 700 Model 33BL79, Waring Products Division, New Hartford, CT) with a 10 cm blending cup by two 30 sec bursts, pausing for 120 sec between bursts to help minimize heat buildup. Water activity was measured at ambient temperature using an AquaLab Series 3 TE (Decagon Devices, Pullman, WA), using standard seven ml disposable sample cups. Measurement of pH was based on a 10 g sample of pulverized sample material, blended with 110 ml distilled, deionized water for two min in a stomacher (Seward Stomacher 400 laboratory blender, Seward Medical, London, UK) and tested immediately with a pH meter (Accumet Portable model AP61 Fisher Scientific, Fair Lawn, NJ). NaCl content was determined using Quantab® titrators for chloride (0.05-1.0% NaCl, Hach Company, Loveland, CO), using five g pulverized sample material combined with 45 ml distilled, deionized water according to manufacturers instructions. Resulting measurements were plotted against one another and mean and standard deviation determined for each of the three parameters, by species and as a full lot. Results from whole muscle versus formed products were not separated. Upon completion of all analyses, each plant received their individual results plotted against results for all plants. Anonymity of plant results was maintained on all but their own sample data.

Results & Discussion

Of the 78 plants contacted, 37 contributed surveys for a 47% response rate. Of these, 33 plants contributed 61 product samples. Analytical results of product samples revealed a mean a_w of 0.74 (SD 0.10); mean NaCl of 6.85% (SD 2.5%); and mean pH of 5.85 (SD 0.33). Specific results broken down by species are shown in Table 1. Some key results garnered from the survey include thermal processing extremes and nearly a complete lack of relative humidity instrumentation. These ranged from processing jerky at 51.6C for 45 min followed by 3C increases every 45 minutes to reach an oven temperature of 62.8C maximum, to processing jerky at 93.3C for 6-7 h. Perhaps the single most important finding is the depth of variability within this industry. The survey tool provided the means to quantify some of the extremes in this variability and in that way will serve as a pivotal source of information for developing challenge studies.

The survey instrument revealed that approximately 56% of all product produced is the whole muscle type and 44% is ground or chunked and formed. Total plant output was not requested nor reported. NaCl concentration in mixes ranged from 1% to more than 85%, and pickup of wet marinade ranged from 3% or less to 100%.

Survey results further revealed that 34 of 37 manufacturers (92%) used only a smokehouse for thermal processing, 3 of 37 (8%) used a commercial oven and one manufacturer (3%) used both a smokehouse and an oven for thermal processing. Within the thermal environment, nine manufacturers determined the wet bulb temperature to yield relative humidity and only one employed a relative humidity instrument. However, 35 of 37 manufacturers claimed they are able to control humidity. Controls employed are closing the dampers (35%),

steam injection (8%), direct addition of water (8%), placing a pan of water in the house (3%) or a combination of these methods (43%). All respondents who claimed a combination of these methods used steam as one component, indicating that 51% of these manufacturers are using steam injection to increase their humidity. Manufacturers reported an average of 44% cooked yield.

Ingredients used in the production of jerky may be grouped into two broad categories, those with a functional purpose besides flavor and those added solely for flavor. Functional ingredients found included sodium nitrite, sodium erythorbate, lactic acid, and potassium sorbate. Thirty-two of 37 respondents (86%) indicated the use of sodium nitrite, however only 15 (41%) also indicated the use of sodium erythorbate as a cure accelerator. Those manufacturers not using a cure accelerator reported time spans ranging from 45 min (wet marinade) up to 7 d (dry cure) between the time seasonings are added until the drying process is begun. Only five (14%) manufacturers reported the use of potassium sorbate for mold inhibition and one (3%) reported using lactic acid to acidify the product. Ingredients used for their seasoning properties included soy sauce (62%), vinegar (8%), worcestershire sauce (16%) and tabasco sauce (5%). Lesser used ingredients included pineapple juice, teriyaki sauce, apple cider, garlic salt, and char oil (3% each).

Respondents indicated a variety of packaging methods were employed including vacuum (78%), no vacuum (32%), and gas flush (14%). Storage, distribution, and marketing were variable as well, with 32% reporting refrigerated storage, 3% frozen and 5 of 13 (38%) reported shelf-stable storage and distribution. However, examination of package markings upon receipt of samples indicated that 12 of 13 (92%) of sample packages lack any precautionary handling markings, and may be interpreted as shelf-stable.

Conclusions

In the Midwest, slightly more than half of the jerky produced by small and very small facilities that responded to the survey was whole muscle, while slightly less than half was ground and formed or chunked and formed. Jerky a_w and pH among different species was similar. NaCl levels among species may be similar, however an insufficient number of turkey samples were tested to conclude this similarity. This data provides sufficient correlation to proceed with lethality studies around a_w 0.74, NaCl 6.85% and pH 5.85, with additional study around upper and lower limits of each parameter in combination to determine any synergistic affect between parameters.

Survey results confirmed the hypothesis that there is widespread variability in thermal processing and most other production methods from one manufacturer to another. Variables included processing time and temperature, processing environment relative humidity and method employed to control humidity. Extremes in thermal processing provide useful baselines under which to proceed with lethality studies. Challenge studies at thermal extremes, in the presence of mean product characteristics, can provide inactivation or protective effects for targeted microorganisms.

The survey revealed that, while a significant proportion of the industry has instituted the use of sodium nitrite into their product, many manufacturers may not be realizing any functional properties of this ingredient. Fifty-three percent of those using nitrite are not using a cure accelerator to speed the curing process. Among this group of products, product is allowed a lower limit of 45 minutes for the curing reaction to occur. With 86% of the industry using cure,

challenge studies should be conducted with cure for most iterations, and without cure in a comparative study at the mean level of product and thermal processing conditions.

Slightly less widespread use of acidic ingredients are used in jerky production. Ingredients such as vinegar (pH 2.41), worcestershire sauce (pH 3.50), soy sauce (pH 4.83) and tabasco sauce (pH 3.20) are used by some manufacturers as flavor additives, primarily as a constituent of a wet marinade process. The pH of a marinade mixture and length of time held in marinade prior to thermal processing should be considered in a challenge study.

Packaging methods employed throughout the industry varied slightly with most manufacturers using vacuum packs or modified atmosphere packaging to extend shelf-life by preventing oxidation or mold growth. The use of potassium sorbate as a mold inhibitor was limited. One consideration in a challenge study would be the impact of packaging and subsequent shelf life on pathogen lethality during storage.

References

USDA, FSIS. Compliance guideline for meat and poultry jerky produced by small and very small plants. 2004. Available at http://www.fsis.usda.gov/pdf/compliance_guideline_jerky.pdf, accessed on 15 Feb 05.

Tables and Figures

Table 1. Mean NaCl content, a_w and pH of whole muscle, ground and formed, and chunked and formed jerky by species from 33 primarily Midwestern companies.

Species	% NaCl	SD[1]	A_w	SD	pH	SD
Beef	6.90% (n=28)	2.54%	0.74 (n=49)	0.10	5.86 (n=49)	0.32
Pork	7.58% (n=5)	2.42%	0.71 (n=6)	0.09	5.87 (n=6)	0.35
Turkey	3.73% *(n=1)*	-	0.77 (n=4)	0.09	5.82 (n=4)	0.46
Buffalo	4.95% *(n=1)*	-	0.79 (n=2)	0.08	5.52 (n=2)	0.16
Maximum for all Products	11.54%		0.90			6.60
Minimum for all Products	2.10%		0.51			5.00

[1] Standard deviation

POSTER: DETERMINATION OF BASELINE PRODUCT CHARACTERISTICS AND PRODUCTION METHODS IN SMALL AND VERY SMALL JERKY PLANTS

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Conclusions

- Beef jerky lethality studies designed to apply to survey respondents should target a_W 0.74. NaCl 6.90%, and pH 5.86.
- There is widespread variability in thermal processing and production methods across manufacturers. Challenge studies at thermal extremes, in the presence of mean product characteristics, would provide inactivation or protective effects for targeted microorganisms.
- With 86% of the industry using cure, challenge studies should be conducted with cure for most iterations, and without cure in a comparative study at the mean level of product and thermal processing conditions.
- The pH of a marinade mixture and length of time held in marinade prior to thermal processing should be considered in a challenge study.
- Packaging methods employed throughout the industry varied slightly, with most manufacturers using vacuum packs or modified atmosphere packaging to extend shelf-life by preventing oxidation or mold growth. One consideration in a challenge study would be the impact of packaging and subsequent shelf life on pathogen lethality during storage.

Objective

Develop a survey tool and collect information from small and very small manufacturers primarily in Kansas, Nebraska, Missouri, and Wisconsin relative to manufacturing equipment, processing techniques, ingredients and end item characteristics typical of jerky products to form a baseline for the design of lethality challenge studies in whole muscle and ground and formed jerky.

Introduction

- In light of a jerky related Salmonellosis outbreak during October 2003, USDA FSIS scrutinized jerky production methods in the U.S.
- USDA FSIS issued “Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants,” which provided thermal processing guidelines, humidity requirements and end product recommendations that may be applied to any jerky production scenario.
- In Spring 2004, USDA solicited proposals to conduct research focused on providing small and very small manufacturing plants a means for validating their HACCP plans, specifically by achieving a 6.5 log reduction in pathogenic microorganisms.
- To develop validation studies, there was a need to determine the current state of the jerky industry in these facilities.

Materials & Methods

- Developed a four page survey tool in conjunction with University of Wisconsin and University of Nebraska.
- Questions directed at detailing equipment, ingredients and processing techniques used in jerky manufacture to gain very specific information providing up to 168 data points.

- Packet mailed to 78 small and very small jerky processors included a cover letter, four page survey, and a request to provide four samples of each jerky product they made for complimentary NaCl, pH and a_W analysis. About one month after surveys were mailed, a follow-up telephone call was made to processors who had not yet returned the completed survey.
- Survey data were compiled and means generated for quantitative data. Qualitative data were compared to determine if consensus existed among industry practices.
- NaCl content was determined using Quantab® titrators for chloride (0.05-1.0% NaCl, Hach Company, Loveland, CO), using five g jerky combined with 45 ml distilled, deionized water.
- a_W was measured at ambient temperature using an AquaLab Series 3 TE (Decagon Devices, Pullman, WA).
- pH was determined on 10 g jerky blended with 100 ml distilled, deionized water and measured with a pH meter (Accumet Portable model AP61 Fisher Scientific, Fair Lawn, NJ).

Results

- Of 78 plants contacted, 37 contributed surveys for a 47% response rate. Of these, 33 plants contributed 61 product samples.
- Whole muscle jerky produced by 33 plants, ground/chunked and formed jerky produced by 26 plants.

Results (continued)

Equipment Used for Jerky Processing:	Relative Humidity Techniques Used:	Common Ingredients Used:	Ingredient Addition Method:
34 (92%): use smokehouse 3 (8%): use commercial oven 1 (3%): use smokehouse and oven Relative Humidity Measurement: 9 (24%): determine wet bulb temperature 1 (3%): use relative humidity instrument *35 (95%): claim they are able to control relative humidity*	13 (35%): close dampers 3 (8%): steam injection 3 (8%): direct addition of water 1 (3%): place pan of water in house 16 (43%): combination of these methods Thermal Processing Schedule Extremes: 93.3°C smokehouse for "entire process" 51.7°C for 45 minutes, followed by 2.2°C increase every 45 minutes to 62.7°C (max)	28 (86%): sodium nitrite 15 (41%): sodium erythorbate 5 (14%): potassium sorbate 23 (62%): soy sauce 3 (8%): vinegar 6 (16%): worcestershire sauce 2 (5%): tabasco sauce	45 products marinated 16 products dry addition 18 products tumbled 2 are vacuum tumbled 1 injected before tumbled 4 soaked before and after tumbled Packaging Type Used for Jerky: 29 (78%): vacuum 12 (32%): no vacuum 5 (14%): gas flush

Table 1. Mean NaCl content, a_w and pH of whole muscle, ground and formed, and chunked and formed jerky by species from 33 primarily Midwestern companies.

Species	% NaCl	SD[1]	a_w	SD	pH	SD
Beef	6.90% (n=28)	2.54 %	0.74 (n=49)	0.10	5.86 (n=49)	0.32
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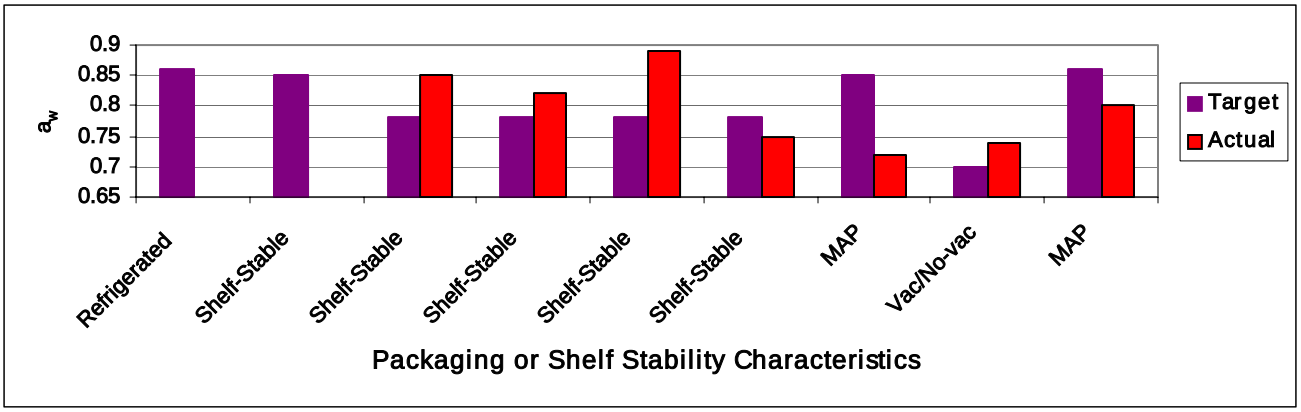
Maximum for all Products	11.54%		0.90			6.60
Minimum for all Products	2.10%		0.51			5.00

Figure 1. Target a_w versus actual a_w in nine jerky samples, relative to packaging



2. Lonnecker, S. M. 2005. Variables affecting lethality of *Escherichia coli* O157:H7 and *Salmonella* species in whole muscle or formed jerky products. M.S. report, Kansas State Univ., Manhattan (abstract follows).

ABSTRACT

In light of several foodborne outbreaks related to jerky products, manufacturers have found themselves in the spotlight of heightened scrutiny by regulatory entities and the consumer public. Industry has found regulations changing and an immediate need to carefully review processing methods and formulations. Regulatory and industry officials have also found that very little scientific information exists that can be directly applied to this jerky products.

The intent of this review is to present direct and indirect scientific information that can be applied to jerky manufacturing schemes, from formulation through thermal processing for the production of a safe product. Studies reviewed in this thesis demonstrate processing methods or ingredients that directly affect lethality of *Salmonella* spp. and *Escherichia coli* O157:H7.

Some key findings of this review include evidence that sodium chloride or fat can support pathogenic survival, evidence on the effect of water activity on lethality and post-processing growth and the remarkable and often unanticipated effect solutes can have, not only on available nutrients but on the osmotic environment surrounding microorganisms. Discussion also focuses on the use of an acid or alkaline environment to reduce pathogen populations and a brief discussion of packaging methods and environments that can introduce storage and distribution concerns.

While specific heat processing schedules are beyond the intended scope of this document, variables that should be considered during thermal treatments are discussed in great detail. These variables include: concepts behind the thermal death of microorganisms, types of heat transfer in gas, liquid and solid media, mass transfer effects and the importance of relative humidity during heat treatment.

This report will provide information that might be implemented not only by industry officials looking for a better understanding of their products or HACCP validation, but also by academia in developing scientific data and further research of relevance to jerky or similarly processed products. Still another potential use is by regulatory officials seeking evidence supporting guideline development or enforcement strategies.

3. Roberts, Michelle N. Evaluation of thermal processing parameters in production of chopped and formed beef jerky for controlling *Escherichia coli* O157:H7 and *Salmonella* species. Chapter on Preliminary Studies. M.S report, Kansas State Univ., Manhattan (December 2006) Co-advisors: Getty, K.J.K. and Fung, D.Y.C. and Committee member: Boyle, E.A.E.

PRELIMINARY STUDIES

NOTE TO READERS: The preliminary studies are a collection of small research studies to develop jerky processing parameters, a process schedule, and inoculation procedures for incorporating microorganisms into the raw jerky batter. These studies were not designed for obtaining process validation information. Therefore, the results presented here should not be used as verification or validation information for determining lethality of jerky processing schedules.

Introduction

The United States Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) has published Compliance Guidelines for the Production of Jerky. The guidelines were issued in part to a 2003 *Salmonella kiambu* outbreak being linked to the consumption of jerky produced by a New Mexico firm, resulting in the recall of 22,000 lbs. of jerky (FSIS, 2003). The guidelines require that processors achieve a water activity level of $\leq$0.70 (FSIS, 2004). In addition, FSIS's standard of identity for jerky is a moisture-to- protein (MPR) ratio of 0.75:1 (AAMP, 2004; Compliance Guideline, 2004). FSIS performance standards for ready-to-eat meat and poultry products, according to a 2001 proposed rule, require that processes achieve a 5.0 log CFU/g reduction of *Escherichia coli* O157:H7 and a 6.5 log CFU/g reduction of *Salmonella* spp.

The basic objectives of the preliminary studies were: 1) to determine if a hand-held jerky strip dispenser with nozzle would produce a product similar to commercial jerky; 2) to evaluate the effectiveness of typical smokehouse schedules for producing commercially chopped and formed all-beef jerky to an appropriate water activity level of $\leq$0.70 and a MPR of 0.75:1; 3) to determine how many strips would be needed to fill the smokehouse to capacity; and 4) to determine inoculum levels needed to achieve greater than 7.0 log CFU/g of *E. coli* O157:H7 or *Salmonella* spp. in raw chopped and formed jerky. Six preliminary studies were conducted and the objectives, materials and methods, and results and discussion of each study are discussed.

Preliminary Study 1

Objectives

1. To determine if a hand-held jerky strip dispenser with a nozzle would produce a product similar to finished commercial jerky product.

2. To evaluate the effectiveness of a typical smokehouse schedule for producing commercially chopped and formed all-beef jerky.

Materials and Methods

Meat Batter Preparation

Fresh chopped and formed all-beef jerky batter (-5°C), was obtained from a commercial processor, immediately frozen at –15 ± 2°C for 1 to 2 weeks and then tempered overnight at 4°C and used the following day. A 0.907 kg (2 lb) batch was used for testing purposes.

Raw jerky batter was transferred to a manual jerky strip dispenser with a 0.19 cm (3/16") by 0.19 cm (3/16") nozzle (Model Nbr. H6253, Grizzly Industrial, Inc., Bellingham, WA). Strips were placed on polyscreen sheets (Excalibur, Sacramento, CA) with four strips per sheet. Each strip was approximately 17.78 cm (7") long with width and depth similar to size of the nozzle. Strip-filled sheets were placed on the seven-smokehouse trays and the trays were placed on a smokehouse cart. Once loaded, the smokehouse cart was placed in a commercial smokehouse (Alkar Model 450-UA, Alkar, Lodi, WI) and thermally processed (Table 1).

Sampling Times

Heat-treated samples were taken at four different times throughout the process [end of Stages 6, 7, 8, and 9] (Table 1). Four strips were taken randomly throughout the smokehouse for each sampling time.

Water Activity (a_w)

Water activity was measured on all samples collected at the different sampling times. A sample consisted of four combined strips. A composite sample was prepared by finely chopping the four strips. Duplicate readings were taken. A water activity meter (Pawkit, Decagon, Pullman, WA) measured water activity. Readings were recorded when the stabilized icon was displayed.

Results and Discussion

Finished strips were approximately 1.27 cm (1/2") by 0.26 cm (1/10") by 10.16 cm (4"). This was too small compared to typical commercial jerky strips having dimensions of approximately1.91 cm (3/4") by 0.32 cm (1/8") by 15.24 cm (6"). It was determined that a larger nozzle would be needed to account for shrinkage during the drying process and to produce a more typical commercial jerky product.

Water activity for samples collected at the end of stage 6, 7, and 8 ranged from 0.76 to 0.71. Final water activity, taken at the end of stage 9, was 0.70 (Table 1). Typical water activity for jerky products should be ≤ 0.70 to ensure lack of microbial growth (FSIS, 2004). A jerky product with a water activity of less than 0.85 is usually considered shelf stable and would not support the growth of pathogenic microorganisms. A water activity of ≤ 0.70 is suggested for the stabilization of jerky product that is to be in contact with air as this water activity level inhibits mold growth (AAMP, 2004).

Table 1. Thermal processing schedule[a], sampling times, and water activity levels for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity
0[b]					Raw	0.94
1	23.9	23.9	90	30 min		
2	43.3	32.2	68	30 min		
3	54.4	32.2	60	30 min		
4	65.6	35.0	48	30 min		
5	68.3	35.0	14	30 min		
6	71.1	40.6	14	30 min	End of stage	0.76
7	71.1	37.8	29	30 min	End of stage	0.72
8	76.7	37.8	21	30 min	End of stage	0.71
9	76.7	35.0	13	30 min	End of stage	0.70
10	76.7	35.0	<10	30 min		

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was not turned on and the dampers were set on automatic to allow for effective control of humidity.
[b]Stage 0 = raw meat batter.

Preliminary Study 2

Objective

1. To determine if a larger nozzle for the jerky strip dispenser would yield a more typical commercial size jerky strip.

Materials and Methods

The same procedures were used for meat batter preparation as described in Preliminary Study 1. However, a larger nozzle of 0.64 cm (1/4") by 2.54 cm (1") nozzle (Model Nr. LEM468D, Allied Kenco, Houston, TX) was attached to the jerky strip dispenser. The sampling times were the same as Preliminary Study 1 and are also defined in Table 2. In addition, water activity levels were measured in a similar manner.

Results and Discussion

Finished strips were approximately 1.91 cm (3/4") by 0.32 cm (1/8") by 15.24 cm (6"). This was comparable to typical commercial jerky strips having dimensions of approximately 1.27-1.91 cm (1/2-3/4") by 0.32-0.64 cm (1/8-1/4") by 15.24-20.32cm (6-8"). Therefore, the larger jerky nozzle allowed for a correct amount of raw product to meet finished product dimensions due to shrinkage during processing.

Due to larger product dimensions, water activity levels at the end of stages 6, 7, and 8 ranged from 0.84 to 0.78 and were approximately 0.4 points higher than for products sampled at the same time in Preliminary Study 1. Final water activity for final product, at end of stage 9, was 0.74 (Table 2). Typical water activity for jerky products should be $\leq$ 0.70 to ensure lack of

microbial growth (FSIS, 2004). The current processing schedule would need to be altered in order to dry the product sufficiently to achieve the desired water activity ≤ 0.70.

Table 2. Thermal processing schedule[a], sampling times, and water activity levels for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity
0[b]					Raw	0.94
1	23.9	23.9	90	30 min		
2	43.3	32.2	68	30 min		
3	54.4	32.2	60	30 min		
4	65.6	35.0	48	30 min		
5	68.3	35.0	14	30 min		
6	71.1	40.6	14	30 min	End of stage	0.84
7	71.1	37.8	29	30 min	End of stage	0.80
8	76.7	37.8	21	30 min	End of stage	0.78
9	76.7	35.0	13	30 min	End of stage	0.74
10	76.7	35.0	<10	30 min		

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was not turned on and the dampers were set on automatic to allow for effective control of humidity.
[b]Stage 0 = raw meat batter.

Preliminary Study 3

Objectives

1. To test a different smokehouse schedule that is more typical of a commercial jerky process.
2. To determine how many strips are needed to completely fill the smokehouse to its capacity.
3. To determine the finished weight of strips for sampling purposes.

Materials and Methods

The same procedures were used for meat batter preparation as described in Preliminary Study 1. However, the raw jerky product was separated into one 1.814 kg (4 lb) batch. Sampling times differed from those listed in Preliminary Studies 1 and 2 in order to test the different smokehouse schedule used in this study. Heat-treated samples were taken at eleven different times throughout the process [end of Stages 6, 7, 8, 10 and at thirty minute intervals throughout Stage 12] (Table 3). Four strips were taken randomly throughout the smokehouse for each sampling time. Water activity was performed in the same manner as in Preliminary Studies 1 and 2.

Results and Discussion

Finished strips were approximately 1.91 cm (3/4") by 0.32 cm (1/8") by 15.24 cm (5.5"). This was comparable to typical commercial jerky strips having dimensions of approximately 1.27-1.91 cm (1/2-3/4") by 0.32-0.64 cm (1/8-1/4") by 12.70-20.32cm (5-8"). Two finished strips weighed approximately 27 g. According to the USDA/FSIS Microbiology Laboratory Guidebook (1998) a typical sample size for microbial analysis is 25g. Therefore, two strips could be used to represent a "sample" in future studies. The 1.814 kg (4 lb) batch made approximately 56 strips and filled 1/3 of the smokehouse. In order to get results typical of a smokehouse running at full capacity, a 5.442 kg (12 lb) batch would need to be used for future studies.

Final water activity for final product, at the end of stage 12 was 0.65 (Table 3) which is below the suggested FSIS requirement of ≤ 0.70 (FSIS, 2004). Water activity reached the desired level 1.5 h into stage 12, so the schedule could be modified to end at this time.

Table 3. Thermal processing schedule[a], sampling times, and water activity levels for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity
0[b]					Raw	0.95
1	55.6	N/A	72	14 min		
2	55.6	37.8	84	16 min		
3	55.6	43.3	43	14 min		
4	77.8	43.3	78	16 min		
5	77.8	48.9	78	14 min		
6	77.8	48.9	78	16 min	End of stage	0.89
7	77.8	48.9	78	14 min	End of stage	0.85
8	77.8	48.9	78	16 min	End of stage	0.86
9	77.8	48.9	78	14 min		
10	77.8	48.9	78	16 min	End of stage	0.81
11	77.8	48.9	78	14 min	End of stage	0.78
12	77.8	N/A	78	3h	30 min into stage 12	0.76
					1 h into stage 12	0.73
					1.5 h into stage 12	0.69
					2 h into stage 12	0.68
					2.5 h into stage 12	0.66
					3 h into stage 12 (End)	0.65

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was not turned on and the dampers were set on automatic to allow for effective control of humidity.
[b]Stage 0 = raw meat batter.

Preliminary Study 4

Objectives

1. To determine the amount of inoculum needed for both *E. coli* O157:H7 and *Salmonella* spp. to achieve > 7.0 log CFU/g in raw batches of product.
2. To determine the effectiveness of the smokehouse schedule for controlling *E. coli* O157:H7 and *Salmonella* spp. inoculated batches of commercially prepared chopped and formed all-beef jerky batter.
3. To analyze the moisture-to-protein ratios (MPR) in finished jerky product.

Materials and Methods

Confirmation of Cultures and Presumptive *E. coli* O157:H7 and *Salmonella* spp. Colonies

All *E. coli* O157:H7 cultures were originally obtained from the American Type Culture Collection (ATCC 43894, ATCC 43889, ATCC 43890, ATCC 43895) (Rockville, MD) and the United States Department of Agriculture/Agricultural Research Service (USDA 380-94) (USDA/ARS, Athens, GA). All *Salmonella* spp. cultures were originally obtained from ATCC [ATCC 35640 (*Salmonella abaetetuba*), ATCC 4931 (*Salmonella enteriditis*), ATCC 13076 (*Salmonella enteriditis*), ATCC 15060 (*Salmonella enteriditis*), ATCC 13311 (*Salmonella typhimurium*). Cultures arrived in commercial bead form and were kept in an -80°C freezer in the KSU Food Safety and Security lab.

Confirmation

All cultures and presumptive *E. coli* O157:H7 and *Salmonella* spp. colonies taken respectively from, phenol red sorbitol agar (PRSA; Becton Dickinson, Franklin Lakes, NJ) and xylose lysine desoxycholate agar (XLD; Becton Dickinson, Franklin Lakes, NJ) plates from raw and heat-treated samples were streaked, using a sterile loop (Fisher Scientific Inc, Pittsburgh, PA) onto tryptic soy agar (TSA; Becton Dickonson, Franklin Lakes, NJ) and incubated at 37ºC for 24 h. Typical colonies were used to inoculate API 20E biochemical identification test strips (bioMerieux Vitek, Inc., Hazelwood, MO) and Enterotubes (Becton Dickinson, Franklin Lakes, NJ).

Preparation of Bacterial Culture

A five-strain cocktail of *E. coli* O157:H7 as described above and a separate five-strain cocktail of *Salmonella* spp. as describe above were prepared. For inoculum preparation of each pathogen strain, one loopful of each culture was transferred into 9 mL of tryptic soy broth (TSB; Difco) and incubated at 35ºC for 18-24 h. Four mL of each culture suspension was then transferred to a sterile bottle (Nalgene, Rochester, NY). Two separate bottles resulted with one containing 20 mL of *E. coli* O157:H7 and one containing 20 mL of *Salmonella* spp.

Meat Batter Preparation and Inoculation

Fresh chopped and formed all-beef jerky batter was obtained from a commercial processor, immediately frozen for 1 to 2 weeks and then tempered overnight at 4ºC and used the following day. The product was separated into three 1.814 kg (4 lb) batches. To the control batch, 15 mL of sterile deionized water was mixed evenly into the meat batter and stored at 4ºC until stuffing and enumeration. To a separate batch, 15 mL of the *E. coli* O157:H7 five-strain inoculum was intermittently pipetted drop-wise over the batter surface and massaged with gloved hands until thoroughly mixed into the jerky batter. The batter was stored at 4ºC until stuffing and enumeration. This procedure was repeated with the last batch of batter, however, 15 mL of the *Salmonella* spp. five-strain inoculum was added instead.

Raw jerky batter (control followed by inoculated batches) was transferred to a manual jerky gun (Model Nbr. H6253, Grizzly Industrial, Inc.) with a 0.64 cm (¼") by 2.54 cm (1") jerky nozzle (Model Nr. LEM468D, Allied Kenco, Houston, TX). Strips were placed on polyscreen sheets (Excalibur, Sacramento, CA) with eight strips per sheet. Each strip was approximately 17.78 cm (7") long. Strip filled sheets were placed on smokehouse trays in a way as to prevent potential cross contamination (Table 4). This allowed elimination of the possibility of competitive exclusion between *E. coli* O157:H7 and *Salmonella* spp. Once loaded, the smokehouse cart was placed in a commercial smokehouse (Alkar Model 450-UA, Alkar, Lodi, WI) and thermally processed (Table 5).

***E. coli* O157:H7 and *Salmonella* spp. Enumeration**

One mL of the *E. coli* O157:H7 inoculum was enumerated using duplicate phenol red sorbitol agar (PRSA; Difco) plates. One mL of the *Salmonella* spp. inoculum was enumerated using duplicate xylose lysine desoxycholate agar (XLD; Difco) plates. Six 1:10 dilutions of each inoculum were made using 0.1% peptone water (PW; Difco) and then spread plated onto their corresponding media in duplicate.

Raw inoculated samples were taken from the inoculated jerky batter to determine the initial inoculum level of the product. Heat-treated samples were taken at five different times [end of Stages 6, 7, 8, 10, and at end of Stage 12] (Table 5). At each sampling time, two samples of each of the three batches were taken. A sample comprised of two strips. Control samples were used for proximate analysis, pH, and water activity analysis. The inoculated samples were used for *E. coli* O157:H7 and *Salmonella* spp. enumeration.

Raw and heat-treated samples were prepared by weighing 25 g in filtered stomacher bags to which 225 mL of PW diluent was added. The samples were pummeled in a stomacher (Stomacher Mix 1 Lab Blender, Microbiology International, Frederick, MD) for 1 min and six serial 1:10 dilutions were prepared using 9 mL 0.1% peptone blanks. Dilutions were spread plated in duplicate onto PRSA for *E. coli* O157:H7 inoculated samples and XLD plates for *Salmonella* spp. inoculated samples. For samples taken at the end of the cycle, a 1:10 dilution was spread-plated (0.25) onto four plates in duplicate, allowing for a sample detection limit of 1 log cfu/g.

pH Analysis

The pH was measured on control samples (2) by weighing 25 g of sample into a filtered stomacher bag and adding 225 mL PW and stomaching for 1 min. A pH meter (Corning Pinnacle 530 pH Meter, Corning, NY) was used to measure pH. The pH readings were recorded when the stabilized icon was displayed (Table 5).

Water Activity (a_w)

Water activity was measured on control samples. Two samples were taken per treatment. Each sample was from a different set of two combined strips. Duplicate readings were taken. A water activity meter (Pawkit, Decagon, Pullman, WA) was used to measure water activity. Readings were recorded when the stabilized icon was displayed (Table 5).

Proximate Analysis

Samples for proximate analysis were taken from non-inoculated raw control, end of Stage 10, and End of Stage 12 (final) product samples (Table 5). Two fifty gram samples of raw inoculated batter, two samples containing two jerky strips each from 1.5 h into Stage 12, and two samples containing two finished jerky strips were vacuum packaged, and placed in frozen storage (-80ºC) prior to analysis. Samples were prepared by freezing in liquid nitrogen and pulverized in a Waring Blender. Moisture (AOAC 985.14) and fat (AOAC 985.15) were determined by a rapid microwave solvent method (CEM, Matthews, NC, U.S.A.). Protein was determined by the LECO method (AOAC 990.03) and salt was analyzed by a volumetric method (AOAC 935.47).

Results and Discussion

Finished strips were approximately 1.91 cm (3/4") by 0.32 cm (1/8") by 15.24 cm (5.5"). This was comparable to typical commercial jerky strips having dimensions of approximately 1.27-1.91 cm (1/2-3/4") by 0.32-0.64 cm (1/8-1/4") by 12.70-20.32cm (5-8"). For all *E. coli* O157:H7 inoculated jerky strips, initial raw batter populations were 5.58-to 5.89 log CFU/g on PRSA. For *Salmonella* spp. inoculated jerky strips, initial raw batter populations ranged from 5.08 to 5.40-log CFU/g on XLD (Table 5). For samples taken at the end of stages 6, 7, 8, and 10, *E. coli* O157:H7 and *Salmonella* spp. ranged from <3 to <5-log CFU/g on all media. Plating was not low enough to catch the dilution. End of Stage 12 product microbial populations were consistently <1 log CFU/g on all media (Table 6).

The dilution factor was not low enough to visibly detect growth on stages 6, 7, 8, and 10, so reductions could not be adequately determined. A $\geq$ 4.24 log CFU/g reduction of both *Salmonella* spp. and *E. coli* O157:H7 was observed on the end product. Due to the inoculum level not being high enough and the detection limit not being low enough, this process was inadequate at determining reduction levels mandated by USDA/FSIS. Therefore, inoculation levels would need to be increased and the detection level lowered to adequately determine log reductions.

Moisture content for control raw product was approximately 51.6% for raw product and ranged from 28.4 to 30.05% for final product. Final product moisture levels fall within the typical range of percent moisture levels for jerky, which is 28-30% (Ricke and Keeton 1997). Protein content for control raw product was approximately 17.0% for raw product and ranged from 27.6 to 28.6% for final product (Table 5). The moisture-to-protein ratio (MPR) ranged from 0.99 to 1.08 for the final product. This ratio is not in compliance with the guideline of an MPR at <0.75:1 that is needed for product to be labeled as jerky (Compliance Guideline, 2004). This product falls within the $\leq$ 2.03:1 and $\geq$0.76:1 MPR range that is typical of kippered beef (AAMP, 2004). The product MPR would need to be driven lower to be labeled as "jerky".

Raw batter pH values ranged from 6.1 to 6.4. Final pH range for all products was 6.1 (Table 5). This falls within the typical pH range of 5.3-6.6 for jerky products products (Lonnecker, 2005; Buege, 2005). This range is of particular concern as it easily supports the growth of gram-negative bacteria. For example, *E. coli* O157:H7 can grow at pH levels <4.4, while some *Salmonella* spp. can grow at pH levels as low as 3.8 (AAMP, 2004).

Final water activity range for all final products was approximately 0.72 (Table 5). Which is slightly higher than the suggested FSIS level of $\leq$ 0.70 to ensure lack of microbial growth (FSIS, 2004). Therefore the smokehouse schedule would need to be modified in order to achieve a water activity of $\leq$ 0.70 to inhibit microbial growth.

Table 4. Order of placement in smokehouse for control (C), *E. coli* O157:H7 (E), and *Salmonella* spp. (S) jerky batches.

C	E	S
C	E	S
C	E	S
C	E	S
C	E	S
C	E	S
C	E	S

Table 5. Thermal processing schedule[a], sampling times, mean water activity levels, mean pH values, and mean proximate analysis for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity	pH	Percent Moisture[c]	Percent Protein	MPR	Percent Fat
0[b]					Raw	0.95	6.23	51.6±0.17	17.0±0.13	3.04:1	18.7±0.27
1	55.6	N/A	72	14 min							
2	55.6	37.8	84	16 min							
3	55.6	43.3	43	14 min							
4	77.8	43.3	78	16 min							
5	77.8	48.9	78	14 min							
6	77.8	48.9	78	16 min	End of stage	0.88	6.26				
7	77.8	48.9	78	14 min	End of stage	0.84	6.21				
8	77.8	48.9	78	16 min	End of stage	0.84	6.29				
9	77.8	48.9	78	14 min							
10	77.8	48.9	78	16 min	End of stage	0.83	6.21	32.1±0.37	25.1±1.07	1.28:1	25.5±0.44
11	77.8	48.9	78	14 min							
12	77.8	N/A	78	1.5 h	End of stage	0.72	6.06	29.2±1.15	28.1±0.74	1.04:1	25.6±0.64

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was not turned on and the dampers were set on automatic to allow for effective control of humidity.

[b]Stage 0 = raw meat batter.

[c]Ranges are due to duplicate sample

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Table 6. *E. coli* O157:H7 and *Salmonella* spp. populations[a] (log CFU/g) at seven sampling times during production of chopped and formed beef jerky.

Stage/Sampling time	Media	
	PRSA	XLD
Raw	5.7±0.22	5.2±0.23
Stage 6	<5	<5
Stage 7	<4	<4
Stage 8	<3	<3
Stage 10	<3	<3
1.5 h into stage 12	<1.48	<1.48
End of stage 12	<0.48	<0.48

[a]Populations are an estimate based on the detection limits.

Preliminary Study 5

Objectives

1. To determine if the addition of 29 mL of an *E. coli* O157:H7 or *Salmonella* spp. inoculum to a 1.814 kg (4 lb) batch of raw jerky batter would allow for >7.0 log CFU/g.
2. To determine if the addition of the exhaust fan at a high blower speed for the first part of the smokehouse cycle would drive down water activity levels and the moisture-to-protein ratio (MPR).
3. To evaluate if a different water activity meter would give more reliable a_w levels.

Materials and Methods

The same procedures were used for meat batter preparation as in Preliminary Study 4, however, 29 mL of sterile deionized water was added to the control batch and 29 mL of the two (*E. coli* O157:H7 or *Salmonella* spp.) inoculums were added to their respective batches. Only the control batch was used to produce strips to undergo the thermal process. *E. coli* O157:H7 and *Salmonella* spp. enumeration were done in a similar fashion, however, only the inoculum and raw samples were enumerated in order to determine the inoculum level in the raw product. The sampling times were the same as in Preliminary Study 3 and are defined in Table 7. One sample, comprised of two strips, was taken randomly throughout the smokehouse for each sampling time. Samples were used for water activity and proximate analysis. The pH levels were not measured in this study.

Water activity was determined as described in Preliminary Study 4, however; only the one sample was analyzed (Table 7). A different water activity (Model CX2, Aqualab, Pullman, WA) was used. Proximate analysis was conducted in a similar fashion; however, one sample of product was taken 1.5 h into stage 12 and at the end of stage 12 (Table 8).

Results and Discussion

Finished strips were approximately 1.91 cm (3/4") by 0.32 cm (1/8") by 13.97 cm (5.5"). This was comparable to typical commercial jerky strips having dimensions of approximately 1.27-1.91 cm (1/2-3/4") by 0.32-0.64 cm (1/8-1/4") by 12.70-20.32cm (5-8"). For *E. coli* O157:H7 inoculated batter, initial raw populations were 9.02-log CFU/g on PRSA. For *Salmonella* spp. inoculated jerky strips, initial raw batter populations were 7.95-log CFU/g on XLD. These levels are high enough to provide detection of the 5-log reduction of *E. coli* O157:H7 and the 6.5-log reduction of *Salmonella* spp. proposed by the USDA/FSIS, provided that the detection limit is lowered from that described in Preliminary 4. This could be achieved by adding 50 mL of peptone diluent to a 25 g sample instead of 225 mL.

Moisture content was 27.63% 1.5 h into stage 12, and 23.43% for final product. This is lower than the typical moisture content for beef jerky, which ranges from 28 to 30% (Ricke and Keeton 1997). Protein content was 28.90% 1.5 h into stage 12, and 30.34% for final product (Table 7). The moisture-to-protein ratio was 0.77 for final product. This ratio is not in compliance with the guideline of an MPR of $\leq$ 0.75:1 needed for product to be labeled as "jerky" (Compliance Guideline, 2004). Therefore, the MPR would need to be driven lower for the product to be considered jerky.

Water activity for final product was 0.71 (Table 7). Typical water activity for jerky products should be $\leq$ 0.70 to ensure lack of microbial growth (FSIS, 2004). The smokehouse schedule would need to be modified to achieve a $\leq$ 0.70 water activity level. This could be achieved by employing the exhaust fan for a longer period of time throughout the smokehouse cycle in order to efficiently dehydrate the product.

Table 7. Thermal processing schedule[a], sampling times, and water activity levels for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity	Percent Moisture	Percent Protein	MPR	Percent Fat
0[b]					Raw	0.94				
1	55.6	N/A	<10	14 min						
2	55.6	37.8	<10	16 min						
3	55.6	43.3	<10	14 min						
4	77.8	43.3	<10	16 min						
5	77.8	48.9	<10	14 min						
6	77.8	48.9	55	16 min	End of stage	0.95				
7	77.8	48.9	65	14 min	End of stage	0.89				
8	77.8	48.9	78	16 min	End of stage	0.84				
9	77.8	48.9	78	14 min						
10	77.8	48.9	78	16 min	End of stage	0.82				
11	77.8	48.9	78	14 min	End of stage	0.79				
12	77.8	N/A	78	3h	30 min into stage 12	0.81				
					1 h into stage 12	0.77				
					1.5 h into stage 12	0.79	27.63	28.90	0.96:1	25.36
					2 h into stage 12	0.74				
					2.5 h into stage 12	0.73				
					3 h into stage 12 (End)	0.71	23.43	30.34	0.77:1	26.82

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was turned on high for the first 1.5 h of the cycle and the dampers were set on automatic to allow for effective control of humidity.
[b]Stage 0 = raw meat batter.

Preliminary Study 6

Objectives

1. To evaluate the effect of using the same schedule as Preliminary Studies 4 and 5, but lengthening the cycle by 2 h accompanied by the addition of the exhaust fan on medium speed for the first 1.5 h of the cycle and increased to high speed for the duration of the cycle.

Materials and Methods

The same procedures were used for meat batter preparation as were used in Preliminary Study 5, however, only one 1.814 kg (4 lb) batch was dispensed into strips. Sampling times differed from those listed in Preliminary Study 5 in order to represent the different smokehouse schedule used in this study. Heat-treated samples were taken at fifteen different times throughout the process [end of Stages 6, 7, 8, 10 and at thirty minute intervals throughout Stage 12] (Table 8). One sample, comprised of two strips, was taken randomly throughout the smokehouse for each sampling time. Samples were used for water activity and proximate analysis. The pH levels were not measured in this study.

Water activity was determined as described in Preliminary Study 4, however; only the one sample was analyzed (Table 8). A different water activity (Model CX2, Aqualab, Pullman, WA) was used. Proximate analysis was conducted in a similar fashion; however, one sample of product was taken 4.5 h into stage 12 and at the end of stage 12 (Table 9).

Results and Discussion

Finished strips were approximately 1.91 cm (3/4") by 0.32 cm (1/8") by 12.70 cm (5"). This was comparable to typical commercial jerky strips having dimensions of approximately 1.27-1.91 cm (1/2-3/4") by 0.32-0.64 cm (1/8-1/4") by 12.70-20.32cm (5-8"). Moisture content was 21.83% 4.5 h into stage 12, and 21.02% for final product. This is lower than the typical moisture content for beef jerky, which ranges from 28 to 30% (Ricke and Keeton, 1997). Protein content was 29.04% 4.5 h into stage 12, and 30.13% for final product (Table 9). The moisture-to-protein ratio was 0.71 for final product. This ratio is in compliance with the guideline of an MPR of $\leq$ 0.75:1 needed for product to be labeled as "jerky" (Compliance Guideline, 2004).

Water activity for final product was 0.69 (Table 8). Typical water activity for jerky products should be $\leq$ 0.70 to ensure lack of microbial growth (FSIS, 2004). This cycle successfully produced product meeting the appropriate MPR and water activity levels.

Table 8. Thermal processing schedule[a], sampling times, and water activity levels for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (°C)	Wet Bulb (W.B.) (°C)	Percent Relative Humidity (R.H.)	Time	Sampling Time	Water Activity
0[b]					Raw	0.94
1	55.6	N/A	<10	14 min		
2	55.6	37.8	<10	16 min		
3	55.6	43.3	<10	14 min		
4	77.8	43.3	<10	16 min		
5	77.8	48.9	<10	14 min		
6	77.8	48.9	<10	16 min	End of stage	0.88
7	77.8	48.9	<10	14 min	End of stage	0.87
8	77.8	48.9	<10	16 min	End of stage	0.86
9	77.8	48.9	<10	14 min		
10	77.8	48.9	<10	16 min	End of stage	0.82
11	77.8	48.9	<10	14 min	End of stage	0.84
12	77.8	N/A	<10	3h	30 min into stage 12	0.81
					1 h into stage 12	0.74
					1.5 h into stage 12	0.77
					2 h into stage 12	0.79
					2.5 h into stage 12	0.73
					3 h into stage 12	0.73
					3.5 h into stage 12	0.71
					4 h into stage 12	0.73
					4.5 h into stage 12	0.71
					5 h into stage 12 (End)	0.69

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. The blower was turned on medium for the first 1.5 h of the cycle and bumped up to high for the duration of the cycle. The dampers were set on automatic to allow for effective control of humidity.
[b]Stage 0 = raw meat batter.

Table 9. Proximate analysis of control chopped and formed beef jerky samples.

Sampling time	Percent Moisture	Percent Protein	MPR	Percent Fat
4.5 h into Stage 12	21.83	29.04	.75:1	29.74
End of Stage 12	21.02	29.70	.71:1	30.13

Conclusions

After conducting six preliminary studies, appropriate processing parameters were developed that allowed for the production of a typical commercial chopped and formed all-beef jerky to an appropriate water activity level of $\leq$ 0.70 and a MPR of 0.75:1. Inoculum methods were developed that allowed for a greater than 7.0 log CFU/g of *E. coli* O157:H7 or *Salmonella* spp. in raw chopped and formed jerky. Appropriate dilution levels were determined for appropriate enumeration and detection of both pathogens throughout the smokehouse schedule. The processing parameters and microbial methods developed from the preliminary studies will be used for a comprehensive validation study.

4. Roberts, M.N., Getty, K.J.K., and Boyle, E.A.E. 2006. Control of *Escherichia coli* O157:H7 and *Salmonella* spp. in chopped and formed beef jerky. Annual Meeting Proceedings, Food Safety Consortium, University of Arkansas, Oct. 1-3. CD format.

CONTROL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. IN CHOPPED AND FORMED BEEF JERKY

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SUMMARY AND IMPLICATIONS

USDA/FSIS issued "Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants," that provides processing parameters for controlling pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. The objective of this study was to determine the effects of typical thermal processing temperatures and times on reducing *E. coli* O157:H7 and *Salmonella* spp. in chopped and formed beef jerky. Initial raw batter *E. coli* O157:H7 and *Salmonella* spp. populations were approximately 7.3 log cfu/g. When the smokehouse dry bulb (D.B.) temperature was 55.6ºC and relative humidity (R.H.) was approximately 10% for 44 min followed by 77.8ºC D.B. and R.H. at approximately 10% for 46 min, ▪▪5 log reductions were observed for both *E. coli* O157:H7 and *Salmonella* spp. populations. However, an additional heating/drying phase of 3 h with smokehouse D.B. at 77.8ºC was required to achieve a moisture-to-protein ratio (MPR) of 0.77:1, which is slightly higher than the 0.75:1 MPR as required by USDA/FSIS for jerky products. Furthermore, additional heating of 3.5 h was needed to achieve *Salmonella* spp. log reductions of ▪▪6.5 as required by USDA/FSIS, which resulted also in MPR values of 0.45:1 and water activity levels of 0.59.

INTRODUCTION

In 2003, the New Mexico Department of Health linked an outbreak of Salmonellosis with consumption of beef jerky (Smelser, 2004). Due to the increasing commonality of foodborne illness associated with dried meats, in 2004, USDA/FSIS published the Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants, which addresses the issues of how to obtain proper lethality, and how to verify proper drying (USDA/FSIS 2004 and Sindelar, 2005). Small meat processors that produce jerky products must validate that their processes achieve a 5-log reduction of *E. coli* O157:H7 and a $\geq$6.5-log reduction of *Salmonella* spp. (Sindelar, 2005). Therefore, the objective of this study was to determine the effects of typical thermal processing temperatures and times on reducing *E. coli* O157:H7 and *Salmonella* spp. in chopped and formed beef jerky.

MATERIALS AND METHODS

Experimental Design: Two treatments, consisting of an *E. coli* O157:H7 inoculated batch and a *Salmonella* spp. inoculated batch were studied. A control batch was also prepared. A replication consisted of both inoculated batches and a control batch placed in the smokehouse simultaneously. The study consisted of three replications. Due to potential cross-contamination from pathogens and potential smokehouse location variations of temperatures, the smokehouse cart was divided in thirds from side-to-side for the two treatments and control. Each batch was

then placed in a different location for each replication (Table 1).

Confirmation of Cultures and Presumptive *E. coli* O157:H7 and *Salmonella* spp. Colonies: All *E. coli* O157:H7 cultures were originally obtained from the American Type Culture Collection (ATCC 43894, ATCC 43889, ATCC 43890 ATCC 43895) (Rockville, MD) and the United States Department of Agriculture/Agricultural Research Service (USDA 380-94) (USDA/ARS, Athens, GA). All *Salmonella* spp. cultures were originally obtained from ATCC [ATCC 35640 (*Salmonella abaetetuba)*, ATCC 4931 (*Salmonella enteriditis)*, ATCC 13076 (*Salmonella enteriditis)*, ATCC 15060 (*Salmonella enteriditis)*, ATCC 13311 (*Salmonella typhimurium*). Cultures arrived in commercial bead form and were kept in an -80ºC freezer in the KSU Food Safety and Security Lab.

Confirmation: All cultures and presumptive *E. coli* O157:H7 and *Salmonella* spp. colonies taken respectively from, phenol red sorbitol agar (PRSA) and xylose lysine desoxycholate agar (XLD; Difco) plates from raw and heat-treated samples were streaked, using a sterile loop (Fisher Scientific Inc, Pittsburgh, PA) onto tryptic soy agar (TSA; Difco) and incubated at 37ºC for 24 h. Typical colonies were used to inoculate API 20E biochemical identification test strips (bioMerieux Vitek, Inc., Hazelwood, MO) and Enterotubes (Becton Dickinson, Franklin Lakes, NJ).

Preparation of Bacterial Culture: A five-strain cocktail of *E. coli* O157:H7 as described above and a separate five-strain cocktail of *Salmonella* spp. as describe above were prepared. For inoculum preparation of each pathogen, one loopful of each culture was transferred into 9 mL of tryptic soy broth (TSB; Difco) and incubated at 35ºC for 18-24 h. Six mL of each culture suspension was then transferred to a sterile bottle (Nalgene, Rochester, NY). Two separate bottles resulted with one containing 30 mL of *E. coli* O157:H7 and one containing 30 mL of *Salmonella* spp.

Meat Batter Preparation and Inoculation: Fresh chopped and formed all-beef jerky batter was obtained from a commercial processor, immediately frozen for 1 to 2 weeks and then tempered overnight at 4ºC and used the following day. The product was separated into three 1.814 kg (4 lb) batches. To the control batch, 29 mL of sterile deionized water was mixed evenly into the meat batter and stored at 4ºC until stuffing and enumeration. To a separate batch, 29 mL of the *E. coli* O157:H7 five-strain inoculum was intermittently pipetted drop-wise over the batter surface and massaged with gloved hands until thoroughly mixed into the jerky batter. The batter was stored at 4ºC until stuffing and enumeration. This procedure was repeated with the last batch of batter, however, 29 mL of the *Salmonella* spp. five-strain inoculum was added instead.

Raw jerky batter (control followed by inoculated batches) was transferred to a manual jerky gun (Model Nbr. H6253, Grizzly Industrial, Inc.) with a 0.64 cm (¼") by 2.54 cm (1") jerky nozzle (Model Nr. LEM468D, Allied Kenco, Houston, TX). Strips were placed on polyscreen sheets (Excalibur, Sacremento, CA) with eight strips per sheet. Each strip was approximately 13 cm (5") long. Strip filled sheets were placed on smokehouse trays with order varying per replication (Table 1). Once loaded, the smokehouse cart was placed in a commercial smokehouse (Alkar Model 450-UA, Alkar, Lodi, WI) and thermally processed as discussed in Table 2.

***E. coli* O157:H7 and *Salmonella* spp. Enumeration:** One mL of the *E. coli* O157:H7 inoculum was enumerated using duplicate phenol red sorbitol agar (PRSA; Difco) plates. One mL of the *Salmonella* spp. inoculum was enumerated using duplicate xylose lysine desoxycholate agar (XLD; Difco) plates and XLD plates with a thin agar layer of trypic soy agar (TAL-XLD; Difco). Six 1:10 dilutions of each inoculum were made using 0.1% peptone water (PW; Difco) and then spread plated onto their corresponding media in duplicate.

Raw inoculated samples were taken from the inoculated jerky batter to determine the initial inoculum level of the product. Heat-treated samples were taken at six different times [end of Stages 6, 7, 8, 10, and 1.5 h into Stage 12, and at the End of the Stage 12] (Table 2). At each sampling time, four strips of each of the three batches were taken. Control samples were used for proximate analysis, pH, and water activity analysis. The inoculated samples were used for *E. coli* O157:H7 and *Salmonella* spp. enumeration.

Raw and heat-treated samples were prepared by weighing 25 g in filtered stomacher bags to which 50 mL of PW diluent was added. The samples were pummeled in a stomacher (Stomacher Mix 1 Lab Blender, Microbiology International, Frederick, MD) for 1 min and two serial 1:10 dilutions were prepared using 9 mL 0.1% peptone blanks. Dilutions were spread plated in duplicate onto PRSA for *E. coli* O157:H7 inoculated samples and XLD and XLD plates with a thin agar layer of trypic soy agar (TAL-XLD; Difco) for *Salmonella* spp. inoculated samples. The 1:10 dilution allowed for a 1.48 log cfu/g detection limit for samples taken at Stages 6, 7, 8, 10, and 1.5 h into Stage 12. For samples taken at the end of the cycle, a 1:10 dilution was spread-plated (0.25) onto four plates in duplicate, allowing for a sample detection limit of 0.48 log cfu/g.

Enrichment: Heat-treated samples were enriched by adding 50 mL modified *E. coli* medium (mEC; Difco) to 25 g of *E. coli* O157:H7 inoculated samples and by adding 50 mL of Preuss Broth (Preuss; Difco) to 25 g of *Salmonella* spp. inoculated samples taken at the End of Stage 12. After incubation at 37ºC for 24 h, the samples were streaked onto PRSA for *E. coli* O157:H7 inoculated samples and XLD and TAL-XLD for *Salmonella* spp. inoculated samples. Plates were incubated at 37ºC for 24 h and observed for typical colonies.

pH Analysis: The pH was measured on control samples (2) by weighing 25 g of sample into a filtered stomacher bag and adding 50 mL PW and stomaching for 1 min. A pH meter (Corning Pinnacle 530 pH Meter, Corning, NY) was used to measure pH. The pH readings were recorded when the stabilized icon was displayed.

Water Activity (a_w): Water activity was measured on control samples. Two samples were taken per treatment. Each sample was from a different set of two combined strips. Duplicate readings were taken. A water activity meter (Model CX2, Aqualab, Pullman, WA) was used to measure water activity. Readings were recorded when the stabilized icon was displayed.

Proximate Analysis: Samples for proximate analysis were taken from non-inoculated raw control, 1.5 h into Stage 12, and End of Stage 12 (final) product samples. Fifty grams of raw sample, two jerky strips from 1.5 h into Stage 12, and two finished jerky strips were vacuum packaged, and placed in frozen storage (-80ºC) prior to analysis. Samples were prepared by freezing in liquid nitrogen and pulverized in a Waring Blender. Moisture (AOAC 985.14) and fat (AOAC 985.15) were determined by a rapid microwave solvent method (CEM, Matthews, NC, U.S.A.). Protein was determined by the LECO method (AOAC 990.03) and salt was analyzed by a volumetric method (AOAC 935.47).

RESULTS AND DISCUSSION

For all *E. coli* O157:H7 inoculated jerky strips, initial raw batter populations ranged from 7.3 to 7.4 log cfu/g on PRSA. For *Salmonella* spp. inoculated jerky strips, initial raw batter populations ranged from 7.1 to 7.5 log cfu/g on both XLD and TAL-XLD media (Table 3). For samples taken at the end of Stages 6, 7, 8, and 10, *E. coli* O157:H7 populations ranged from <1.48 (detection limit) to 2.68 log cfu/g on PRSA and *Salmonella* spp. populations ranged from <1.48 to 2.08 log cfu/g on both XLD and TAL-XLD. By 1.5 h into Stage 12, populations for both pathogens were consistently <1.48 log cfu/g on all media. In addition, End of Stage 12

product populations were consistently <0.48 log cfu/g on all media (Table 3).

All sampling times indicate a ≥ 5.0 log cfu/g reduction of *E. coli* O157:H7 as required by USDA/FSIS with the most consistent reductions occurring at the end of Stage 7 to the End of Stage 12 (Figure 1). A ≥ 6.5 log cfu/g reduction of *Salmonella* spp. as mandated by USDA/FSIS was observed 1.5 h into Stage 12 and at the End of Stage 12. For End of Stage samples, log reductions for both *E. coli* O157:H7 and *Salmonella* spp. were well above those mandated by USDA/FSIS.

Enrichment results for samples from 1.5 h into Stage 12 and End of Stage 12 samples showed negative confirmation for all samples tested, confirming the likelihood that pathogens are dead as opposed to heat-injured. Thus indicating •• 7.0 log cfu/g reductions of both pathogens.

Moisture content for control raw product ranged from 52.4 to 56.0% and 15.1 to 19.8% for final product. Protein content for control raw product ranged from 16.0 to 17.0% for raw product and 34.19 to 37.66% for final product (Table 4). Salt contents for control raw product ranged from 2.2 to 2.3% and from 4.2 to 5.2% for final product. The moisture-to-protein ratio ranged from 0.40 to 0.58 for final product. This ratio is in compliance with the guideline of an MPR <0.75:1 needed for product to be labeled as jerky (Compliance Guideline, 2004).

Raw batter pH values ranged from 6.0 to 6.2. Final pH range for all products was 5.1 to 5.3 (Table 5). It should be noted that a lowered pH was not a determining factor for the reduction of *E. coli* O157:H7 or *Salmonella* spp.

Final water activity range for all final product was 0.570 to 0.625 (Table 5). Typical water activity for jerky products should be ≤ 0.70 to ensure lack of microbial growth (FSIS, 2004).

REFERENCES

New Mexico Department of Health. 2004. Outbreak of Salmonellosis Associated with Beef Jerky in New Mexico, 2004. New Mexico Epidemiology Report. Vol. 2004, Nbr. 3

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Table 1. Order of placement in smokehouse for control (C), *E. coli* O157:H7 (E), and *Salmonella* spp. (S) jerky batches.

Replication #1	Replication #2	Replication #3
E S C	C E S	S C E
E S C	C E S	S C E
E S C	C E S	S C E
E S C	C E S	S C E
E S C	C E S	S C E
E S C	C E S	S C E
E S C	C E S	S C E

Table 2. Thermal processing schedule and sampling times for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (ºC)[a]	Time	Blower Speed	Exhaust Fan	Sampling Time
1	55.6	14 min	Medium	Yes	
2	55.6	16 min	Medium	Yes	
3	55.6	14 min	Medium	Yes	
4	77.8	16 min	Medium	Yes	
5	77.8	14 min	Medium	Yes	
6	77.8	16 min	Medium	Yes	End of stage
7	77.8	14 min	Fast	Yes	End of stage
8	77.8	16 min	Fast	Yes	End of stage
9	77.8	14 min	Fast	Yes	
10	77.8	16 min	Fast	Yes	End of stage
11	77.8	14 min	Fast	Yes	
12	77.8	5 h	Fast	Yes	1.5 h into stage
End					End of stage 12

[a]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. Percent relative humidity remained at <10 throughout the entire smokehouse cycle.

Table 3. *E. coli* O157:H7 populations (log CFU/g) recovered on PRSA and *Salmonella* spp. populations recovered on XLD and TAL-XLD at seven sampling times during production of chopped and formed beef jerky.

Stage/Sampling time	Media		
	PRSA	XLD	TAL-XLD
Raw	7.3±0.1^{a}	7.2±0.1^{ax}	7.3±0.1^{ax}
Stage 6	2.3±0.4^{b}	1.7±0.1^{bx}	1.9±0.2^{bx}
Stage 7	2.0±0.3^{c}	1.7±0.3^{bx}	1.9±0.1^{bx}
Stage 8	1.8±0.2^{d}	1.7±0.1^{bx}	1.8±0.1^{bx}
Stage 10	1.6±0.0^{e}	1.6±0.0^{cx}	1.7±0.0^{bx}
Stage 12 (1.5 h into stage)	<1.48^{f}	<1.48^{dx}	<1.48^{cx}
End of stage 12	<0.48^{g}	<0.48^{ex}	<0.48^{dx}

[a-f]Indicates significant differences in a column within the heating process.
[x] Indicate significant differences between XLD and TAL-XLD counts for that sampling period.

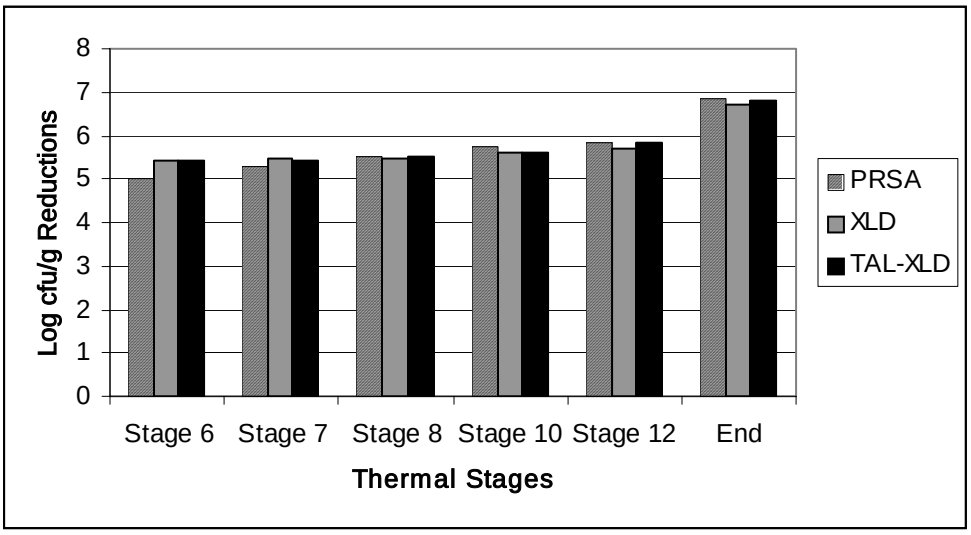

Figure 1. *E. coli* O157:H7 log CFU/g reductions on PRSA and *Salmonella* spp. reductions on XLD and TAL-XLD media at seven sampling times during production of chopped and formed beef jerky.

Table 4. Proximate analysis means and standard deviations of control chopped and formed beef jerky samples.

Sampling time	*Percent Moisture*	Percent Protein	MPR	Percent Fat	Percent Salt
Raw	53.7±1.4	16.5±0.4	3.25:1	17.4±1.8	2.2±0.0
Stage 12 (1.5 h)	24.8±1.5	32.0±0.9	0.77:1	24.5±1.4	4.3±0.3
End of Stage 12	16.8±2.2	36.2±1.4	0.46:1	25.6±1.4	4.5±0.5

[1]Moisture-to-protein ratios.

Table 5. pH and water activity means and standard deviations of control chopped and formed beef jerky samples.

	pH Value	Water Activity
Raw	6.1±0.1	0.936±0.02
Stage 6	6.0±0.0	0.846±0.01
Stage 7	6.0±0.1	0.829±0.02
Stage 8	6.0±0.0	0.843±0.02
Stage 10	6.0±0.1	0.819±0.02
Stage 12 (1.5 h)	5.7±0.1	0.670±0.02
End of Stage 12	5.2±0.1	0.594±0.02

6. Roberts, Michelle N. 2006. Evaluation of thermal processing parameters in production of chopped and formed beef jerky for controlling *Escherichia coli* O157:H7 and *Salmonella* species. Abstract and Chapter on Validation Study. M.S report, Kansas State Univ., Manhattan, KS. Co-advisors: Getty, K.J.K. and Fung, D.Y.C. and Committee member: Boyle, E.A.E.

ABSTRACT

Due to a 2003 salmonellosis outbreak being linked to consumption of beef jerky produced by a New Mexico Firm, United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) issued, "Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants." The objectives of this study were: 1) to conduct preliminary studies to determine proper processing parameters for producing typical commercial jerky, 2) to determine the effects of typical thermal processing temperature/time combinations on reducing *E. coli* O157:H7 and *Salmonella* spp. in chopped and formed jerky, and 3) to determine whether xylose lysine desoxycholate (XLD) alone, or thin agar layer (TAL-XLD) method incorporating selective media (XLD) and nonselective media (TSA) would provide a better detection of injured cells. Both *E. coli* O157:H7 inoculated and *Salmonella* spp. inoculated raw jerky batter (ca. 7.0 $\log_{10}$ CFU/g) was dispensed into 2.54 cm by 0.64 cm by 15.24 cm strips. Strips were processed for 6.75 h and heat treated samples were collected at six different sampling times. Greater than or equal to 5.0 $\log_{10}$ CFU/g reductions were seen for both pathogenic populations after 45 min of processing at dry bulb temperatures of 55.6°C and relative humidity (R.H.) at <10%, followed by 45 min with dry bulb temperatures at 77.8°C and R.H. at <10%. However, product was dried further at 77.8°C for an additional 3.25 h to achieve moisture-to-protein ratios of approximately 0.75:1 and water activity levels of $\leq$ 0.70 characteristic of shelf stable jerky. *Salmonella* spp. counts on XLD and TAL-XLD were similar ($p > 0.05$) throughout the whole process. At the end of the thermal process, approximately 7.2 $\log_{10}$ CFU/g reductions of both *E. coli* O157:H7 and *Salmonella* spp. were observed, thus meeting USDA/FSIS validation guidelines for being deemed safe and shelf stable.

CHAPTER IV
EVALUATION OF THERMAL PROCESSING PARAMETERS IN PRODUCTION OF CHOPPED AND FORMED BEEF JERKY FOR CONTROLLING *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.

Introduction and Objectives

In 2003, the New Mexico Department of Health (NMDOH) linked an outbreak of Salmonellosis, which impacted 26 individuals, with consumption of beef jerky. This outbreak, caused by a rare strain, *Salmonella* Kiambu, resulted in the recall of 22,000 lbs. of beef jerky (FSIS, 2003). Due to the increasing commonality of foodborne illness associated with dried meats, in 2004, the United States Department of Agriculture/Food Safety and Inspection Service, published "Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants," which addresses the issues of how to obtain proper lethality, and how to verify proper drying (Sindelar, 2005; FSIS, 2004).

According to this publication, jerky processors must meet the performance lethality for *Salmonella*, a reduction of $\geq$ 6.5 $\log_{10}$ CFU/g. However, more recent literature acknowledged by the USDA/FSIS demonstrates that a 5.0 $\log_{10}$ CFU/g reduction of both *Salmonella* spp. and *E. coli* O157:H7 will now be accepted as indication of a "safe" product (FSIS, 2001; Uhler, 2005; Ingram, 2006). The Compliance Guideline outlines specific thermal processing parameters, such as humidity and wet bulb settings for jerky processors to utilize in order to achieve proper reductions.

Validation and challenge studies have recently become very important as a means to determine whether or not individual thermal processes are capable of producing a safe product. Researchers at many universities have conducted studies on both restructured and whole-muscle jerky processed in both home-style dehydrators, as well as commercial smokehouses (Harrison and Harrison, 1996; Harrison, et al., 1996; Faith, et al., 1998; Buege, et al., 2006).

Prior to conducting a validation study, six preliminary studies were conducted to determine proper processing parameters in order to obtain typical commercially prepared chopped and formed beef jerky. The objectives of the validation study were to determine the effects of typical thermal processing temperatures and times on reducing *E. coli* O157:H7 and *Salmonella* spp. in chopped and formed beef jerky and to determine whether xylose lysine desoxycholate (XLD) alone, or thin agar layer (TAL) method utilizing XLD with a thin top layer of Tryptic Soy Agar (TSA) would provide a better detection of injured cells.

Materials and Methods

Experimental Design:

Two treatments, consisted of an *E. coli* O157:H7 inoculated batch and a *Salmonella* spp. inoculated batch, as well as control batches of jerky were prepared. A replication consisted of both inoculated batches and a control batch placed in the smokehouse simultaneously. This study consisted of three replications.

Confirmation of Cultures and Presumptive *E. coli* O157:H7 and *Salmonella* spp. Colonies:

E. coli O157:H7 cultures used in this study were originally obtained from the American Type Culture Collection (ATCC 43890, ATCC 43894 and 43895; Rockville, MD), and Centers for Disease Control and Prevention (KSU-01 and KSU-02; CDC, Atlanta, GA). *Salmonella* spp. cultures were originally obtained from the ATCC (ATCC 4931, ATCC 35640, ATCC 13076, and ATCC 13311), and United States Department of Agriculture/Agricultural Research Service (USDA 15060; USDA/ARS, Athens GA). *Salmonella* spp. cultures included, three strains of *Salmonella enterica* subsp. *enterica* serovar Enteriditis, one strain of *Salmonella enterica* subsp. *enterica* serovar Abaetetuba, and one strain of *Salmonella enterica* subsp. *enterica* serovar Typhimurium. *E. coli* O157:H7 isolate ATCC 43894 was obtained from a human who experienced hemorrhagic colitis. ATCC 43895 was isolated from raw ground beef implicated in a case of hemorrhagic colitis. Isolates ATCC 43890, KSU-01 and KSU-02 were obtained from humans with *E. coli* O157:H7 infections. *S*. Enteriditis isolates ATCC 4931, ATCC 13076, and USDA 15060 and *S*. Typhimurium isolate ATCC 13311 were obtained from humans experiencing salmonellosis, while *S*. Abaetetuba isolate ATCC 35640 was obtained from creek water in Zaiman, Argentina.

For confirmation, all cultures and presumptive *E. coli* O157:H7 and *Salmonella* spp. colonies taken respectively from, phenol red sorbitol agar (PRSA; Difco, Detroit, MI) with 1% sorbitol (Fisher Scientific, Pittsburgh, PA) and xylose lysine desoxycholate agar (XLD; Remel, Lenexa, KS) plates from raw and heat-treated samples were streaked, using a sterile loop (Fisher Scientific Inc, Pittsburgh, PA) onto tryptic soy agar (TSA; Remel) and incubated at 37ºC for 24 h. Typical colonies were used to inoculate API 20E biochemical identification test strips (bioMerieux Vitek, Inc., Hazelwood, MO) and Enterotubes (Becton Dickinson, Franklin Lakes, NJ).

Preparation of Bacterial Culture:

A five-strain cocktail of *E. coli* O157:H7 (ATCC 43890, ATCC 43894, ATCC 43895, KSU-01 and KSU-02) and a separate five-strain cocktail of *Salmonella* spp. (ATCC 4931, ATCC 35640, ATCC 13076, ATCC 13311, and USDA 15060) were prepared. For inoculum preparation, one loopful of each culture was transferred into 9 mL of tryptic soy broth (TSB; Difco) and incubated at 35°C for 18-24 h. Six mL of each culture suspension was then transferred to a sterile bottle (Nalgene, Rochester, NY). Two separate bottles resulted with one containing 30 mL of *E. coli* O157:H7 and one containing 30 mL of *Salmonella* spp. One mL of each separate inoculum was saved for inoculum enumeration.

Meat Batter Preparation and Inoculation:

Fresh chopped and formed all-beef jerky batter containing: 83% lean beef, dextrose, flavorings, beef stock, salt, soy, corn protein, smoke flavoring, and curing agents was obtained from a commercial processor, immediately frozen for 1 to 2 weeks and then tempered overnight at 4ºC and used the following day. The product was separated into three 1.814 kg (4 lb) batches.

To the control batch, 29 mL of sterile deionized water was mixed evenly into the meat batter and stored at 4ºC until stuffing and enumeration. To a separate batch, 29 mL

of the *E. coli* O157:H7 five-strain inoculum was intermittently pipetted drop-wise over the batter surface and massaged with gloved hands until thoroughly mixed into the jerky batter. The batter was stored at 4ºC until stuffing and enumeration. This procedure was repeated with the last batch of batter; however, 29 mL of the *Salmonella* spp. five-strain inoculum was added instead.

Raw jerky batter (control followed by inoculated batches) was transferred to a manual jerky strip dispenser (Model Nbr. H6253, Grizzly Industrial, Inc.) with a 0.64 cm (¼") by 2.54 cm (1") jerky nozzle (Model Nr. LEM468D, Allied Kenco, Houston, TX). Strips were placed on polyscreen sheets (Excalibur, Sacremento, CA) with eight strips per sheet. Each strip was approximately 2.54 cm (1") by 0.64 cm (1/4") by 15.24 cm (6"). Strip filled sheets were placed on smokehouse trays with order varying per replication (Table 10). Once loaded, the smokehouse cart was placed in a commercial smokehouse (Alkar Model 450-UA, Alkar, Lodi, WI) and processed, using a typical commercial thermal processing procedure as discussed in Table 11.

Table 10. Order of placement in smokehouse (three replications) for control (C), *E. coli* O157:H7 (E), and *Salmonella* spp. (S) jerky batches.

Replication 1			**Replication 2**			**Replication 3**		
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E
E	S	C	C	E	S	S	C	E

Table 11. Thermal processing schedule[1] and sampling times for chopped and formed beef jerky.

Stage	Dry Bulb (D.B.) (ºC)	Time	Blower Speed	Sampling Time
1	55.6	14 min	Medium	
2	55.6	16 min	Medium	
3	55.6	14 min	Medium	
4	77.8	16 min	Medium	
5	77.8	14 min	Medium	
6	77.8	16 min	Medium	End of stage
7	77.8	14 min	Fast	End of stage
8	77.8	16 min	Fast	End of stage
9	77.8	14 min	Fast	
10	77.8	16 min	Fast	End of stage
11	77.8	14 min	Fast	
12	77.8	5 h	Fast	1.5 h into stage
End				End of stage 12

[1]The smokehouse has an automated damper system and the ability to inject steam humidity as needed to control humidity. Percent relative humidity remained at <10% throughout the entire smokehouse cycle. The exhaust fan was activated during the entire cycle.

E. coli O157:H7 and _Salmonella_ spp. Enumeration:

Phenol red sorbitol agar (PRSA) plates were used for enumeration of *E. coli* O17:H7 inoculated samples, while typically prepared XLD plates and plates containing 25 mL XLD with a 14 mL top layer of TSA (TAL-XLD) were used for the enumeration of *Salmonella* spp. inoculated samples (Wu and Fung, 2003). One mL of the *E. coli* O157:H7 inoculum was enumerated using duplicate PRSA plates. One mL of *Salmonella* spp. inoculum was enumerated using duplicate XLD and duplicate TAL-XLD plates. Six serial 1:10 dilutions of each inoculum were made using 0.1% peptone water (PW; Difco). Dilutions were then spread plated onto their corresponding media in duplicate.

Raw inoculated samples were taken from the inoculated jerky batter to determine the initial inoculum level of the product. Heat-treated samples were taken at six different times [after Stages 6, 7, 8, 10, 1.5 h into Stage 12, and at the end of the cycle]. At each sampling time, two samples (comprised of two strips) were taken from each of the three batches. Control samples were used for proximate analysis, pH, and water activity. The inoculated samples were used for *E. coli* O157:H7 and *Salmonella* spp. enumeration.

Two strips from each raw and heat-treated sample were cut into small pieces and combined together. Twenty-five gram samples were deposited into filtered stomacher bags to which 50 mL of PW diluent was added. The samples were pummeled in a stomacher (Stomacher Mix 1 Lab Blender, Microbiology International, Frederick, MD) for one min and two serial 1:10 dilutions were prepared using 9 mL 0.1% PW blanks. Dilutions were spread plated in duplicate onto PRSA for *E. coli* O157:H7 inoculated samples and XLD and TAL-XLD for *Salmonella* spp. inoculated samples.

A 1:3 dilution allowed for a 1.5 $\log_{10}$ CFU/g detection limit for samples pulled at the end of Stages 6, 7, 8, 10, and 1.5 h into Stage 12. For samples taken at the end of the cycle, a 1:3 dilution was spread-plated (0.25) onto four plates in duplicate, allowing for a sample detection limit of 0.5 $\log_{10}$ CFU/g. All media was incubated at 37°C for 24 h.

E. coli O157:H7 and _Salmonella_ spp. Enrichment:

Heat-treated samples were enriched by adding 225 mL of modified *E. coli* medium (mEC; Difco) containing sodium novobiocin (Sigma Chemical Co.; St. Louis, MO) to 25 g of *E. coli* O157:H7 inoculated samples and by adding 225 mL of Preuss broth (Preuss; Difco) to 25 g of *Salmonella* spp. inoculated samples taken 1.5 h into Stage 12, and at the end of the cycle. All samples were stomached for 1 min. After incubation at 37°C for 24 h, the enriched samples were streaked onto PRSA for *E. coli* O157:H7 inoculated samples and XLD and TAL-XLD for *Salmonella* spp. inoculated samples. Plates were incubated at 37°C for 24 h and observed for typical colonies (PRSA-colorless colonies; XLD/TAL-XLD-black colonies). Presumptive colonies were then confirmed using API 20E test kits and Enterotubes.

pH and Water Activity (a_w):

The pH level was measured on raw and heat-treated control samples (2) by weighing 25 g of sample into a filtered stomacher bag and adding 50 mL PW and stomaching for 1 min. A pH meter (Corning Pinnacle 530 pH Meter, Corning, NY) was used to measure pH. Readings were recorded when the stabilized icon was displayed.

Water activity was measured on raw and heat-treated control samples. Two samples were taken per treatment. Each sample was from a different set of combined strips. Duplicate readings were taken. A water activity meter (Model CX2, Aqualab, Pullman, WA) was used to measure water activity. Readings were recorded when the stabilized icon was displayed.

Proximate Analysis:

Samples for proximate analysis were taken from non-inoculated raw control, 1.5 h into Stage 12, and End of Stage 12 (Final) product samples. Fifty grams of raw sample, two jerky strips from 1.5 h into Stage 12, and two finished jerky strips were vacuum packaged and placed in frozen storage (-80°C) prior to analysis. Samples were prepared by freezing in liquid nitrogen and pulverized in a Waring Blender. Moisture (AOAC 985.14) and fat (AOAC 985.15) were determined by a rapid microwave solvent method (CEM, Matthews, NC, U.S.A.). Protein was determined by the LECO method (AOAC 990.03) and salt was analyzed by a volumetric method (AOAC 935.47).

Statistical Analysis:

The experimental design was a strip-split plot and data were analyzed using PROC MIXED in SAS Version 9.0 (SAS Institute, 2002). The fixed effects were media types, sampling time, and bacteria types along with all combinations of these effects. The random effects were replication, replication by sampling time by bacteria type, replication by sampling time, and replication by bacteria type. Significant interactions ($p \leq 0.05$) were sampling time by bacteria type and media type. LS Means were used to compare sampling time by bacteria type and media type. Least significant differences

(LSD) were used to compare LS Means of sampling times, bacteria types, and media. Proximate analysis, pH, and water activity mean values and standard deviations were calculated by Microsoft Excel.

To determine statistical differences between pathogen counts at different sampling times, duplicate plate counts were averaged. For product pulled at the end of Stages 6, 7, 8, 10, and 1.5 h into Stage 12, when duplicate plate counts had no colonies, they were assigned a $\log_{10}$ CFU/g value of 0.8. This assumed that the actual value was between zero and the detection limit, 1.5 $\log_{10}$ CFU/g. Similarly, for samples pulled at the end of the smokehouse cycle, when duplicate plate counts had no colonies, they were assigned a $\log_{10}$ CFU/g value of 0.3, which assumed that the actual value was between zero and the detection limit, 0.5 $\log_{10}$ CFU/g. However, for determining log reductions only the detection limit will be used as a value.

Results and Discussion

***E. coli* O157:H7 and *Salmonella* spp. Enumeration and Enrichment:**

Initial levels of the five-strain inoculum of *E. coli* O157:H7 and *Salmonella* spp. averaged on PRSA, XLD and TAL-XLD respectively, over three populations, were, 9.3, 9.0 and 9.2 $\log_{10}$ CFU/g. There were no statistical differences ($p > 0.05$) between the two media types used to enumerate inoculum level of *Salmonella* spp. Initial level of bacterial populations in raw inoculated meat was similar ($p > 0.05$) for both pathogens with raw batter counts ranging from 7.2 to 7.4 $\log_{10}$ CFU/g on PRSA and 7.1 to 7.4 $\log_{10}$ CFU/g on XLD and TAL-XLD (Table 12). When product reached Stages 6, 7, 8, and 10, counts ranged from <1.5 (detection limit) to 2.7 $\log_{10}$ CFU/g on PRSA. During these stages, populations recovered for *E. coli* O157:H7 were consistently decreasing ($p \leq 0.05$). For XLD and TAL-XLD, Stages 6, 7, 8, and 10 achieved similar ($p > 0.05$) recoveries with counts ranging from 1.4 to 2.1 $\log_{10}$ CFU/g.

When processed at dry bulb temperatures as high as 55.6°C (132°F) for 45 min followed by dry bulb temperatures of 77.8°C (172°F), and with relative humidity levels at < 10%, $\geq$ 5.0 $\log_{10}$ CFU/g reductions (Figure 1) of both pathogen populations were seen in as little time as 1.5 h (Stage 6) into the 6 h and 45 min cycle, meeting guidelines suggested by USDA/FSIS. At all sampling times, internal product temperatures leveled out at 78.9°C (174°F), meeting the USDA/FSIS recommendation of heating jerky product to an internal temperature of 71.1°C (160°F), also noted as a temperature that eliminates bacterial growth (FSIS, 2000). Although the appropriate log reductions were achieved by the end of Stage 6, product was dried further in order to meet Compliance Guideline recommendations suggesting that a water of activity of $\leq$ 0.70 be met in order for jerky to be considered shelf stable, as well as the USDA/FSIS requirement of a moisture-to-protein ratio (MPR) of $\leq$ 0.75:1.

Table 12. Mean *E. coli* O157:H7 populations (log_{10} CFU/g) recovered on PRSA and mean *Salmonella* spp. populations recovered on XLD and TAL-XLD at seven sampling times during production of chopped and formed beef jerky.

	Media		
Stage/Sampling time	**PRSA**	**XLD**	**TAL-XLD**
Raw	7.3 ± 0.1^{a}	7.2 ± 0.1^{ax}	7.3 ± 0.1^{ax}
Stage 6	2.3 ± 0.4^{b}	1.7 ± 0.1^{bx}	1.9 ± 0.2^{bx}
Stage 7	2.0 ± 0.3^{c}	1.7 ± 0.3^{bx}	1.9 ± 0.1^{bx}
Stage 8	1.8 ± 0.2^{d}	1.7 ± 0.1^{bx}	1.8 ± 0.1^{bx}
Stage 10	1.6 ± 0.0^{e}	1.6 ± 0.0^{bx}	1.7 ± 0.0^{bx}
Stage 12 (1.5 h into stage)	$<1.48^{f}$	$<1.48^{cx}$	$<1.48^{cx}$
End of stage 12	$<0.48^{g}$	$<0.48^{dx}$	$<0.48^{dx}$

[a-f] Indicates significant differences in a column within the heating process.
[xy] Indicates significant differences between XLD and TAL-XLD counts for that sampling period.

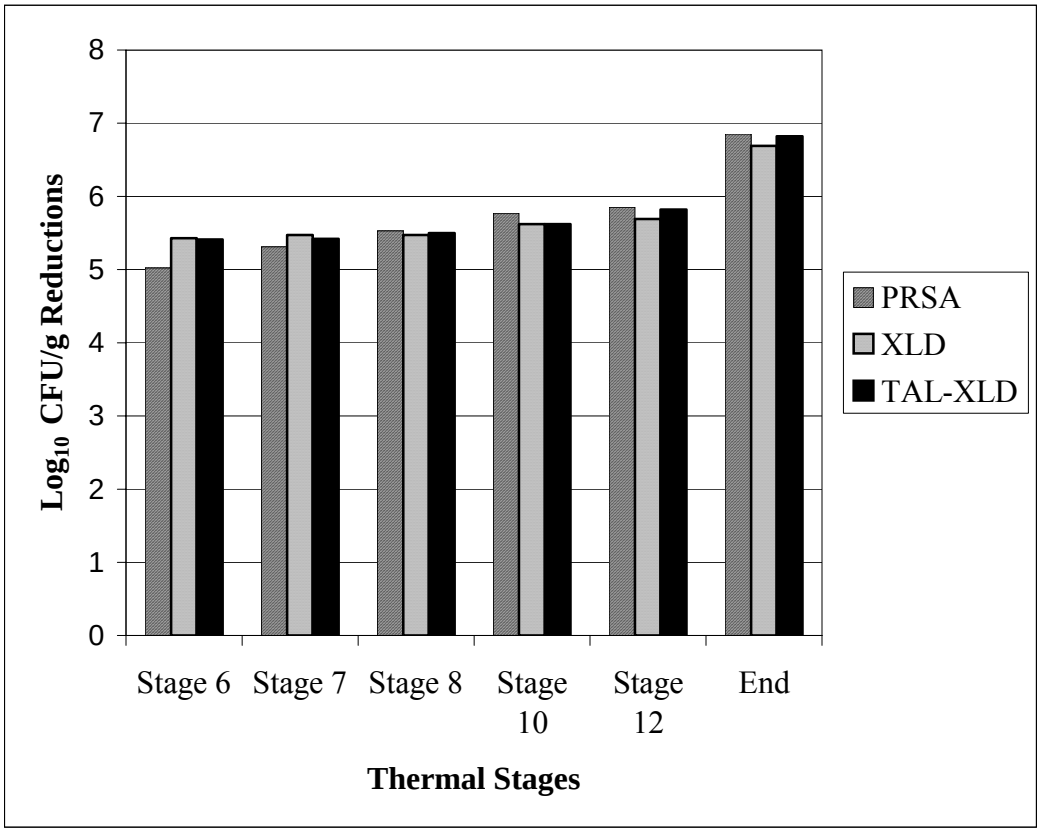

Figure 1. Average log reductions (log_{10} CFU/g) for chopped and formed beef jerky inoculated with either *E. coli* O157:H7 or *Salmonella* spp.

By 1.5 h into Stage 12, there were no populations recovered for any of the pathogens, with counts reported as < 1.5 log_{10} CFU/g (detection limit). At the end of the

cycle, finished product counts were consistently <0.5 $\log_{10}$ CFU/g on all media types (Table 12) averaging final reductions of $\geq$ 6.5 $\log_{10}$ CFU/g for both *E. coli* O157:H7 and *Salmonella* spp. (Figure 1), meeting Appendix A lethality performance standards for *Salmonella* spp. (FSIS, 1999) and guidance specified by USDA/FSIS stating that $\geq$ 5.0 $\log_{10}$ CFU/g reductions of both *E. coli* O157:H7 and *Salmonella* spp. are indicative of safe jerky processes (FSIS, 2001; Uhler, 2005).

Enrichment results for samples from 1.5 h into Stage 12 and end product samples showed negative confirmation for all 24 samples tested, confirming the likelihood that pathogens were dead as opposed to being heat-injured. Thus, indicating an even greater reduction of approximately 7.2 $\log_{10}$ CFU/g for both pathogens.

Results indicate that high humidity, such as the $\geq$ 90% relative humidity stated in the Compliance Guideline, is not necessarily needed to achieve appropriate $\log_{10}$ reductions. This is important as resentment towards the Compliance Guideline is due to the fact that many small and very small processors argue that their equipment is not capable of achieving the specified high humidity levels (AAMP, 2004).

Other research in which similar results as ours were obtained include a study conducted by Faith, et al. (1998), in which home dehydrators were used to process *E. coli* Ol57:H7 inoculated, restructured jerky. Batches of inoculated strips were dried at several different temperature/time combinations, with no added humidity. Results indicated 5 $\log_{10}$ CFU/g reductions for jerky processed at lower dry bulb temperatures, such as 52°C (125°F) when processed for longer periods of time, i.e., 8-10 h, and when processed at higher dry bulb temperatures, such as 68°C (155°F) for shorter periods of time, i.e., 4-6 h.

In addition to this, a study by Buege et al. (2006), in which a commercial smokehouse was used to process whole-muscle beef jerky inoculated with *Salmonella* serovars and *E. coli* O157:H7 showed that while processing parameters with wet bulb spikes, resulting in increased humidity, produced $\geq$ 5.0 $\log_{10}$ CFU/g reductions of both pathogens, a four-hour drying cycle processing at 48.9ºC (120F) for 60 min, 54.4ºC (130ºF) for 60 min, 60ºC (140ºF) for 60 min, and 76.7ºC (170ºF) for 60 min, without added humidity, was also capable of achieving sufficient reductions.

Although TAL-XLD was generally able to recover $\geq$ 0.1 $\log_{10}$ CFU/g more than XLD, results were not significantly different ($p > 0.05$) for samples collected at all sampling times. Although these results do not necessarily support the supposition that the thin agar layer (TAL) method recovers a greater portion of heat-injured cells than selective media alone, other research has provided evidence to support this theory.

In a 2001 study by Wu and Fung, the thin agar layer (TAL) method was used to enumerate four heat-injured foodborne pathogens including, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Yersinia enterocolitica*. One mL of pathogen cocktail was added to peptone water (PW) that had been preheated to 55°C. The suspension was then heated for ten minutes in a shaking water bath at 55°C. After heating, the suspension was immediately cooled to room temperature (25°C) and enumerated on both TAL agar plates and pathogen-selective agar plates. Results showed TAL to have significantly greater ($p \leq 0.05$) recovery of all five pathogens than pathogen-selective media alone.

pH and Water Activity (a_w):

Raw batter pH values ranged from 6.0 to 6.2. Final pH range for all products was 5.1 to 5.3 (Table 13). This falls below the typical pH range of 5.3-6.6 for jerky products (Lonnecker, 2005; Buege, 2005). This range is of particular concern as it easily supports the growth of gram-negative bacteria. For example, *E. coli* O157:H7 can grow at pH levels $\leq$ 4.4, while some *Salmonella* spp. can grow at pH levels as low as 3.8 (AAMP, 2004).

Although $\geq$ 5 $\log_{10}$ CFU/g reductions were achieved at the end of Stage 6, water activity levels ranged from 0.833 to 0.859. These values did not consistently fall under the level of $\leq$ 0.85, which excludes most pathogenic growth. Product was dried further in order to achieve a water activity of $\leq$ 0.70 which excludes mold growth, deeming the product shelf stable. Water activity 1.5 h into Stage 12 ranged from 0.619 to 0.692, while water activity range for all final products was 0.570 to 0.625 (Table 13). These values fall within the level of $\leq$ 0.70 suggested by FSIS to ensure lack of microbial growth (FSIS, 2004).

Proximate Analysis:

The FSIS's standard of identity for jerky is a moisture-to-protein ratio (MPR) of $\leq$ 0.75:1 (FSIS, 2004). For all final products, MPR was approximately 0.46:1. This ratio is in compliance with the FSIS standard. At 1.5 h into Stage 12, the MPR was approximately 0.77:1. Although product pulled at this time cannot be considered jerky, it does fall within the range of $\leq$ 2.03:1 and $\geq$ 0.76:1 typical of a kippered beef product (AAMP, 2004). With $\geq$5 $\log_{10}$ CFU/g reductions were clearly met at this point, processors producing kippered beef could use this thermal process as well to produce safe product.

Moisture content ranged from 52.3 to 56.0% for raw product and ranged from 14.6 to 19.0% for final product. Final product moisture levels fall below the typical range of percent moisture levels for jerky, which is 28-30% (Ricke and Keeton, 1997). Protein content for control raw product ranged from 16.1 to 16.9% and ranged from 34.8 to 37.6% for final product. Salt contents for raw treatments were approximately 2.2% and from 4.0 to 5.0% for final product (Table 13). These levels fall within the typical salt range for jerky which is 3.7 -11.4% (Lonnecker et al. 2005).

Although product pulled at the end of the cycle clearly met the requirements typical of shelf-stable jerky, product pulled at this point may lack pleasing palatability characteristics, as moisture and protein levels were below those typical of jerky. In order to achieve a more pleasing product, end product should be pulled between 1.5 h into Stage 12 and at the end of Stage 12. By keeping the MPR closer to 0.75:1 and the water activity as close to 0.70, a processor will also have decreased product costs, as water is an inexpensive ingredient. Jerky produced with these parameters will also be safe from a microbial standpoint.

Table 13 . pH and water activity means and standard deviations of control chopped and formed beef jerky at different sampling times.

Stage/Sampling Time	**pH Value**	**Water Activity**	**Percent Moisture**	**Percent Protein**	**MPR**	**Percent Fat**	**Percent Salt**
Raw	6.1±0.1	0.936±0.016	53.7±1.4	16.5±0.4	3.25:1	17.4±1.8	2.2±0.0
Stage 6	6.0±0.0	0.846±0.013					
Stage 7	6.0±0.1	0.829±0.024					
Stage 8	6.0±0.0	0.843±0.021					
Stage 10	6.0±0.1	0.819±0.017					
Stage 12 (1.5 h)	5.7±0.1	0.670±0.032	24.8±1.5	32.0±0.9	0.77:1	24.5±1.4	4.3±0.3
End of Stage 12	5.2±0.1	0.594±0.020	16.8±2.2	36.2±1.4	0.46:1	25.6±1.4	4.5±0.5

Conclusions

The thermal process used in the production of chopped and formed beef jerky resulted in approximately 7.2 $\log_{10}$ CFU/g reductions for both *E. coli* O157:H7 and *Salmonella* spp for product collected at the end of the process. No statistical differences were observed for *Salmonella* spp. counts on XLD and TAL-XLD. Reductions of $\geq$ 5.0 $\log_{10}$ CFU/g were achieved for both pathogenic populations after 45 min of processing at dry bulb temperatures of 55.6°C and relative humidity (R.H.) at < 10%, followed by 45 min with dry bulb temperatures at 77.8°C and R.H. of < 10%. However, product was dried further at 77.8°C in order to achieve moisture-to-protein ratios of $\leq$ 0.75:1 and water activity levels of $\leq$ 0.70 characteristic of a microbiologically safe, shelf stable jerky. With achievement of $\geq$ 5.0 $\log_{10}$ CFU/g reductions for both pathogenic populations, according to USDA/FSIS guideline, this process produced product considered safe and shelf-stable.

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NOTE TO READERS: *This report contains specific information about processing times and temperatures and is the most appropriate document to use for validation. It was reviewed by two meat scientists and is in layman's language that is easily understood by small and very small processors. It can also be easily accessed from the web* at www.asi.ksu.edu/cattlemensday under Publications and then pages 122-124 of Beef Cattle Research 2007.

Beef Cattle Research — 2007

THERMAL PROCESS FOR JERKY PROVIDES PROPER LETHALITY FOR CONTROLLING PATHOGENS

M. N. Roberts, K.J.K. Getty, and E.A.E. Boyle

Introduction

In 2003, the New Mexico Department of Health linked an outbreak of Salmonellosis with consumption of beef jerky. Due to the increasing commonality of foodborne illness associated with dried meats, in 2004 USDA/FSIS published the Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants, which addresses the issues of how to obtain adequate lethality and verify adequate drying. Small meat businesses that produce jerky products must validate that their processes achieve a 5-log reduction of *E. coli* O157:H7 and a $\geq$ 6.5-log reduction of *Salmonella*. The objective of this study was to determine the effects of thermal processing temperatures and times on reducing *E. coli* O157:H7 and *Salmonella* in chopped and formed beef jerky.

Experimental Procedures

Meat Batter Preparation and Inoculation. Fresh chopped and formed all-beef jerky batter was obtained from a commercial processor. The product was separated into three 4-lb batches. Two treatments, consisting of an *E. coli* O157:H7-inoculated batch and a *Salmonella*-inoculated batch, were prepared by adding an *E. coli* O157:H7 five-strain inoculum or *Salmonella* five-strain inoculum and thoroughly mixing into the jerky batter. A control batch was prepared by adding sterile deionized water into the meat batter.

Batter was extruded using a manual jerky gun with a 1/4-inch by 1-inch nozzle onto polyscreen sheets and then thermally processed in a commercial smokehouse (Table 1). A replication consisted of both inoculated batches and a control batch placed in the smokehouse simultaneously. Three replications were conducted.

***E. coli* O157:H7 and *Salmonella* Enumeration**. Raw inoculated samples were taken from the inoculated jerky batter. Heat-treated samples were taken at six different times (end of stages 6, 7, 8, 10; 1.5 hours into stage 12; and at the end of the stage 12; Table 1). Population levels of *E. coli* O157:H7 and *Salmonella* were determined for both raw and heat-treated samples. In addition, heat-treated samples with counts below the detection limit were tested for a positive or negative level of either *E. coli* O157:H7 or *Salmonella*.

Water Activity (a_w), pH, Proximate Analysis, and Salt. Water activity and pH levels were determined on control samples. Samples for proximate analysis (moisture, fat, and protein) and salt content were taken from the non-inoculated raw control batch 1.5 hours into stage 12 and at the end of stage 12 (final).

Results and Discussion

For all *E. coli* O157:H7- and *Salmonella*-inoculated jerky strips, initial raw batter populations ranged from 7.3 to 7.4 log cfu/g and 7.1 to 7.5 log cfu/g, respectively. When the product reached stages 6, 7, 8, and 10, *E. coli* O157:H7 populations ranged from less than 1.48 (detection limit) to 2.68 log cfu/g and *Salmonella* counts ranged from less than 1.5 to 2.1 log cfu/g. By 1.5 hours into stage 12, counts were consistently less than 1.5 log cfu/g on all media. End-product *E. coli* O157:H7 and *Salmonella* populations were consistently <0.5 log cfu/g.

There was ≥ 5.0 log cfu/g reduction of *E. coli* O157:H7 at all sampling times as required by USDA/FSIS, with the most consistent reductions being after stage 7. A ≥ 6.5 log cfu/g reduction of *Salmonella*, as mandated by USDA/FSIS, was seen in stage 12 and at the end of the cycle (Figure 1). End product populations for both *E. coli* O157:H7 and *Salmonella* show reductions well above those mandated by USDA/FSIS.

Samples from 1.5 hours into stage 12 and end-product samples showed negative populations for both *E. coli* O157:H7 and *Salmonella* for all samples tested, confirming the likelihood that pathogens are dead as opposed to heat-injured.

Moisture content ranged from 52.4 to 56.0% for raw product and 15.1 to 19.8% for the final product. Protein content ranged from 15.9 to 17.0% for raw product and 34.2 to 37.7% for the final product. Salt contents for raw products ranged from 2.2 to 2.3% and from 4.2 to 5.2% for final product. The moisture-to-protein ratio ranged from 0.4 to 0.6 for the final product. This ratio is in compliance with the requirement of an MPR less than 0.75:1 needed for the product to be labeled as "jerky".

Raw batter pH values ranged from 6.0 to 6.2. The final pH range for all products was 5.1 to 5.3. It should be noted that a lowered pH was not a determining factor for the reduction of *E. coli* O157:H7 or *Salmonella* populations.

Water activity range for all final products was 0.570 to 0.625. According to the USDA/FSIS Jerky Compliance Guidelines, water activity for jerky products should be ≤ 0.80 to ensure lack of microbial growth.

Implications

A thermal process for producing chopped and formed jerky provided proper lethality to control pathogens such as *E. coli* O157:H7 and *Salmonella* and provides a process that will produce safe jerky for consumers.

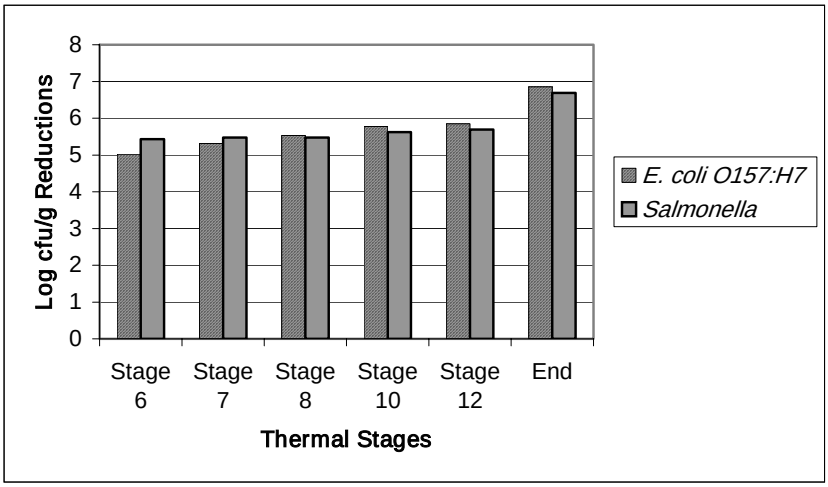

Figure 1. *E. coli* O157:H7 log CFU/g Reductions and *Salmonella* Reductions at Six Thermal Stages[a] during Production of Chopped and Formed Beef Jerky.

[a]Times and dry bulb smokehouse temperatures for thermal stages: stage 6 – 44 min at 132ºF and 46 min at 172ºF, stage 7 – 44 min at 132ºF and 1 hour at 172ºF, Stage 8 – 44 min at 132ºF and 1 hour 16 min at 172ºF, stage 10 – 44 min at 132ºF and 1 hour 46 min at 172ºF, stage 12 – 44 min at 132ºF and 3 hours 30 min at 172ºF, End – 44 min at 132ºF and 7 hours at 172ºF.

Table 1. Thermal Processing Schedule[a] and Sampling Times for Chopped and Formed Beef Jerky

Stage	Dry Bulb (D.B.) (ºF)[a]	Time	Blower Speed	Sampling Time	Cumulative Times and Temperatures at Each Sampling Time
1	132	14 min	Medium		
2	132	16 min	Medium		
3	132	14 min	Medium		
4	172	16 min	Medium		
5	172	14 min	Medium		
6	172	16 min	Medium	End of stage	44 min at 132ºF and 46 min at 172ºF
7	172	14 min	Fast	End of stage	44 min at 5132ºF and 1 h at 172ºF
8	172	16 min	Fast	End of stage	44 min at 132ºF and 1 h 16 min at 172ºF
9	172	14 min	Fast		
10	172	16 min	Fast	End of stage	44 min at 132ºF and 1 h 46 min at 172ºF
11	172	14 min	Fast		
12	172	5 h	Fast	1.5 h into stage	44 min at 132ºF and 3 h 30 min at 172ºF
End				End of stage 12	44 min at 132ºF and 7 h at 172ºF

[a]The smokehouse has an automated damper system and the ability to inject steam as needed to control humidity and the exhaust fan was running during the whole process. Percent relative humidity remained at less than 10% throughout the entire smokehouse cycle. Blower speed: Medium=788.8 ± 52.7 ft/min and fast speed = 1141.5 ± 111.9 ft/min.