Effect of natural antimicrobials against *Clostridium perfringens* outgrowth during cooling of turkey breast

by

Kristen Irene Peterson

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Major Professor
Elizabeth A. E. Boyle

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Abstract

The demand for natural, uncured processed meats has increased dramatically, resulting in the industry needing to find alternate ingredients to inhibit the outgrowth of *Clostridium perfringens.* The efficacy of natural antimicrobials to inhibit *C. perfringens* in processed meat products is not widely known. Two natural antimicrobials were evaluated in an uncured deli-style turkey breast product (1.5% salt) to determine inhibition of *C. perfringens* outgrowth during 15 h of chilling and to assess consumer acceptability. Four treatments of ground turkey breast were evaluated with the following formulations: control with no antimicrobials, 1.0% fruit/spice extract only, 1.0% dried vinegar only, and a combination of 1.0% fruit/spice extract and 1.0% dried vinegar. Treatments were inoculated with a three-strain mixture of *C. perfringens* spores to a targeted inoculation level of 2.0 log CFU/g. Individual 11 g portions were vacuum packaged, cooked to 71°C, and chilled from 54.4°C to 26.7°C in 5 h and from 26.7°C to 7.2°C in an additional 10 h. Triplicate samples were analyzed for growth of *C. perfringens* every 5 h by plating on tryptose-sulfite-cycloserine agar. A consumer panel (N=96) was also conducted to evaluate product for liking and acceptability. An interaction ($P<0.05$) between hour and treatment was observed for *C. perfringens* growth. The combination of fruit/spice extract and dried vinegar was found to be more effective ($P<0.05$) inhibiting outgrowth after 15 h than when these ingredients were used individually. Inclusion of antimicrobials did not affect ($P>0.05$) consumer ratings for liking. There were differences found ($P<0.05$) for consumer acceptability of flavor and flavor expectation, with the fruit/spice extract treatment being more acceptable to consumers than control. These results show that there is a synergistic effect against the outgrowth of *C. perfringens* when using both fruit/spice extract and

dried vinegar together. It also shows that the use of either natural antimicrobial at these concentrations does not diminish the eating quality of the final product for consumers.

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Chapter 1 - Introduction

Clostridium perfringens is a significant foodborne pathogen, commonly found on meat and poultry. It is the causative agent for one of the most common foodborne illnesses, causing roughly 1 million cases each year (Centers for Disease Control and Prevention [CDC], 2018). *Clostridium perfringens* is an anaerobic spore-forming bacterium that can survive at temperatures ranging from 12°C - 60°C (54°F - 140°F), growing rapidly between 43°C - 47°C (109°F - 117°F) (CDC, 2018). Due to the robustness of the pathogen, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) requires inspected facilities to mitigate *C. perfringens* risk via controlled chilling. This is outlined in a document commonly known as Appendix B (USDA-FSIS, 2017).

The demand for natural and organic processed meat products has grown dramatically (Sebranek and Bacus, 2007). However, traditional antimicrobials such as lactates and diacetates cannot be used in the formulation of natural processed meats. The USDA-FSIS defined the term "natural" in the Food Standards and Label Policy Book (USDA-FSIS, 2005) as follows:

"…does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient…"

This definition poses a challenge to manufacturers to ensure the food safety of naturally processed meat products, specifically with respect to the growth inhibition of *C. perfringens*. Incorporation of sodium nitrite in a formulation enhances the efficacy of antimicrobials against *C. perfringens* outgrowth (King et al., 2015). The use of nitrite allows manufacturers a longer cooling time for products. However, without it, this cooling time shortens from 15 h to 6 h (USDA-FSIS, 2017). In 2019, Consumer Reports and Center for Science in the Public Interest filed a petition with the USDA-FSIS to redefine labeling of "uncured" or "no nitrates or nitrites

added" on products (Consumer Reports, 2019). Currently, products with these claims can have a natural source of nitrite, typically from vegetable powders. If USDA-FSIS considers prohibiting the use of nitrite in products with claims such as "uncured" or "no nitrates or nitrites added", manufacturers will have to find natural alternatives of antimicrobials that can inhibit *C. perfringens* outgrowth in uncured products over an extended cooling cycle.

Therefore, research needs to be conducted on natural antimicrobials that could potentially inhibit the growth of *C. perfringens* in uncured meat products. Certain vinegars and fruit extracts have been shown to inhibit *C. perfringens* growth, with or without a natural source of nitrite (King et al., 2015). In addition, what is the impact of these ingredients from a consumer acceptability perspective? The objective of this study was to evaluate the effectiveness of two natural antimicrobials used separately and together against the outgrowth of *C. perfringens* in an uncured turkey breast product over a 15 h cooling cycle. An additional objective of this study was to evaluate the effects of the natural antimicrobials on product sensory attributes, including palatability.

Chapter 2 - Review of Literature

Turkey Industry

Over the last 50 years, the turkey industry has grown and evolved to meet the needs of consumers as another protein option alongside beef and pork. Since 1970, turkey production has increased 110% (National Turkey Federation, 2019). Turkey consumption continues to remain elevated, with people consuming 7.35 kg annually in 2018 (National Turkey Federation, 2019). The availability of turkey per capita has doubled in the last five decades, while the availability of beef has decreased, and the availability of pork has flatlined (Bentley, 2019).

Eating habits have also changed with consumers. Many are eating at home less often, and eating more at quick serve restaurants, full serve restaurants, and ready-to-eat (RTE) locations. Turkey has evolved from being a traditional dinner protein to one being consumed during other times of the day. In 2017, 45% of consumers reported consuming turkey as a snack, and 75% consumed turkey for lunch (Technomic, 2017). These trends have put turkey at the forefront as one of the preferred proteins for all eating occasions.

Clostridium perfringens

Clostridium perfringens is a Gram-positive, anaerobic, spore-forming rod (Fruin, 1977). There are five types (A through E) of this organism. Type A causes foodborne illnesses in humans (Fruin, 1977). *Clostridium perfringens* is found in the environment, specifically in soil and sediment, but can also be found in the normal intestinal microflora of animals and humans.

Therefore, it can also be found in areas prone to sewage contamination (Schneider et al., 2017). Because of the potential for cross-contamination, it is also commonly found on raw meat and poultry. Other common food sources include beef, gravies, and dried or pre-cooked foods high in protein (Schneider et al., 2017). This bacterium can survive high temperatures, up to 60°C. It replicates at optimal temperatures of 43°C - 47°C, having a generation time of less than 10 min (King et al., 2015).

Public Health Impact of *C. perfringens*

Clostridium perfringens is a foodborne pathogen that causes 10% of all reported cases of foodborne illnesses in the United States annually (Scallan et al., 2011). The vegetative cells of *C. perfringens* produce a toxin that can cause illness in humans (Grass et al., 2013). From 1998 to 2008, 536 outbreaks were reported from *C. perfringens*. This was found to be the most common pathogen causing illness when compared to *Staphylococcus aureus* and *Bacillus cereus* (Bennett et al., 2013). *Clostridium perfringens* also caused eight deaths during this 10-year period, more than the other two pathogens combined. Similarly, outbreaks caused by *C. perfringens* affected more people, with an average of seven per outbreak (Bennett et al., 2013).

Meat and poultry dishes were the most common foods associated with *C. perfringens* outbreaks. Of these dishes, 47% were from a restaurant or deli. Based on this data, it is important to be cautious with any meat or poultry item at a restaurant or deli, as it could be susceptible to *C. perfringens* (Bennett et al., 2013).

USDA-FSIS Guidelines for *C. perfringens*

According to the Centers for Disease Control and Prevention (2018), *C. perfringens* populations of at least 6 log can cause illness in humans. It is presumed that, with *C. perfringens* populations commonly found on raw meat and poultry, the raw material may have a 2 - 3 log concentration (USDA-FSIS, 2017). In June 2017, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) released updated guidelines for cooling of fully and partially heat-treated RTE and not-ready-to-eat (NRTE) meat and poultry products, also commonly known as Appendix B (USDA-FSIS, 2017). This guideline describes procedures to minimize the risk of *C. perfringens* outgrowth by cooling products quickly and avoiding environments that would permit *C. perfringens* growth. Four options for cooling were provided by USDA-FSIS (2017) to minimize growth to <1.0 log in thermally processed meat and poultry products. These options are as follows:

***Option 1 (for uncured products)*:** Product internal temperature should not remain between 54.4°C (130°F) and 26.7°C (80°F) for more than 1.5 h or between 26.7°C (80°F) and 4.4°C (40°F) for more than 5 h (6.5 h total cooling time).

Option 2 (for uncured products): Chilling should begin within 90 min after cooking cycle is complete. Product should be chilled from 48.9°C (120°F) to 26.7°C (80°F) in 1 h and from 26.7°C (80°F) to 12.7°C (55°F) in 5 h (6 h total cooling time).

Option 3 (for cured products): Product internal temperature should not remain between 54.4°C (130°F) and 26.7°C (80°F) for more than 5 h or between 26.7°C (80°F) and 7.2°C (45°F) for more than 10 h (15 h total cooling time). Product must contain at least 100 ppm ingoing sodium nitrite and 250 ppm sodium erythorbate or ascorbate.

Option 4 (for cured products): Product internal temperature should not remain between 48.9°C (120°F) and 4.4°C (40°F) for more than 20 h, and the cooling process should have a constant drop in product temperature or controls the product's temperature, so it does not stay between 48.9°C (120°F) and 26.7°C (80°F) for more than 2 h.

Antimicrobials Against *C. perfringens*

Nitrite

Sodium nitrite is the curing agent typically added to meat products as a preservative to inhibit growth of *Clostridium botulinum* and its deadly neurotoxin production (Juneja et al., 2010). It has been shown that *C. perfringens* can be resistant to nitrites, but nitrite is effective against *C. perfringens* when used in conjunction with salt above 2% (Juneja et al., 2010). Roberts and Derrick (1978) discovered the amount of sodium nitrite necessary to inhibit *C. perfringens* growth dropped from 300 ppm to 25 ppm when NaCl increased from 3% to 6%. This shows that there is a synergistic effect when nitrite is used in combination with salt.

With the increased demand for natural processed meats, the research community has been investigating natural sources of nitrate/nitrite as alternatives to sodium nitrite. Examples of these natural sources include vegetable powders (Sebranek and Bacus, 2007). These ingredients, when used with a nitrate-reducing bacterial culture, can provide a natural source of nitrite in the meat product. King et al. (2015) tested the efficacy of a natural nitrite source (cultured celery juice powder) against *C. perfringens* outgrowth in deli style turkey breast. At 50 ppm, they determined that cultured celery juice powder alone did not control outgrowth over a 15 h cooling

time, as they saw an increase in the *C. perfringens* population of 4.2 log. However, they found greater efficacy when used in conjunction with 0.7% dried vinegar, observing only a 1.5 log increase in *C. perfringens* over 15 h. They also observed that the cultured celery juice powder did not improve inhibition when used with 1% fruit extract or 1% cultured sugar-vinegar blend, as these ingredients were effective at controlling *C. perfringens* populations on their own (King et al., 2015). This research concluded that it is possible to inhibit *C. perfringens* outgrowth with or without nitrite sources. This is important to note, as consumers may not comprehend the definition of "naturally cured" or "uncured" based on current regulations (Sebranek and Bacus, 2007). This confusion has become even more evident in recent years. In 2019, Consumer Reports and the Center for Science in the Public Interest (CSPI) filed a petition to the USDA to redefine the labeling rule that allows the use of "uncured" or "no nitrate or nitrite added" on labels of natural meat products (Consumer Reports, 2019). With the attention drawn to the use of nitrite, regardless if it is from chemical or natural sources, manufacturers could be forced to remove it from processed meat products defined as natural. Therefore, research needs to be focused on non-nitrate/nitrite alternative ingredients that have the potential to inhibit outgrowth of *C. perfringens*.

Fruit and Spice Extracts

As the demand for natural meat products continues to grow, additional antimicrobial ingredients need to be identified and evaluated that can meet natural claims. Plant extracts and essential oils have been reported to inhibit *Listeria monocytogenes* in food applications (McDonnell et al., 2013). Juneja and Friedman (2006) investigated the efficacy of plant extracts

against *C. perfringens.* They evaluated carvacrol, cinnamaldehyde, thymol, and oregano oil at 0.1, 0.5, 1.0, or 2.0% (wt/wt) in ground turkey over cooling times from 12 h to 21 h. They observed that there was inhibition ($P<0.05$) for all oils tested in 12 h at all concentrations. They also observed that high concentrations of oils were needed to inhibit outgrowth for longer chilling times. Finally, they determined that cinnamaldehyde was more effective ($P<0.05$) than the other compounds tested.

Citrus fruits, like lemon, lime, and grapefruit, can have antimicrobial activity due to their high citric acid content (King et al., 2015). Fruits also contain phenolic compounds, which have shown antimicrobial activity (Cosansu and Juneja, 2018). Grapeseed extract (0 - 3%) was tested in *sous vide* cooked ground beef held at 15, 20, and 25°C to evaluate *C. perfringens* outgrowth (Cosansu and Juneja, 2018). They determined that the addition of grapeseed extract extended the time needed to reach 6 log CFU/g of *C. perfringens* 2.5-fold at 15°C.

King et al. (2015) evaluated the effect of a fruit extract blend in turkey by itself and in conjunction with a natural curing agent on *C. perfringens* outgrowth. Their results showed that 1% fruit extract was effective at controlling populations of *C. perfringens*, regardless if a natural curing agent was used. This demonstrates that the fruit extract blend can be used alone in a natural product to control *C. perfringens* outgrowth. It was unclear which fruit extracts in the blend contained the active components that inhibited the growth of *C. perfringens*. The authors stated that further testing needs to be conducted to determine what active ingredients are providing the antimicrobial properties.

These studies support the concept that fruit extracts can inhibit growth of *C. perfringens* in natural processed meat products. Little research has been conducted on the sensory impact when these fruit extracts are used in natural meat products. Therefore, commercial fruit extract

blends need to be further evaluated, not only for their efficacy against *C. perfringens* in specific substrates, but also for the impact they have on sensory attributes.

Vinegar

Vinegar is a natural source of acetic acid, which is an organic acid shown to inhibit outgrowth of *C. perfringens* during chilling in meat products like uncured turkey, roast beef, and ham (King et al., 2015). Organic acids can have an antimicrobial effect due to lowering the internal pH of the cell, resulting in metabolic inhibition by the undissociated or ionized acidic molecules (Sabah et al., 2003). The efficacy of vinegar against *C. perfringens* has been researched (King et al., 2015; Valenzuela-Martinez et al., 2010). King et al. (2015) tested dried vinegar, lemon-vinegar blend, and cultured sugar-vinegar blend at 0.7%, 2%, and 1%, respectively, calculated on total batch weight basis in turkey breast. They found that of the three types of vinegars, the cultured sugar-vinegar blend controlled the population of *C. perfringens* to at or below initial levels. In comparison, the dried vinegar and lemon-vinegar blend allowed for 2.0 and 2.5 log increases, respectively. King et al. (2015) also tested these vinegars in combination with a natural curing ingredient, cultured celery powder. They observed that the cultured celery powder did not ($P>0.05$) have a synergistic effect on *C. perfringens* outgrowth when used with the cultured sugar-vinegar blend. However, they did find a difference ($P<0.05$) when the lemon-vinegar blend was combined with the cultured celery powder versus the lemon-vinegar blend treatment alone (1.46 log and 2.52 log, respectively). Valenzuela-Martinez et al. (2010) tested the use of a buffered vinegar at 0.75%, 1.25%, or 2.5% and a buffered lemon juice-vinegar blend at 1.5%, 2.5%, or 3.5% in ground turkey roast to determine its potential effect

against the growth of *C. perfringens* over extended cooling cycles, ranging from 6.5 h to 21 h. They found that buffered vinegar at 2.5% and buffered lemon juice-vinegar blend at 3.5% were effective at inhibiting growth to <1.0 log CFU/g within 21 h. These results demonstrate that vinegar, whether used alone or in conjunction with other ingredients, could be an effective antimicrobial against *C. perfringens* in natural meat products.

Research assessing the sensory attributes resulting from incorporation of a vinegar source in turkey products is limited. Since vinegar is an acid, there is concern on how vinegar would affect the overall taste and quality of a processed meat. Valenzuela-Martinez et al. (2010) emphasized the need for investigation of vinegar-based ingredients on the sensory acceptability and shelf stability of meat products.

Salt and Salts of Organic Acids

Sodium and potassium salts are commonly used in meat and poultry products for flavor enhancement or to extend the microbiological shelf stability (Thippareddi et al., 2002). When used at levels above 5% (wt/vol), salt in the form of NaCl in media inhibits the outgrowth of *C. perfringens* (Juneja et al., 2010). However, these salt levels are higher than what is typically found in processed meats. Similarly, increased levels of salt for food safety is not desired in meat products due to health concerns (Li et al., 2012).

Research has been done to evaluate the efficacy of sodium and potassium salts of organic acids against *C. perfringens* outgrowth (Kennedy et al., 2013; Li et al., 2012; Thippareddi et al., 2002; Valenzuela-Martinez et al., 2010). Kennedy et al. (2013) determined that 2% potassium lactate was effective at inhibiting growth of *C. perfringens* in uncured turkey when cooled for 10

or 12 h. Thippareddi et al. (2002) showed that sodium citrate at or above 1% was effective at controlling *C. perfringens* populations in roast beef and injected pork during a 21 h cooling cycle. While these ingredients are effective, they cannot be used in products defined as natural (Valenzuela-Martinez et al., 2010). Therefore, other ingredients need to be used to help inhibit *C. perfringens* outgrowth. These ingredients could prove more effective than salt alone. Li et al. (2012) studied varying salt concentrations at 1%, 1.5%, and 2% (w/w) with 0%, 2%, or 2.5% lemon juice-vinegar blend concentrations in roast beef against *C. perfringens* outgrowth from 6 h to 21 h of cooling. Their study showed that the incorporation of the lemon juice-vinegar blend at either 2% or 2.5% inhibited the outgrowth of *C. perfringens* to <1.0 log CFU/g, regardless of salt concentration or cooling time. With the demand for natural meat products, processors will be looking to these other natural ingredients to enhance product safety without increasing the sodium content of their meat products.

Labeling Regulations for Natural Food Products

The labels on food products provide information to the consumer. Manufacturers of food products have to follow certain regulations provided by USDA-FSIS as it relates to the labeling information, including claims such as "natural" for processed meat products (USDA-FSIS, 2005). In 1982, the USDA drew up a policy, providing initial guidance on the term "natural" for processed meat products (USDA-FSIS, 2009). Policy Memo 055 stated that "natural" could be used for labeling of meat and poultry products provided that the product did not contain artificial flavors, colors, or chemical preservatives (as defined in 21 CFR 101.22), and that the product and all its ingredients were minimally processed (USDA-FSIS, 2009).

In 2005, USDA-FSIS rescinded Policy Memo 055 and updated the guidance on "natural" claims based on case by case decisions from past years, placing the new guidance in the Food Standards and Labeling Policy Book (USDA-FSIS, 2005). This update included additional clarification on the definition of natural ingredients, stating that "sugar, sodium lactate (from a corn source) [at certain levels], and natural flavorings from oleoresins or extractives are acceptable for 'all natural' claims." (USDA-FSIS, 2009). At the time, the agency approved sodium lactate as a natural ingredient as it was considered a natural flavoring, not a preservative. In 2006, the USDA received information indicating sodium lactate may provide antimicrobial effects at levels approved for flavoring. This antimicrobial effect could extend the shelf life of the product, classifying it as a "chemical preservative" in 21 CFR 101.22 (USDA-FSIS, 2009). At the end of 2006, the USDA modified the policy, removing the allowance of lactates to be used in products with a natural claim (USDA-FSIS, 2009).

The labeling regulations for "uncured" and "no nitrates or nitrites added" are described in 9 CFR 317.17 (CFR, 2020). A product can be labeled "uncured" if it is prepared without nitrates or nitrites and the descriptive or common name of the product is permitted or required to include nitrates or nitrites (CFR, 2020). If a product is labeled "uncured", the product also needs to be labeled "no nitrate or nitrite added" (CFR, 2020). The USDA has not defined maximum nitrate or nitrite levels in products labeled as "uncured". Some ingredients may contain nitrates or nitrites, like beet juice powder, celery juice powder, or sea salt (Sindelar, 2017). If the USDA Labeling Division determines an ingredient may contain nitrates or nitrites, a disclaimer must be added, stating "no nitrates or nitrites added except for those naturally occurring in…" (Sindelar, 2017). While these terms are defined verbally by USDA, there is no official definition and clarification in the USDA Food Standards and Labeling Policy Book (Sindelar, 2017).

Sporulation of *C. perfringens*

Many variations of sporulation media have been developed for *C. perfringens*, but there is not one media that works for all strains (de Jong et al., 2002). Duncan and Strong (1968) conducted research to investigate these different mediums. They compared three mediums that showed effective sporulation for different *C. perfringens* strains. However, these mediums produced spores that were unable to survive at temperatures above 100°C (Duncan and Strong, 1968). A new media was developed to optimize the heat resistance of the spores in conjunction with producing an abundant crop. The results from this study showed that the new media was more effective at producing heat resistant spores for more strains of *C. perfringens* compared to the other common mediums used (Duncan and Strong, 1968).

Juneja et al. (1993) further optimized the Duncan Strong media to improve sporulation yield. They revisited the original formulation and experimented with the addition of caffeine and other related methylxanthines to promote sporulation. In this study, it was determined that the addition of caffeine increased spore yield 51-fold. The carbohydrate source was also reviewed and optimized. Raffinose replaced starch in the original formula. By implementing these two changes to the original Duncan Strong medium, sporulation increased for two of three *C. perfringens* strains tested (Juneja et al., 1993).

Sensory Evaluation of Natural Antimicrobials

There is minimal research evaluating consumer preference of processed meat products formulated with natural antimicrobials. As the industry continues to focus more on natural

antimicrobials to extend shelf life and food safety of meat products, more research is needed to evaluate consumer acceptability. Research has been conducted using trained panels to determine the impact of fruit extracts and vinegar on processed meat products (Bradley et al., 2011; Lee and Ahn, 2005). Lee and Ahn (2005) evaluated the impact of plum extract in turkey rolls at 1%, 2%, and 3% for odor, color, and texture using a ten-member trained panel. The panelists denoted product treated with plum extract was darker in color compared to control ($P<0.05$) but found texture and odor to be similar ($P>0.05$) between all treatments (Lee and Ahn, 2005). Pomegranate juice was tested at 1% in cooked chicken patties and evaluated by a trained sensory panel for appearance, juiciness, and overall palatability (Naveena et al., 2008). The trained panel found the treated chicken patty similar ($P>0.05$) to control for all characteristics. Acetic acid, the main component of vinegar, has also been the subject of trained sensory panels in processed meat products. Bradley et al. (2011) tested buffered vinegar at 2.5% and a 52% sodium lactate-48% vinegar blend at 2.5% in ground pork patties. The patties were evaluated for appearance, aroma, texture, taste, flavor and overall integrity by a trained eight-member sensory panel. The panelists found the buffered vinegar treatment scored higher ($P<0.05$) in juiciness compared to control but did not differ ($P>0.05$) in rancid aroma, texture, taste, flavor, or overall integrity (Bradley et al., 2011). Similarly, the lactate/vinegar blend did not differ ($P>0.05$) in juiciness or overall integrity compared to control. In addition to the trained panel, Bradley et al. (2011) conducted a consumer panel ($N=60$) to compare a control with a lactate/vinegar blend for consumer acceptability. The decision to test these two treatments was based upon color, microbial, and sensory descriptive data (Bradley et al., 2011). Consumers found the two treatments were similar ($P>0.05$) for acceptability of appearance, aroma, texture, flavor and overall acceptability. Samant et al. (2015) summarized research conducted on the sensory

effects of chemical and natural antimicrobials in poultry products. They found the most common natural antimicrobials came from essential oils (Samant et al., 2015). These oils, such as oregano, rosemary, and clove, have their own distinct flavor and odor which could mask off flavors or odors of poultry products. Similarly, these oils could potentially reduce consumer acceptability of the product. Samant et al. (2015) concluded by stating that further research is needed to determine the sensory impact of natural antimicrobials in poultry products.

After review of the current research, further studies need to be conducted to evaluate the efficacy of natural antimicrobials in processed meats against *C. perfringens* outgrowth as well as the impact the natural antimicrobials have on consumer preference.

Chapter 3 - Evaluation of Deli Style Turkey Breast

Introduction and Objectives

Clostridium perfringens is a significant foodborne pathogen, commonly found on meat and poultry (Centers for Disease Control and Prevention [CDC], 2018). It is the causative agent for one of the most common foodborne illnesses, causing approximately 1 million cases each year (CDC, 2018). *Clostridium perfringens* is an anaerobic spore-forming bacterium that can survive at temperatures ranging from 12°C - 60°C (54°F - 140°F), growing rapidly between 43°C - 47°C (109°F - 117°F) (CDC, 2018). Due to the robustness of the pathogen, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) requires inspected facilities producing meat products to mitigate *C. perfringens* risk via controlled chilling. This is outlined in a document commonly known as Appendix B (USDA-FSIS, 2017).

The demand for natural and organic processed meat products has grown nearly 20% each year from 1990-2006 (Sebranek and Bacus, 2007). However, traditional antimicrobials such as lactates and diacetates cannot be used in the formulation of natural processed meat. This poses a challenge to manufacturers to ensure the food safety of naturally processed meat products, specifically the growth inhibition of *C. perfringens.* The incorporation of sodium nitrite in a formulation contributes to inhibition of *C. perfringens,* allowing manufacturers a longer cooling time for products. However, without the use of sodium nitrite, this cooling time drastically shortens from 15 h to 6 h (USDA-FSIS, 2017).

Therefore, research needs to be conducted on natural antimicrobials that could potentially inhibit the growth of *C. perfringens* in natural meat products. Certain vinegars and fruit extracts

have been shown to inhibit growth in conjunction with a natural source of nitrite (King et al., 2015). In addition, it needs to be understood how natural antimicrobials affect the overall quality and palatability to the consumer. The objective of this study was to evaluate the effectiveness of two natural antimicrobials against the outgrowth of *C. perfringens* in an uncured turkey breast over a 15 h cooling cycle. This cooling cycle was chosen for this study to showcase the efficacy of the antimicrobials against *C. perfringens* outgrowth. According to Appendix B, the product evaluated in this study would fall under a 6 h cooling cycle to inhibit *C. perfringens* outgrowth to <1.0 log. Therefore, extending the cooling cycle to 15 h could increase *C. perfringens* populations to >1.0 log, allowing evaluation of a "worst-case" scenario to challenge the efficacy of natural antimicrobials to inhibit the outgrowth. An additional objective of this study was to evaluate the effects of the natural antimicrobials on product sensory attributes.

Materials and Methods

Experimental Design

This study evaluated four natural antimicrobial treatments incorporated into a low sodium, deli-style turkey breast product having standardized levels of 1.5% salt and 1% sugar in finished product. The treatments included a control (no antimicrobial added), or the addition of 1% dried vinegar (Kerry Ingredients, Beloit, WI), 1% fruit/spice extract (Prosur Inc., Naperville, IL), or 1% each of the dried vinegar and fruit/spice extract into the deli style turkey breast formulation. The ingredient percentages were based on finished product weight per the recommendation of the manufacturers. The fruit/spice extract ingredient also contained 50%

salt, so adjustments were made by reducing free salt by the same amount in the treatments containing fruit/spice extract. All treatments were inoculated with *C. perfringens* spores, vacuum packaged, and held at 4.5°C for 12 -18 h. The treatments were then heat shocked in a water bath to 71°C and placed in an air incubator with refrigeration capabilities set at 60°C. Once all treatments were transferred, the incubator was programmed to follow a 15 h cooling cycle, from 54.4°C to 26.7°C in 5 h, and 26.7°C to 7.2°C in 10 h. Samples were pulled every 5 h which corresponded to temperatures of 26.7°C, 12.8°C, and 7.2°C. For each formulation and timepoint, three samples were evaluated for *C. perfringens* populations on tryptose-sulfite-cycloserine (TSC) media (Oxoid Microbiology Products, Hampshire, United Kingdom). The study was replicated three times.

A consumer panel was conducted at the Kansas State University Meat Science Sensory Laboratory. Panelists (N=96) were recruited from Manhattan, KS and the surrounding communities. Each panelist evaluated 4 samples (1/treatment) in random order and were asked to rate each sample for traits of appearance, texture, flavor, aftertaste, and overall liking on a 100 point line scale. Anchors were located at 0 and 100, with 0 labeled as dislike extremely and 100 labeled as like extremely. Each scale also had a midpoint at 50 labeled as neither like nor dislike. Finally, panelists evaluated each trait as either acceptable or unacceptable.

Product Preparation

Four product formulations (Table 1) were made using water, salt (Cargill, Inc., Wayzata, MN), sugar (United Sugars Corporation, Bloomington, MN), fruit/spice extract, and dried vinegar. Fresh turkey breast meat (<3 days postmortem) was sourced from a commercial

supplier (Jennie-O Turkey Store, Wilmar, MN), and received at <4.5°C. Product was immediately ground through a 6.35 mm plate using a grinder (Biro G58483, Biro Manufacturing Co., Marblehead, OH). Individual brines were made for each treatment by mixing salt and chilled water (0°C to 4.45°C) in a sanitized plastic tub until the salt was completely dissolved. Sugar, fruit and spice extract, and/or dried vinegar were then added in that order. The ingredient amounts were calculated based on the target percent desired in finished product. This calculation accounts for the product retention and yield at packaging. The brines were added to the ground turkey at 20% enhancement to achieve a product retention at 20% and an overall percent yield of 120%, as the product was cooked in a cook-in-bag style casing. The temperature of the brine for all treatments was between 0°C and 7.2°C. The slurry was mixed for 4 min on low speed (Hobart N-50, Troy, OH). The slurry was then placed in a 22.86 cm x 55.88 cm bag (Sealed Air Cryovac BH4670 series, Duncan, SC, water transmission rate 0.5-0.6 g/645.16 cm^2/24 h @ 37.78°C, 100% relative humidity; oxygen transmission rate 3-6 cm^3/m^2/24 h @ 4.45°C, 0% relative humidity), vacuum sealed (Multivac A300, Wolfertschwenden, Germany), and placed in a 4.5°C cooler for 24 h before inoculation.

Table 1. Formulation of four treatments for deli style turkey breast (in percent).

Treatment	Skinless, boneless, turkey breast meat	Water	Salt	Sugar	FS[1]	DV[2]
Control	83.3%	14.2%	1.5%	1.0%	-	-
Fruit/Spice Extract	83.3%	13.7%	1.0%	1.0%	1.0%	-
Dried Vinegar	83.3%	13.2%	1.5%	1.0%	-	1.0%
FS/DV	83.3%	12.7%	1.0%	1.0%	1.0%	1.0%

[1]Fruit and Spice Extract.
[2]Dried Vinegar.

For the sensory portion of the research, remaining slurry from each treatment was stuffed (Handtmann VF608) in a 109 mm x 270 mm plastic casing (Viscofan USA Inc., Montgomery, AL), clipped with 400G clip (Tipper Tie, Apex, NC) and held for 12 h at 4.5°C. Each chub was stuffed to a target weight of 3.63 kg. Product was then steam cooked following a programmed cycle (Alkar RapidPak, Lodi, WI), set at 57°C for 60 min, 65.5°C for 60 min, 73.9°C for 60 min, then 79.4°C until product reached an internal temperature of 73.8°C (Appendix A). A spare chub was used to monitor product internal temperature during the cook cycle and the temperatures were recorded (Appendix B). A temperature logger associated with the oven was inserted into the center of the chub. The product temperature was monitored until it reached 73.8°C, and then the product was then placed in a cooler set at 3.8°C. After 72 h, the product was hand peeled and sliced to 3.175 mm thickness using a deli slicer (Hobart 1712E, Troy, OH). The slices were placed 10 per package into a 21.59 cm x 35.56 cm bag (Sealed Air Cryovac B2460 Series, Duncan, SC, water transmission rate 0.5-0.6 g/645.16 cm^2/24 h @ 37.78°C, 100% relative humidity; oxygen transmission rate 3-6 cc/m^2/24 h @ 4.45°C, 0% relative humidity) and vacuum sealed. Product was then high pressure pasteurized (Flow Pressure Systems, Sweden) at 600 mPa for 3 min to eliminate any post-process bacterial contaminants. Finished product was shipped overnight in refrigerated coolers with ice packs to Kansas State University for consumer sensory evaluation.

Clostridium perfringens Culture Preparation and Confirmation

Strains ATCC 13124, ATCC 12915, and ATCC 12916 of *C. perfringens* were obtained from Microbiologics (Microbiologics, St. Cloud, MN). Strain 13124 was sourced from a patient

infected with gas gangrene (Mollby and Holme, 1976). Strains 12915 and 12916 were sourced from outbreaks in London in the 1940s and 1950s (Hobbs et al., 1953). They were prepared separately using procedures modified from Kennedy et al. (2013). A 100 µl aliquot of stock culture was grown in 10 ml of a cooked meat medium (BD Diagnostic Systems, Sparks, MD) containing heart tissue granules and peptic digest of animal tissue, and incubated anaerobically for 24 h at 35°C. From this culture,100 µl was transferred to 10 ml of freshly steamed fluid thioglycolate medium (BD Diagnostic Systems, Sparks, MD) and incubated anaerobically for 24 h at 35°C. This cycle was repeated twice more to increase the spore crop. Next, 2.5 ml of the fluid thioglycolate culture was inoculated into 250 ml of freshly steamed, modified Duncan Strong medium with raffinose and caffeine (Hi-Media Laboratories LLC, West Chester, PA) and incubated anaerobically for 48 h at 35°C. Final spore levels were determined by pour plating prior to harvesting. Spore cultures were centrifuged (Model RC-3B, Sorvall, Wilmington, DE) at 4,700 x g for 20 min. The supernatant was discarded, and the pellet was suspended in 10 ml of 95% ethanol. After homogenizing with a vortex (Model G560, Scientific Industries, Bohemia, NY), the suspension was stored at 4.5°C for 2 h. It was centrifuged again at 4,700 x g for 20 min, the supernatant discarded, and the pellet rinsed with 10 ml of 0.85% saline solution (BD Diagnostic Systems, Sparks, MD). The pellet was homogenized in the saline solution with a vortex and centrifuged again at 4,700 x g for 20 min. The supernatant was discarded, and the pellet was rinsed again with 10 ml of 0.85% saline solution. This procedure was followed one more time.

Spores were enumerated through heat shocking 1.5 ml of each strain in a recirculating oil bath (Model 1157P, Polyscience, Niles, IL) set at 80°C for 10 min to kill vegetative cells, followed by serial dilution in Butterfield's Phosphate (World Bioproducts, Libertyville, IL) and

then pour plating with tryptose-sulfite-cycloserine (TSC) (Oxoid Microbiology Products, Hampshire, United Kingdom), adding a thin agar overlay of TSC after initial agar was set. The culture was incubated for 24 h at 35°C under anaerobic conditions.

Individual strains of spore crops were subsequently stored in 10 ml of 0.85% saline at -20°C for up to 6 weeks before preparing spore cocktails for inoculation. For each trial, fresh inoculum of the spore cocktail was prepared by adding approximately equal levels of the three strains to yield approximately 2.5 - 3 log CFU/g.

Inoculation

The four meat slurry treatments were portioned into 11 g samples. A negative control sample of each treatment for each time point was immediately vacuum sealed. Three samples of each treatment for each time point were aseptically inoculated with 110 µl of freshly prepared *C. perfringens* spore cocktail for a target inoculation level of 2.5 - 3.0 log. The inoculated samples were then vacuum sealed in a 15.24 cm x 30.48 cm impermeable pouch (UltraSource 3 ml vacuum pouch, Kansas City, MO, water transmission rate 6-0.75 g/m^2/24 h @ 25°C, 90% relative humidity; oxygen transmission rate 50-70 cc/m^2/24 h @ 25°C, 60% relative humidity) using a vacuum packaging machine (Multivac C400, Wolfertschwenden, Germany). To ensure proper distribution of the inoculum and consistent temperature profiles between treatments, the packages were manually massaged for one min using a T-spreader, then flattened by hand to ~ 2 mm thickness before vacuum sealing. Treatments were held for 12 - 16 h at 4.5°C before cooking and cooling.

Cooking, Cooling, and Sampling

Inoculated samples and the control were immersed in a 75°C water bath (Anova Precision Cooker, Anova Culinary, San Francisco, CA) and heated until the internal temperature of representative packages reached 71°C, which heat shocked the spores and killed any vegetative cells. Internal temperature of a representative package was monitored using a digital thermometer (Traceable Products, Webster, TX). The time to reach the target cook temperature was manually recorded, taking 75 sec. Cooked samples were then placed into an air incubator set at 60°C until all samples were transferred, which took about 20 min. The incubator was then programmed to follow a 15 h cooling cycle as defined by USDA-FSIS Appendix B (54.4°C to 26.7°C in 5 h and 26.7°C to 7.2°C in 10 h) (USDA-FSIS, 2017). A probe (Temperature Data Logger, DataTrace, Mesa Labs, Lakewood, CO) was placed in a representative product sample to measure product temperature. A second probe was placed in the incubator to measure environmental temperature. Both probes were programmed to collect data every 5 min throughout the duration of the cooling cycle (Appendix C).

Enumeration

Triplicate samples from each treatment group were pulled after heat shock, and at 0, 5, 10, and 15 h during the cooling cycle. An 11 g sample was diluted in 99 ml of Butterfield's phosphate buffer in a 15.24 cm x 30.48 cm impermeable pouch (UltraSource 3 ml vacuum pouch, Kansas City, MO, water transmission rate 6-0.75 g/m^2/24 h @ 25°C, 90% relative humidity; oxygen transmission rate 50-70 cc/m^2/24 h @ 25°C, 60% relative humidity) and

homogenized with a stomacher (AESAP1064, Biomerieux, Marcy-l'Étoile, France) for 1 min. Homogenates were serially diluted in Butterfield's phosphate buffer, and 1 ml of the sample was pour plated with TSC agar. An overlay of TSC agar was added once set. Plates were incubated anaerobically for 36 - 48 h at 35°C.

Chemistry Analysis

The meat slurries of all four treatments were analyzed for pH, salt, sugar, moisture, nitrate, nitrite and protein content, and the water used in the enhancement solution was analyzed for nitrate and nitrite. All samples were analyzed at an accredited lab (Hormel Foods Laboratory, Austin, MN). The lab is ISO certified (ISO/IEC 17025:2017) for salt, moisture and protein determination.

Treatment pH was measured using the method described in the USDA-FSIS Microbiology Laboratory Guidebook (USDA-FSIS, 1998). Salt was measured using the 4500-Cl, automated ferricyanide method as described in Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Sugar, moisture, nitrate/nitrite and protein were determined using AOAC methods 71:6, 950.46B(b), 993.30, and 992.15(6.25), respectively (AOAC International, 2012). All treatments were submitted for each repetition of the study.

Consumer Panel Evaluation

Consumers (N=96) were recruited from Manhattan, KS and the surrounding communities and monetarily compensated for participation. Consumer panels were conducted at the KSU

Meat Science Sensory Laboratory and approved by the Institutional Review Board (Appendix D). Panelists were seated roughly 1.8 m apart in a classroom auditorium. A total of 12 panels were conducted with eight consumers per session and lasted approximately 30 min. Panelists were provided with a ballot, toothpick, napkin, fork, knife, expectorant cup, and water, apple juice, and unsalted crackers for palate cleansers. Each ballot contained an informational sheet (Appendix E), a demographic questionnaire, a purchasing motivator sheet, and survey ballots for each sample to be evaluated (Appendix F). Prior to the start of each panel, instructions were given to consumers about how to fill out the ballot sheets and the testing procedures.

Each panelist evaluated four samples (one/treatment) in random order and recorded ratings on an electronic tablet (Model 5709 HP Stream 7; Hewlett - Packard, Palo Alto, CA) using a digital survey (Version 2417833; Qualtrics Software, Provo, UT). Panelists were asked to rate each sample for traits of appearance, texture, flavor, aftertaste, and overall liking on a 100 point line scale. All attributes were rated with anchors at 0 and 100, with 0 labeled as dislike extremely and 100 labeled as like extremely. Each scale also had a midpoint at 50 labeled as neither like nor dislike. Panelists also evaluated each trait as either acceptable or unacceptable based on their personal food habits and attitudes towards uncured sliced turkey breast meat. Finally, the panelists determined if the product met their expectation for flavor of a sliced turkey breast lunchmeat.

Statistical Design

A randomized complete block design with sample as the experimental unit was used for microbial data. Sampling time was used as a repeated measure. Analysis of variance (ANOVA)

was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) (Appendix G). Least square means were calculated for each independent variable. Statistical significance was set at $P<0.05$.

The chemistry data was analyzed via the PROC GLM procedure in SAS (SAS Inst. Inc., Cary, NC) (Appendix G). Least square means were calculated for each independent variable. Statistical significance was set at $P<0.05$.

Comparisons among treatments for the consumer panels were evaluated for significance using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) (Appendix G). The model was evaluated as a completely randomized design. The panel session number was included as a random effect. Consumer acceptability data was analyzed with a model that included a binomial error distribution.

Results and Discussion

Clostridium perfringens Outgrowth

Initial *C. perfringens* populations from when the product was heat shocked in the water bath are shown in Table 2. The target inoculation level was 2.0 log CFU/g, and all treatments had a similar initial inoculation level ($P>0.05$).

Table 2. Initial *C. perfringens* populations at heat shock in uncured turkey breast formulated with natural antimicrobial ingredients[1].

Treatment[2]	Log CFU/g
Control	2.22[a]
FS	2.06[a]
DV	1.89[a]
FS & DV	2.02[a]

[a] No significant difference (P>0.05).
[1] Standard error of the mean = 0.10.
[2] FS - fruit and spice extract, DV - dried vinegar.

There was a treatment by hour interaction (P<0.05) for mean log growth CFU/g of *C. perfringens* on TSC (Table 3). There was a 4.45 log increase of *C. perfringens* in the control treatment over 15 h of cooling. King et al. (2015) saw a population increase of 4.62 log in an uncured turkey breast containing 1.4% salt. The largest population difference was observed in the first 5 h of the cooling cycle (54.4°C to 26.7°C), with a 3.47 log increase in the control. The combination of fruit/spice extract and dried vinegar was found more effective (P<0.05) than when these ingredients were used individually after 15 h of cooling; however, all treatments containing antimicrobials met Appendix B guidelines, as the increase in *C. perfringens* populations were limited to <1.0 log. The dried vinegar treatment had a 0.81 log increase of *C. perfringens* over 15 h, with a 0.44 log increase observed in the first 5 h. In comparison to the other treatments containing antimicrobials, dried vinegar had the least antimicrobial activity against *C. perfringens* outgrowth (P<0.05). The fruit and spice extract treatment had a 0.07 log increase of *C. perfringens* over 15 h with a 0.32 log decrease in the first 5 h, and an increase of 0.44 log in 10 h. Finally, the treatment with both fruit/spice extract and dried vinegar saw a 0.67 log decrease of *C. perfringens* over 15 h, with a decrease of 0.46 log in the first 5 h.

Table 3. Treatment by time interaction on *C. perfringens* populations (log CFU/g) during a 15 h cooling cycle in uncured turkey breast formulated with natural antimicrobial ingredients[1].

	Chilling time (h)			
Treatment[2]	**0**	**5**	**10**	**15**
Control	1.92 ± 0.15^{gfh}	5.39 ± 0.51^{b}	6.42 ± 0.48^{a}	6.37 ± 0.22^{a}
FS	1.89 ± 0.14^{gfh}	1.56 ± 0.57^{ih}	2.33 ± 0.86^{ed}	1.96 ± 0.94^{gf}
DV	2.10 ± 0.16^{ef}	2.54 ± 0.32^{d}	3.10 ± 0.27^{c}	2.91 ± 0.15^{c}
FS & DV	2.06 ± 0.22^{ef}	1.60 ± 0.22^{gih}	1.59 ± 0.12^{gih}	1.39 ± 0.20^{i}

[1]Standard error of mean = 0.14.
[2] FS - fruit/spice extract, DV - dried vinegar.
[a-i]Least square means without a common superscript within table differ (P<0.05).

Between 5 and 10 h, the control treatment saw an increase of 1.03 log in *C. perfringens* population, while the fruit and spice extract treatment only saw an increase of 0.77 log and the dried vinegar treatment saw an increase of 0.56 log. The *C. perfringens* population in the combination treatment did not change (P>0.05) between 5 and 10 h. Between 10 and 15 h, the fruit and spice extract treatment had a 0.37 log decrease (P<0.05) in the *C. perfringens* population. All other treatments did not change (P>0.05) in populations during this time period. The combination treatment containing fruit/spice extract and dried vinegar was more effective at inhibiting *C. perfringens* outgrowth after 15 h of cooling than when these antimicrobials were used separately, demonstrating a synergistic effect. It is important to note that all treatments containing antimicrobials met Appendix B guidelines with <1.0 log growth at 15 h.

Research has demonstrated the efficacy of these antimicrobials against *C. perfringens* outgrowth (Juneja et al., 2004; King et al., 2015). Fruit and spice extract and dried vinegar contain naturally occurring organic acids or phenolic compounds. While the exact composition of the antimicrobial ingredients used in this study is not known, dried vinegar is a natural source of acetic acid. Phenolic compounds have been shown to have an inhibitory effect on Gram-

positive bacteria (Tiwari et al., 2009). The respective salts of acetic acid, sodium acetate and sodium diacetate at 1% each have been shown to inhibit growth of *C. perfringens* over 15 h of cooling in turkey breast (Juneja and Thippareddi, 2004). Conversely, King et al. (2015) found a 1.98 log increase in *C. perfringens* over 15 h of cooling with 0.7% dried vinegar in turkey breast. While King et al. (2015) used 0.7% dried vinegar in turkey breast, the usage level in this study was 1.0%. Another difference between the current study and the study conducted by King et al. (2015) is the salt content, with the current study targeting 1.5% salt in finished product and King et al. (2015) targeting 1.4%. Finally, King et al. (2015) included sodium tripolyphosphate and modified food starch in their formula, which can affect the overall moisture of the product compared to the current study. The inhibition of *C. perfringens* shown in this study and research by Juneja and Thippareddi (2004) supports the feasibility that natural sources of organic acids and other functioning compounds are effective antimicrobial agents in ready-to-eat meat.

Since the fruit/spice extract composition was not known, it is challenging to determine what active ingredients in this extract functioned to inhibit *C. perfringens* outgrowth. Other fruit extracts have been evaluated for their antimicrobial activity. Citrus fruits, like lemon, have a high citric acid content. Citric acid has been shown to inhibit *C. perfringens* in ground turkey roast (Valenzuela-Martinez et al., 2010). Extract from grapefruit, another fruit high in citric acid, was shown to inhibit *C. perfringens* outgrowth in a sous-vide chicken product when used at 200 ppm (60% grapefruit extract) in the product (Juneja et al., 2006). The spice in the fruit/spice extract may also play a role in contributing to antimicrobial properties. Essential oils of plants can inhibit the production of secondary metabolites in Gram-positive bacteria, like *C. perfringens* (Tiwari et al., 2009). A study testing the efficacy of certain plant oils like cinnamon, thyme, and oregano showed inhibition of *C. perfringens* outgrowth over 15 h in a ground turkey

product at 0.1%, 0.5%, 1.0% or 2.0% (wt/wt) (Juneja et al., 2007). Based on the results of the current study, it is possible that the fruit/spice extract ingredient is composed of a unique blend with active ingredients containing citric acid and essential oils to be an effective antimicrobial agent.

Chemistry Results

Chemistry results of the four treatments are shown in Table 4. Treatment pH, and salt and protein content were similar ($P>0.05$). The pH, salt concentration, and protein content of the treatments averaged 5.9, 1.5% and 19.2%, respectively. The fruit/spice extract treatment was higher in residual nitrite ($P<0.05$) than the control and dried vinegar treatment at 5.3 ppm; however, these values are consistent with observations from past research for uncured meats, with values found as high as 9.2 ppm (Sindelar et al., 2007). The combination treatment was higher ($P<0.05$) in residual nitrate compared to the control and fruit/spice extract treatment, but similar ($P>0.05$) to the dried vinegar treatment. These values are higher compared to past research for uncured meats, with values ranging from 10 - 17.6 ppm (Sindelar et al., 2007). This may be due to the residual nitrate found in the water source, which was found to have 17 ppm residual nitrate. The dried vinegar treatment and combination treatment were lower in moisture than the control ($P<0.05$) by 0.9% and 1.4%, respectively. The moisture content of the fruit/spice extract treatment was higher than the combination treatment ($P<0.05$) by 1.1%. This treatment was similar in moisture ($P>0.05$) to the control and dried vinegar treatments. This is attributed to the reduction of water in the formulation to account for the addition of the fruit/spice extract and dried vinegar ingredients. The formulations of the combination and

control treatment (Table 1), show the amount of water to be 12.7% and 14.2%, respectively. This explains the variation in the moisture content of the treatments. A reduced moisture content could inhibit initial growth of *C. perfringens* (Strong et al., 1970). Therefore, this difference could have marginally impacted the outgrowth of *C. perfringens* during the cooling cycle. The sugar content of the fruit and spice extract treatment was 0.9%, which was 0.2% lower ($P<0.05$) than the other three treatments. The lower level of sugar in the fruit and spice extract treatment potentially inhibited outgrowth of *C. perfringens* in the first 5 h of the cooling cycle, as a 0.33 log decrease was observed. Sacks (1983) found sucrose to be a better growth substrate for *C. perfringens* compared to other saccharides. Since there was a lower concentration of that growth substrate available, it is possible that it took more time for the *C. perfringens* populations to grow.

Table 4. Chemistry results of four treatments.

Treatment[1]	pH	Salt	Sugar	Moisture	Protein	Nitrate (ppm)	Nitrite (ppm)
Control	5.9	1.6%	1.1%[a]	76.9%[a]	19.2%	23.0[a]	0.3[a]
Fruit/Spice Extract	5.9	1.5%	0.9%[b]	76.6%[ab]	19.1%	23.0[a]	5.3[b]
Dried Vinegar	6.0	1.5%	1.1%[a]	76.0%[bc]	19.2%	25.7[ab]	0.0[a]
FS/DV	6.0	1.5%	1.1%[a]	75.5%[c]	19.2%	33.7[b]	1.0[ab]

[1]FS - fruit and spice extract; DV - dried vinegar.
[a-c] Least square means without a common superscript within columns differ ($P<0.05$).

Demographics of Consumer Sensory Panel

The consumer demographic data obtained from the sensory analysis survey is shown in Table 5. Of the 96 consumers in the study, 63.54% were male and panelists were predominately Caucasian. Additionally, 48.96% of panelists were <20 to 39 years old, and 51.04% were over the age of 40. Furthermore, 46.88% of participants indicated they had obtained a college degree, and 71.88% indicated they had an annual household income of more than $50,000 per year. Of the 96 panelists, 75.01% indicated they consume lunchmeat 1 - 5 times a week.

Table 5. Demographic characteristics of consumers (N=96) who participated in consumer sensory panels.

Characteristic	Response	Percentage of panel respondents
Gender	Male	63.54
	Female	36.46
Age	Under 20	21.88
	20 to 29 years old	19.79
	30 to 39 years old	7.29
	40 to 49 years old	11.46
	50 to 59 years old	27.08
	Over 60	12.50
Ethnic origin	Caucasian/White	91.67
	Hispanic	3.13
	African American	1.04
	Mixed Race	2.08
	Native American	1.04
	Other	1.04
Marital status	Single	45.83
	Married	54.17
Household size	1 person	20.83
	2 people	20.83
	3 people	21.88
	4 people	22.92
	5 people	7.29
	6 people	2.08
	>6 people	4.17
Income	<$25,000	16.67
	$25,000 - $34,999	5.21
	$35,000 - $49,999	2.08
	$50,000 - $74,999	15.63
	$75,000 - $99,999	18.75
	$100,000 - $149,999	16.67
	$150,000 - $199,999	14.58
	>$199,999	6.25
	Prefer not to answer	4.17
Education Level	Non-high school graduate	2.08
	High school graduate	14.58
	Some college/technical school	33.33
	College graduate	29.17
	Post college graduate	17.71
	Prefer not to answer	3.13
How many times a week do you consume luncheon meat?	0	2.08
	1-5	75.01
	6-10	12.50
	11-15	2.08
	Prefer not to answer	8.33

Consumer Sensory Evaluation

Results of the consumer sensory panel evaluation indicated there were no differences ($P>0.05$) among the treatments for degree of liking of flavor, appearance, texture, aftertaste, and overall liking (Table 6).

Consumers were also asked to indicate if each sample was acceptable for each trait based on their personal food habits and attitudes towards uncured sliced turkey breast. There were differences found ($P<0.05$) for consumer acceptability of flavor and expectations for flavor (Table 7). Of the panelists, 88.5% found the fruit/spice extract treatment acceptable for flavor. In comparison, only 76.0% found the control treatment acceptable for flavor. The dried vinegar treatment ranked similarly ($P>0.05$) to the control, and the combination treatment ranked similarly ($P>0.05$) with all treatments. For expectation of flavor, over 70% of panelists found the fruit/spice and combination treatments acceptable, while the control treatment only had 56.3% of panelists determining it acceptable. The dried vinegar treatment ranked similarly ($P>0.05$) to all treatments.

Table 6. Least square means for consumer (N=96) degree of liking[1] of uncured turkey breast meat with natural antimicrobial treatments.

Treatment[2]	Flavor	Appearance	Texture	Aftertaste	Overall
Control	57.6	56.8	62.5	55.1	57.0
FS	60.6	57.8	60.8	59.3	60.0
DV	56.9	59.7	62.4	55.2	57.9
FS & DV	63.9	56.3	67.2	59.6	63.8
SEM[3]	2.27	2.29	1.96	2.16	2.26
P-value	0.12	0.73	0.12	0.27	0.15

[1]Sensory scores: 0=dislike extremely; 100=like extremely.
[2] FS - fruit and spice extract, DV - dried vinegar.
[3]SEM - Standard error of mean.

Table 7. Least square means for percentage of consumers (N=96) who indicated traits of uncured turkey breast formulated with natural antimicrobial ingredients were acceptable.

Treatment[1]	Flavor	Appearance	Texture	Aftertaste	Overall	Expectation
Control	76.0[b]	82.3	91.7	74.0	76.0	56.3[b]
FS	88.5[a]	82.3	87.5	86.5	83.3	70.8[a]
DV	74.0[b]	88.5	94.8	76.0	76.0	64.6[ab]
FS & DV	82.3[ab]	81.3	87.5	81.3	80.2	74.0[a]
SEM[2]	0.04	0.04	0.03	0.04	0.04	0.05
P-value	0.04	0.52	0.21	0.11	0.50	0.05

[1] FS - fruit and spice extract, DV - dried vinegar.
[2]SEM - Standard error of mean.
[a,b] Least square means in the same column without a common superscript differ (P<0.05).

While the composition of the fruit/spice extract used in this study is not known, previous research has evaluated the impact fruit and spice compounds have on sensory traits in processed meat products. Plum extract was added to turkey rolls at 1%, 2%, and 3%, and evaluated for odor, color, and texture by a ten - member trained panel (Lee and Ahn, 2005). The panelists denoted a difference in color compared to control (P<0.05) but found texture and odor to be

similar (P>0.05) between all treatments (Lee and Ahn, 2005). Pomegranate juice was tested at 1% in cooked chicken patties and evaluated by a trained sensory panel for appearance, juiciness, and overall palatability (Naveena et al., 2008). The trained panel found the treated chicken patty similar to control (P>0.05) for all characteristics. In another study, Rojas and Brewer (2007) evaluated the sensory impact of grapeseed extract at 0.01% and 0.02% in ground beef and ground pork. A ten - member trained panel determined that grapeseed extract treatments were similar to control treatments (P>0.05) for rancidity, herbal, grassy, sulfur, and wet cardboard characteristics (Rojas and Brewer, 2007). Green tea is another compound that has been tested due to the polyphenol content (Jo et al., 2002). Green tea leaf extract at 0.1% in raw and cooked pork patties was evaluated by a trained sensory panel. Panelists did not denote a color preference among raw samples (P>0.05) or a preference in odor, taste, and tenderness in cooked pork patties (P>0.05). It is important to note that the current study used a consumer panel to evaluate liking. The previous research cited used a trained panel to evaluate differences among treatments. Despite the method to evaluate sensory characteristics, the data is supported that fruit extracts do not significantly affect quality attributes like flavor and texture. The study conducted by Lee and Ahn (2005) found a difference in color (P<0.05) when using plum extract in turkey. The current study did not have consumers compare the appearance across treatments. However, the data shows that consumers found all antimicrobial treatments as acceptable in appearance (P>0.05) as the control.

The active component of the dried vinegar ingredient used in this study is acetic acid. Research has been conducted on the sensory implication of acetic acid in processed meat products. Bradley et al. (2011) tested buffered vinegar at 2.5% and a 52% sodium lactate-48% vinegar blend at 2.5% in ground pork patties. The patties were evaluated for appearance, aroma,

texture, taste, flavor and overall integrity by a trained eight-member sensory panel. The panelists found the buffered vinegar treatment scored higher ($P<0.05$) in juiciness compared to control but did not differ ($P>0.05$) in rancid aroma (Bradley et al., 2011). Similarly, the lactate/vinegar blend did not differ ($P>0.05$) in juiciness or overall integrity compared to control. In addition to the trained panel, Bradley et al. (2011) conducted a consumer panel ($N=60$) to compare control and the lactate/vinegar blend for consumer acceptability. The decision to test these two treatments was based upon color, microbial, and sensory descriptive data (Bradley et al., 2011). They found consumer panel scores for the two treatments to be similar ($P>0.05$) for acceptability of appearance, aroma, texture, flavor and overall acceptability. Another study was conducted by Ponrajan et al. (2012) evaluating the sensory impact of 2% buffered vinegar in a beef top sirloin. An eight-member trained sensory panel evaluated steaks for tenderness, beef flavor, overall juiciness, and off flavor (Ponrajan et al., 2012). The panelists found the vinegar-treated sirloin less tender and less juicy ($P<0.05$) than control. They also determined the vinegar treatment had more off flavor ($P<0.05$) than control. Finally, they found the treatments similar ($P>0.05$) for beef flavor (Ponrajan et al., 2012). A final study by Stelzleni et al. (2013) compared the sensory impact of liquid and powder buffered vinegar (2% and 2.5%, respectively) in ground beef patties. The eight-member trained sensory panel found both treatments to be similar ($P>0.05$) to control for juiciness but found the treatments to rank lower for beef flavor intensity ($P<0.05$) compared to control. One point of difference between the past research cited and the current study is the concentration of dried vinegar used, where the current study used 1% dried vinegar. This reduced concentration could impact the consumer preference of the product. Similarly, the current study used consumers to evaluate liking, where the past research used trained panels to evaluate differences. The past research denotes that vinegar can affect texture and flavor, but not

necessarily appearance or odor. Given that the dried vinegar treatment of the current study showed *C. perfringens* outgrowth of 0.81 log at 15 h, it would be beneficial to evaluate a higher concentration of dried vinegar in a future study and determine the impact it has on consumer palatability and acceptability.

Chapter 4 - Conclusions

The use of the natural antimicrobials was effective in inhibiting the growth of *Clostridium perfringens* during an extended 15 h cooling cycle. While using dried vinegar or fruit and spice extract alone controlled outgrowth, using both ingredients together provided a synergistic effect after 15 h of cooling. Results of this study indicate that *C. perfringens* populations can be inhibited without the inclusion of a nitrite source if the natural antimicrobials used in this study are incorporated into the formulation.

The results of the consumer panel showed that consumers did not find differences in overall liking and acceptability when these natural antimicrobials were incorporated into a turkey formulation. Therefore, quality would not be negatively impacted when these antimicrobials are used at 1% in finished product.

It is important to note that further research should be conducted to evaluate these antimicrobials in other proteins to determine the potential effect on *C. perfringens* outgrowth and impact on quality attributes. It is also recommended to evaluate different concentrations of these two antimicrobials or other natural antimicrobials in turkey breast. As the demand for uncured, natural processed meats continues to increase, the industry will need to respond. It is necessary to evaluate the efficacy of natural antimicrobials against *C. perfringens* outgrowth to provide a safe, wholesome product to consumers.

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Appendix A - Turkey Cook Cycle

RECIPE NAME: NA - Nat Tky **OPERATOR:** kmi

STEP NO.	FUNC NO.	FUNCTION DE SCRIPTION (These fields update only after the Controller is Download)	STEP TIME HRS : MINS	DRY BULB DEG F	WET BULB DEG F	PROD TEMP DEG F	TEMP PREF. 0=IT 1=TIME	FAN SPEED 0=OFF 1=LOW 2=MED 3=HI	COOK TYPE 0=ELEC 1=GAS 2=STEAM	DAMPERS 1=AUTO 0=CLOSED	EXH FAN 1=ON 0=OFF	HUMIDITY 1=STEAM 2=WATER
1	1	COOK	1 : 00	135	135	0	1	2	2	1	0	1
2	1	XSTEP	1 : 00	150	150	0	1	2	2	1	0	1
3	1	XSTEP	1 : 00	165	165	0	1	2	2	1	0	1
4	1	XSTEP	0 : 01	175	175	165	0	2	2	1	0	1

Figure A-1. Cook cycle for turkey breast logs.

Appendix B - Turkey Cook Chart

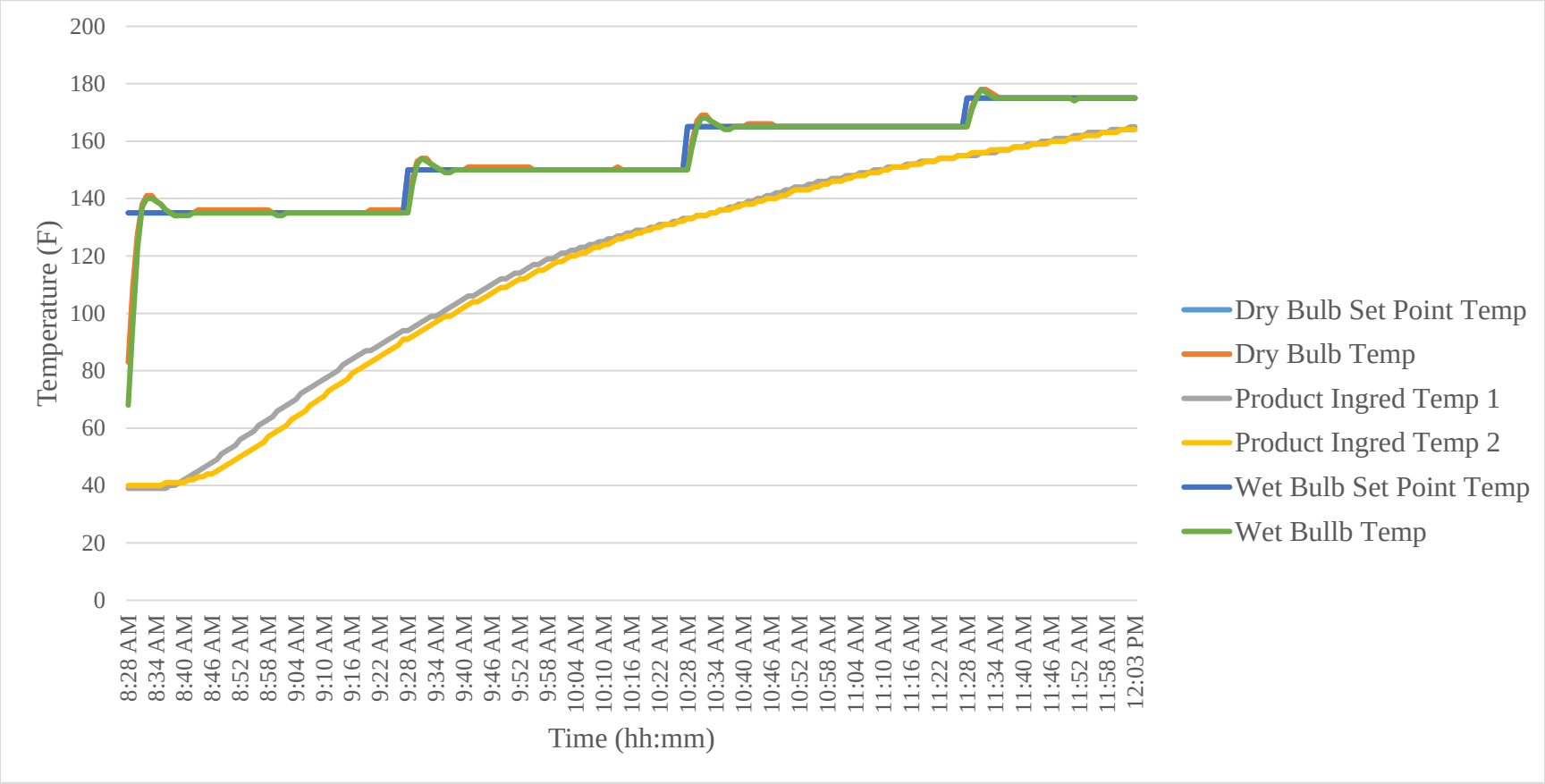

Figure B-1. Cook chart for turkey breast logs.

Appendix C - Cooling Cycle Chart

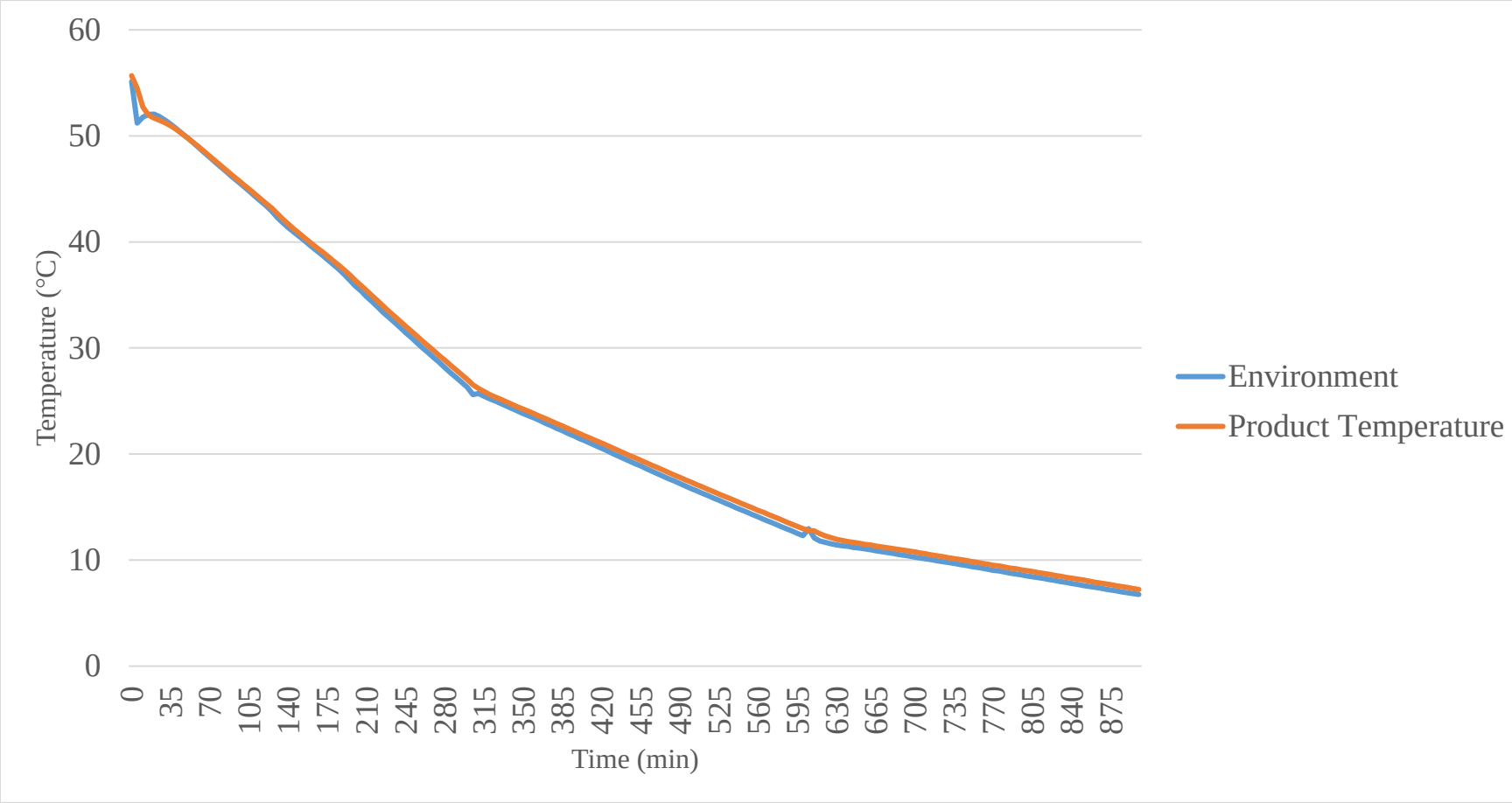

Figure C-1. Cooling cycle chart for product temperature and environment.

Appendix D - IRB Form for Sensory Consumer Panel

IRB Application Page 2

ADMINISTRATIVE INFORMATION:

Title of Project/Course: Consumer and Trained Panel Evaluation of Meat for AS&I

Type of Application: (check one box)
☐ New / Renewal ☐ Revision (to a pending new application)
☑ Modification to an existing approved application #: 7440

Principal Investigator Details: (must be a KSU faculty member):

Name: Travis O'Quinn Degree/Title: Ph.D.

Department: Animal Sciences and Industry Campus Phone: 532-3469

Campus Address: 244 Weber Hall

E-mail: travisoquinn@ksu.edu Fax #:

Responsible Graduate Student: (Person to contact for questions/problems with the form):

Name: Travis O'Quinn Campus Phone: 532-3469

E-mail: travisoquinn@ksu.edu

Does this project involve any collaborators not part of the faculty/staff at KSU? (projects with non-KSU collaborators may require additional coordination and approvals):

☑ No ☐ Yes

Project Classification (Is this project part of one of the following?):

☑ Thesis ☑ Dissertation ☑ Faculty Research

☑ Other: Service Projects

Note: Class Projects should use the short form application for class projects.

Copy of the Consent Form: ☑ Copy will be submitted to comply@ksu.edu with this application ☐ Consent form not used

Funding Source: ☑ Federal ☑ State ☑ Internal ☑ Other

Funding Agency: Please give name of Funding Agency. (You will also need to provide a copy of the sponsor's grant application or contract as submitted to the funding agency. Submit documents to comply@ksu.edu with your application.)

Varies from project to project

Based upon criteria found in 45 CFR 46 – and the overview of projects that may qualify for exemption explained at http://www.hhs.gov/ohrp/policy/checklists/decisioncharts.html, I believe that my project using human subjects should be determined by the IRB to be exempt from IRB review:

☐ No ☑ Yes (If yes, please provide the category of "Exemption" in the space below)

Exempt Projects: 45 CFR 46 identifies six categories of research involving human subjects that may be exempt from IRB review. The categories for exemption are listed here: **http://www.hhs.gov/ohrp/policy/checklists/decisioncharts.html#c2** If you believe that your project qualifies for exemption, please indicate which exemption category applies (1-6). Please remember that only the IRB can make the final determination whether a project is exempt from IRB review, or not.

Exemption Category: Exemption 45 CFR 46.101(b)(6) - Food is wholesome and without additives or the additives are FDA/USDA approved and included at levels determined to be safe for human consumption.

MODIFICATION:

Is this a modification of an approved protocol? ☐ **No** ☑ **Yes** **If yes, please comply with the following:**

If you are requesting a modification or a change to an IRB approved protocol, please provide a concise description of all of the changes that you are proposing in the following block. Additionally, please highlight or bold the proposed changes in the body of the protocol where appropriate, so that it is clearly discernible to the IRB reviewers what and where the proposed changes are. This will greatly help the committee and facilitate the review.

Adding new graduate students to the approved protocol (#7440)

I. NON-TECHNICAL SYNOPSIS (Please provide a brief narrative description of proposal. This should typically be less than 75 words and be easily understood by nonscientists):

Research will be for sensory evaluation of meat for human consumption.

II. BACKGROUND (concise narrative review of the literature and basis for the study):

Literature will be variable with each project covered herein.

III. PROJECT/STUDY DESCRIPTION

(Please provide a concise narrative description of the proposed activity in terms that will allow the IRB or other interested parties to clearly understand what it is that you propose to do that involves human subjects. This description must be in enough detail so that IRB members can make an informed decision about the proposal).

See attached

IV. OBJECTIVE

(Briefly state the objective of the research – what you hope to learn from the study).

To determine consumer and trained panelist perceptions of fresh and processed beef, pork, lamb, poultry, and goat tenderness, juiciness, flavor, overall liking, acceptability, color and odor

V. DESIGN AND PROCEDURES (succinctly outline formal plan for study)

A. List all sites where this research will be conducted:

Rm 107-108, 111, 121, 263 Weber Hall, KSU; various (attached)

B. Variables to be studied: Tenderness, juiciness, flavor, color, odor, and overall acceptability of fresh and processed meats

C. Data collection methods: (surveys, instruments, etc - **copies must submitted to comply@k-state.edu**).

Possible evaluation forms are attached

D. List any factors that might lead to a subject dropping out or withdrawing from a study. These might include, but are not limited to emotional or physical stress, pain, inconvenience, etc.

All participation is voluntary. Panelists may drop out due to any reason including, but not limited to: time conflicts or dislike of product

E. List all biological samples taken: (if any)

None

Describe storage and disposition of biological samples: (How long will samples be kept, will samples be used for other purposes, how will samples be destroyed)

N/A

Will whole genome sequencing be used:

☑ **No**

☐ **Yes**

F. Debriefing procedures for participants:

Verbal debriefing is given following the sensory panels

VI. RESEARCH SUBJECTS:

A. Source:

Meat consumers from the community and Faculty, Staff, and Graduate students in ASI

B. Number: (provide a brief rationale for your sample size)

Dependent on the study (approximately 8 - 300)

C. Inclusion criteria: (List any unique qualifiers desirable for research subject participation)

Consumers must regularly consume the meat product tested; Trained panelists will be selected by screening and training in sensory evaluation

D. Exclusion criteria: (list any unique disqualifiers for research subject participation)

Consumers will be recruited from groups in the community (churches, clubs, etc) and asked to participate and will be paid cash for their participation. Sample recruitment letter is attached. Additionally, some consumer groups will be identified and recruited via on-line newsletters (K-State Today) advertisement using the attached recruiting paragraph.

Trained panelists will be asked to participate and given candy and fruit as an inducement

E. Recruitment procedures:
How will subjects be identified?

From groups in the community who have expressed an interest in particpating

How will subjects be recruited (advertisement, associates, etc.) ?

Using the attached recruiting letter or the attached recruiting paragraph for newsletters

How will subjects be enrolled?

By signing up for an available time slot

Describe any follow-up recruitment procedures: (reminder emails, mailings, etc.)

A reminder email will be sent approximately 1 week in advance of the scheduled time slot

VII. RISK - PROTECTION - BENEFITS: The answers for the three questions below are central to human subjects research. You must demonstrate a reasonable balance between anticipated risks to research participants, protection strategies, and anticipated benefits to participants or others.

A. Risk for Subjects: (check all that apply)

- ☐ Exposure to infectious diseases
- ☐ Use of confidential records
- ☐ Exposure to radiation
- ☐ Manipulation of psychological or social variables such as sensory deprivation, social isolation, psychological stressors
- ☐ Examining for personal or sensitive information in surveys or interviews
- ☐ Presentation of materials which subjects might consider sensitive, offensive, threatening, or degrading
- ☐ Invasion of privacy of subject or family
- ☐ Social or economic risk
- ☐ Risk associated with exercise or physical exertion
- ☐ Legal risk
- ☐ Review of medical records
- ☐ Review of criminal records
- ☐ HIV/AIDS or other STD's
- ☐ Employment/occupational risk
- ☐ Others – Please explain below (Indirect risks, risk to individuals who are not the primary subjects):

No known risks

B. Minimizing Risk: (Describe specific measures used to minimize or protect subjects from anticipated risks.)

HACCP is used for handling and cooking of products to be tested

C. Benefits: (Describe any reasonably expected benefits for research participants, a class of participants, or to society as a whole.)

Panelists become experienced in sensory evaluation; Consumer panelists receive cash for their participation

D. More than Minimal Risk? In your opinion, does the research involve more than minimal risk to subjects? ("Minimal risk" means that "the risks of harm anticipated in the proposed research are not greater, considering probability and magnitude, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.")

☐ Yes ☑ No

VIII. CONFIDENTIALITY: Confidentiality is the formal treatment of information that an individual has disclosed to you in a relationship of trust and with the expectation that it will not be divulged to others without permission in ways that are inconsistent with the understanding of the original disclosure. Consequently, it is your responsibility to protect information that you gather from human research subjects in a way that is consistent with your agreement with the volunteer and with their expectations.

A) Explain the type of data that will be collected (electronic, hard copy, video, specimens, etc.):

Electronic, hard copy

B) Explain where the data will be stores:

When data is entered into the computer, a random number is assigned to panelists to keep their identity unknown

C) Explain the time frame of the data storage, to include how data will be distroyed:

Data will be maintained for approximately 1 year following the study until the study is published

D) Explain who will have access to the data, and privacy/security provisions (password protection, encryption, etc.):

Only the PI and overseeing graduate student will have access to the data

IX. **INFORMED CONSENT:** Informed consent is a critical component of human subjects research - it is your responsibility to make sure that any potential subject knows exactly what the project that you are planning is about, and what his/her potential role is. (There may be projects where some forms of "deception" of the subject is necessary for the execution of the study, but it must be carefully justified to and approved by the IRB). A schematic for determining when a waiver or alteration of informed consent may be considered by the IRB is found at **http://www.hhs.gov/ohrp/policy/checklists/decisioncharts.html#c10**

Even if your proposed activity does qualify for a waiver of informed consent, you must still provide potential participants with basic information that informs them of their rights as subjects, i.e. explanation that the project is research and the purpose of the research, length of study, study procedures, debriefing issues to include anticipated benefits, study and administrative contact information, confidentiality strategy, and the fact that participation is entirely voluntary and can be terminated at any time without penalty, etc. Even if your potential subjects are completely anonymous, you are obliged to provide them (and the IRB) with basic information about your project. See informed consent example on the URCO website. It is a federal requirement to maintain informed consent forms for 3 years after the study completion.

Answer the following questions about the informed consent procedures.

☑ Yes ☐ No **A.** Are you using a written informed consent form? If "yes," include a copy with this application. If "no" see B.

☐ Yes ☑ No **B.** In accordance with guidance in 45 CFR 46, I am requesting a waiver or alteration of informed consent elements (see section VIII above). If "yes," provide a basis and/or justification for your request.

☐ Yes ☑ No **C.** Are you using the online Consent Form Template provided by the URCO? If "no," does your Informed Consent document have all the minimum required elements of informed consent found in the Consent Form Template? (Please explain)

☐ Yes ☑ No **D.** Are your research subjects anonymous? If they are anonymous, you will not have access to any information that will allow you to determine the identity of the research subjects in your study, or to link research data to a specific individual in any way. Anonymity is a powerful protection for potential research subjects. (An anonymous subject is one whose identity is unknown even to the researcher, or the data or information collected cannot be linked in any way to a specific person).

☑ Yes ☐ No **E.** Are subjects debriefed about the purposes, consequences, and benefits of the research? Debriefing refers to a mechanism for informing the research subjects of the results or conclusions, after the data is collected and analyzed, and the study is over. (If "no" explain why.) **Copy of debriefing statement to be utilized should be submitted to comply@k-state.edu with your application.**

F. Describe the Informed Consent Process:

Who is obtaining the consent? (i.e. Principle Investigator, Graduate Student, etc.)

Principle Investigator and/or graduate students

When and where will consent be obtained?

The panelists will sign an informed consent form prior to participation

If assent (for minors) is required, please describe who will obtain the assent? (Assent means a child's affirmative agreement to participate in research)

N/A

If assent (for minors) is required, when and where will assent be obtained?

N/A

How will consent be obtained from non-English speaking participants? (a translated written form, orally, identify the name and qualifications of the individual providing the translation)

N/A

Informed Consent Checklist

Items	YES	NO	N/A
Does the title appear at the top of the consent/assent form?	☑	☐	☐
Is the consent/assent form written toward the subject?	☑	☐	☐
Is there a statement that explains that the study is *research*?	☑	☐	☐
Is there a statement that explains the *purpose* of the research?	☑	☐	☐
Are the procedures to be followed explained clearly and adequately?	☑	☐	☐
Does the consent document describe *risks or discomforts* to subjects as a result of participating in the research?	☑	☐	☐
Is the consent/assent form written in the *native language* of the potential subject?	☑	☐	☐

Are participants compensated?	☑	☐	☐
If the subjects' identity is known to the PI, does the form detail how confidentiality of records will be maintained?	☐	☐	☑
Is contact information for both the PI and the URCO/IRB office included?	☑	☐	☐
Does the consent document indicate to the participant that he/she can withdraw at any time from the project without penalty or loss of benefit?	☑	☐	☐
Are there probable circumstances which would require the PI to terminate a subject's participation regardless of his or her consent?	☐	☑	☐
A statement that identifiers might be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent	☐	☐	☑
A statement that the subject's information or biospecimens collected as part of the research, even if identifiers are removed, will not be used or distributed for future research studies.	☐	☐	☑
A statement that biospecimens (even after identifiers are removed) may (or may not) be used for commercial profit.and whether subjects will or will not share in the profit.	☐	☐	☑
A statement that clinically relevant research results will or will not be provided to subjects. .	☐	☐	☑
A statement indicating whether or not the research project will or will not include whole genome sequencing. .	☐	☐	☑
Is the consent document written in lay language (Recommended 8th grade level)?	☑	☐	☐

X. PROJECT INFORMATION: (If you answer Yes to any of the questions below, you should explain them in one of the paragraphs above)

☐ Yes	☑ No	A.	Deception of subjects? If "YES" explain why this is necessary.
☐ Yes	☑ No	B.	Shock or other forms of punishment
☐ Yes	☑ No	C.	Sexually explicit materials or sexual experience
☐ Yes	☑ No	D.	Sexual orientation
☐ Yes	☑ No	E.	Sexual abuse
☐ Yes	☑ No	F.	Handling of money or other valuable commodities
☐ Yes	☑ No	G.	Extraction or use of blood, other bodily fluids, or tissues (if "yes', you must comply with facility and handling protections detailed in the 5th Edition of the Biosafety in Biomedical Laboratories (BMBL))
☐ Yes	☑ No	H.	Questions about any kind of illegal or illicit activity
☐ Yes	☑ No	I.	Questions about protected health information as defined by HIPAA
☐ Yes	☑ No	J.	Purposeful creation of anxiety
☐ Yes	☑ No	K.	Any procedure that might be viewed as invasion of privacy
☐ Yes	☑ No	L.	Physical exercise or stress
☑ Yes	☐ No	M.	Administration of substances (food, drugs, etc.) to subjects
☐ Yes	☑ No	N.	Any procedure that might place subjects at risk
☐ Yes	☑ No	O.	Will there be any use of Radioactive materials and/or use of Radioactive producing machines
☐ Yes	☑ No	P.	Any form of potential abuse; i.e., psychological, physical, sexual

☑ Yes	☐ No	Q.	Is there potential for the data from this project to be published in a journal, presented at a conference, etc?
☑ Yes	☐ No	R.	Use of surveys or questionnaires for data collection. **Copies should be submitted to comply@k-state.edu with your application.**
☐ Yes	☑ No	S.	Is this a Clinical Trial? (one or more human subjects are prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of the interventions on biomedical or behavioral health-related outcomes**.)**

XI. SUBJECT INFORMATION: (If you answer yes to any of the questions below, you should explain them in one of the paragraphs above)

☐ Yes	☑ No	a.	Under 18 years of age (these subjects require parental or guardian consent)
☐ Yes	☑ No	b.	Over 65 years of age
☐ Yes	☐ No	c.	Minorities as target population
☐ Yes	☑ No	d.	Physically or mentally disabled
☐ Yes	☐ No	e.	Economically or educationally disadvantaged
☐ Yes	☑ No	f.	Unable to provide their own legal informed consent
☐ Yes	☑ No	g.	Pregnant females as target population
☐ Yes	☑ No	h.	Victims
☐ Yes	☑ No	i.	Subjects in institutions (e.g., prisons, nursing homes, halfway houses)
☐ Yes	☑ No	j.	Are subjects likely to be vulnerable to coercion or undue influence
☐ Yes	☑ No	k.	Is this international research? If yes, provide details as to if OHRP regulations apply in or near the area you intend to conduct research or if you have contacted individuals for applicable regulations to human subject research.
☑ Yes	☐ No	l.	Are research subjects in this activity students recruited from university classes or volunteer pools? If so, do you have a reasonable alternative(s) to participation as a research subject in your project, i.e., another activity such as writing or reading that would serve to protect students from unfair pressure or coercion to participate in this project? If you answered this question "Yes," explain any alternatives options for class credit for potential human subject volunteers in your study. (It is also important to remember that: Students must be free to choose not to participate in research that they have signed up for at any time without penalty. Communication of their decision can be conveyed in any manner, to include simply not showing up for the research.)

Panelists and recruits for screening may choose to not participate

☐ Yes	☑ No	m.	Is audio from the subjects recorded? If yes, how do you plan to protect the recorded information and mitigate any additional risks?

☐ Yes	☑ No	n.	Are research subjects' images being recorded (video taped, digitally recorded, photographed)? If yes, how do you plan to protect the recorded information and mitigate any additional risks?

XII. FDA ACTIVITIES: Answer the following questions about potential FDA regulated activities:

☐ Yes	☑ No	a.	Is this a Clinical Trial?
☐ Yes	☑ No	b.	Are you using an FDA approved drug/device/diagnostic test?
☐ Yes	☑ No	c.	Does this activity involve the use of FDA-Regulated products? (biological products, color additives, food additives, human drugs, etc.)
☐ Yes	☑ No	d.	Has the protocol been submitted to the FDA, or are there plans to submit it to the FDA?
☐ Yes	☑ No	e.	Have you submitted an FDA form 3454 or 3455 (conflict of interest)?

XIII. CONFLICT OF INTEREST: Concerns have been growing that financial interests in research may threaten the safety and rights of human research subjects. Financial interests are not in them selves prohibited and may well be appropriate and legitimate. Not all financial interests cause Conflict of Interest (COI) or harm to human subjects. However, to the extent that financial interests may affect the welfare of human subjects in research, IRB's, institutions, and investigators must consider what actions regarding financial interests may be necessary to protect human subjects. Please answer the following questions:

☐ Yes ☑ No a. Do you or the institution have any proprietary interest in a potential product of this research, including patents, trademarks, copyrights, or licensing agreements?

☐ Yes ☑ No b. Do you have an equity interest in the research sponsor (publicly held or a non-publicly held company)?

☑ Yes ☐ No c. Do you receive significant payments of other sorts, eg., grants, equipment, retainers for consultation and/or honoraria from the sponsor of this research?

☐ Yes ☑ No d. Do you receive payment per participant or incentive payments?

e. If you answered **yes** to any of the above questions, please provide adequate explanatory information so the IRB can assess any potential COI indicated above.

The research conducted is often part of externally funded research projects and grants

XIV. PROJECT COLLABORATORS:

A. KSU Collaborators: List anyone affiliated with KSU who is collecting or analyzing data: (list all collaborators on the project, including co-principal investigators, undergraduate and graduate students).

Name:	Department:	Campus Phone:	Campus E-mail:
See attached list			

Add Row | Delete Row

B. Non-KSU Collaborators: List all collaborators on your human subjects research project not affiliated with KSU in the spaces below. KSU has negotiated an Assurance with the Office for Human Research Protections (OHRP), the federal office responsible for oversight of research involving human subjects.

Name:	Organization:	Phone:	Institutional E-mail:
None			

Add Row | Delete Row

C. Does your non-KSU collaborator's organization have an Assurance with OHRP? (for Federalwide Assurance listings of other institutions, please reference the OHRP website under Assurance Information at: **http://ohrp.cit.nih.gov/search**).

☐ Yes ☐ No If yes, Collaborator's FWA #

Is your non-KSU collaborator's IRB reviewing this proposal?

☐ Yes ☐ No If yes, IRB approval #

Describe the non-KSU collaborator's role in the research activity.

XV. IRB Training:

A. The URCO must have a copy of the Unaffiliated Investigator Agreement on file for each non-KSU collaborator who is not covered by their own IRB and assurance with OHRP. When research involving human subjects includes collaborators who are not employees or agents of KSU the activities of those unaffiliated individuals may be covered under the KSU Assurance only in accordance with a formal, written agreement of commitment to relevant human subject protection policies and IRB oversight. The Unaffiliated Investigators Agreement can be found and downloaded at **http://www.k-state.edu/research/comply/irb/forms**

Online Training

TRAINING REQUIREMENTS HAVE RECENTLY CHANGED

The IRB has mandatory training requirements prior to protocol approval. Training is now offered through the Collaborative Institutional Training Initiative (CITI) Program. Instructions for registration and access to training are on the URCO website http://www.k-state.edu/research/comply/.

Use the check boxes below to select the training courses that apply to this application. If you have any questions about training, contact URCO at comply@ksu.edu, or (785) 532-3224.

Mandatory Training

Required for all Principal Investigators, research staff and students

☒ Responsible Conduct of Research

☒ IRB core modules (IRB Researchers and personnel on IRB protocols)

Required (Provost-mandated) for all full-time K-State employees

☑ Export Compliance

Required procedure-specific training (check all that apply to this protocol):

☐ International Research ☐ Research in Public Elementary and Secondary Schools ☐ Research with Children

☐ Research with Prisoners ☐ Internet Research ☐ Vulnerable Subjects - Research Involving Workers/Employees

☐ Research with Subjects with Physical Disabilities and Impairments ☐ Illegal Activities or Undocument Status in Human Research

☐ Gender and Sexuality Diversity in Human Research ☐ Research with human blood, body fluids, or tissues

☐ Research with Older Adults

All new personnel or personnel with expired training are required to register for CITI and take the new training requirements. If you previously completed online IRB modules, your training status will remain current until it expires. URCO will verify training from the previous system as well as the new system prior to approval of any protocol.

INVESTIGATOR ASSURANCE FOR RESEARCH INVOLVING HUMAN SUBJECTS

(Print this page separately because it requires a signature by the PI.)

P.I. Name: Travis O'Quinn

Title of Project: Consumer and Trained Panel Evaluation of Meat for AS&I

XVI. ASSURANCES: As the Principal Investigator on this protocol, I provide assurances for the following:

A. Research Involving Human Subjects: This project will be performed in the manner described in this proposal, and in accordance with the Federalwide Assurance FWA00000865 approved for Kansas State University available at http://www.hhs.gov/ohrp/assurances/forms/filasurt.html, applicable laws, regulations, and guidelines. Any proposed deviation or modification from the procedures detailed herein must be submitted to the IRB, and be approved by the Committee for Research Involving Human Subjects (IRB) prior to implementation.

B. Training: I assure that all personnel working with human subjects described in this protocol are technically competent for the role described for them, and have completed the required IRB training accessed via the URCO website at: http://www.k-state.edu/research/comply/irb/training. I understand that no proposals will receive final IRB approval until the URCO has documentation of completion of training by all appropriate personnel.

C. Extramural Funding: If funded by an extramural source, I assure that this application accurately reflects all procedures involving human subjects as described in the grant/contract proposal to the funding agency. I also assure that I will notify the IRB/URCO, the KSU PreAward Services, and the funding/contract entity if there are modifications or changes made to the protocol after the initial submission to the funding agency.

D. Study Duration: I understand that it is the responsibility of the Committee for Research Involving Human Subjects (IRB) to perform continuing reviews of human subjects research as necessary. I also understand that as continuing reviews are conducted, it is my responsibility to provide timely and accurate review or update information when requested, to include notification of the IRB/URCO when my study is changed or completed.

E. Conflict of Interest: I assure that I have accurately described (in this application) any potential Conflict of Interest that my collaborators, the University, or I may have in association with this proposed research activity.

F. Adverse Event Reporting: I assure that I will promptly report to the IRB / URCO any unanticipated problems involving risks to subjects or others that involve the protocol as approved. Unanticipated or Adverse Event Form is located on the URCO website at: http://www.k-state.edu/research/comply/irb/forms. In the case of a serious event, the Unanticipated or Adverse Events Form may follow a phone call or email contact with the URCO.

G. Accuracy: I assure that the information herein provided to the Committee for Human Subjects Research is to the best of my knowledge complete and accurate.

You may sign this form using a digital signature. DO NOT sign the form until it has been completed.
You cannot edit the form entries once the form has been digitally signed. If you are making revisions to a previously signed form, right-click the digital signature and select Clear to remove the signature (this can only be done by the person who originally digitally signed the form). Forms that have not been signed will not be accepted.

P.I. Signature: Date:

Appendix E - Consumer Evaluation Form

INFORMED CONSENT STATEMENT

1. I volunteer to participate in research involving Sensory Evaluation of Meat. This research will be conducted by personnel in the Department of Animal Sciences and Industry at Kansas State University.

2. I fully understand the purpose of the research is for the evaluation of beef steaks, pork chops, lamb chops, goat meat, poultry meat, ground meat, and processed meat products from the previously mentioned species for the sensory traits of tenderness, juiciness, flavor intensity, connective tissue amount, off flavor presence, odor, and color and sensory evaluation will last approximately one hour.

3. I understand that there are minimal risks associated with participating and that those risks are related to possible food allergies. All meat products will be USDA inspected and all ingredients are GRAS (generally accepted as safe) by FDA.

4. I understand that my performance as an individual will be treated as research data and will in no way be associated with me for other than identification purposes, thereby assuring confidentiality of my performance and responses.

5. My participation in this study is purely voluntary; I understand that my refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled and that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled.

6. If I have any questions concerning my rights as a research subject, injuries or emergencies resulting from my participation, I understand that I can contact the Committee on Research Involving Human Subjects, 203 Fairchild Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3224.

7. If I have questions about the rationale or method of the study, I understand that I may contact, Dr. Travis O'Quinn, 247 Weber Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3469 or Sally Stroda, 107 Weber Hall, at 785-532-1273.

I have read the Subject Orientation and Test Procedure statement and signed this informed consent statement, this _____ day of _____, _____.

_____ _____

Printed name Signature

Appendix F - Sensory Questionnaire

Sensory Demographic Questions

Big Panel 1 - Red

Please tell us a little about yourself.

Panelist Number

Gender

- ○ Male
- ○ Female

Age

- ○ Under 20
- ○ 20 to 29 years old
- ○ 30 to 39 years old
- ○ 40 to 49 years old
- ○ 50 to 59 years old
- ○ over 60

Ethnic Origin

- ○ African American
- ○ Asian

- ◯ Caucasian/White
- ◯ Hispanic
- ◯ Native American
- ◯ Other
- ◯ Mixed Race

Marital Status

- ◯ Single
- ◯ Married

Household Size

- ◯ 1 person
- ◯ 2 People
- ◯ 3 People
- ◯ 4 People
- ◯ 5 People
- ◯ 6 People
- ◯ > 6 People

Annual Household Income

- ◯ < $25,000
- ◯ $25,000 - $34,999
- ◯ $35,000 - $49,999
- ◯ $50,000 - $74,999
- ◯ $75,000 - $99,999
- ◯ $100,000 - $149,999
- ◯ $150,000 - $199,999
- ◯ > $199,999

Highest Level of Education Completed

- ◯ Non-High School Graduate
- ◯ High School Graduate
- ◯ Some College / Technical School
- ◯ College Graduate
- ◯ Post-College Graduate

How many times a week do you consume **luncheon meat**?

0 3 6 9 12 15 18 21

None

→

Sensory Questions

Sample Number

- ○ 7110

Appearance

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Was the sample acceptable for appearance?

- ○ Acceptable
- ○ Unacceptable

Texture

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Was the sample acceptable for texture?

- ○ Acceptable
- ○ Unacceptable

Flavor

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Was the sample acceptable for flavor?

- ◯ Acceptable
- ◯ Unacceptable

Did this sample meet your expectations for flavor?

- ◯ Yes
- ◯ No

Aftertaste

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Was the sample acceptable for aftertaste?

- ◯ Acceptable
- ◯ Unacceptable

Overall Liking

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Was the sample acceptable overall?

- ◯ Acceptable
- ◯ Unacceptable

→

Appendix G - SAS Statistical Code

Statistical code in SAS to run analysis on micro data

```
data chill;
input trt $ rep hour $ sample log;
datalines;

data chill2;
      set chill;
      if hour='hs';
run;

data chill3;
set chill;
if hour='hs' then delete;
run;

proc mixed data=chill2;
class trt rep hour sample;
model log=trt hour trt*hour;
random sample rep(sample trt);
lsmeans trt/pdiff;
lsmeans hour/pdiff;
lsmeans trt*hour/pdiff;
run;

proc mixed data=chill3;
class trt rep hour sample;
model log=trt hour trt*hour;
random sample rep(sample trt);
lsmeans trt hour trt*hour/pdiff;
store out=chill5;
run;
proc plm restore=chill5;
lsmeans trt hour trt*hour/linestable;
run;
```

Statistical code in SAS to run analysis on sensory data

```
data sensorydata;
input panel trt app aacc text tacc flav flavacc exp aft aftacc oa oaacc @@;
datalines;

proc glimmix data=sensorydata;
class trt panel;
model oaacc=trt/ddfm=kenwardroger;
random panel;
lsmeans trt/pdiff;
store out="";
run;
proc plm restore="";
lsmeans trt/linestable;
run;
```

Replace overall acceptability with the other acceptability ratings.

Statistical code in SAS to run analysis on chemistry data

```
data chem;
input trt ph salt sugar moisture protein nitrate nitrite;
      datalines;

proc glm data=chem;
class trt;
model ph salt sugar moisture protein nitrate nitrite = trt;
lsmeans trt/pdiff;
run;
```