

THE EFFECT OF INTERNAL ENDPOINT TEMPERATURE ON SMOKED SAUSAGE  
QUALITY STORED UNDER LIGHT EMITTING DIODE AND FLUORESCENT LIGHTING

by

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B.S., Kansas State University, 2010

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences & Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2012

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## Abstract

Quality attributes of vacuum packaged, skinless smoked sausage made with a combination of pork, turkey, and beef, cooked to 64, 68, or 72°C internal endpoint temperature following USDA FSIS Appendix A, and displayed at 4°C for up to 120 days under light emitting diode (LED) and fluorescent (FLS) lighting were evaluated. External color, pH, thiobarbituric acid reactive substances (TBARS), proximate analysis, reheat yield, and sensory attributes were measured on day 0, 90, and 120 of display. Purge amount and color were measured on day 10, 90, and 120. Product was displayed in LED or FLS retail display cases set to the same operational and temperature profiles.

Lighting type had no effect ( $P>0.05$ ) on any of the measured attributes. Instrumental external color was less ( $P<0.05$ ) red by 0.63 units in product thermally processed to 64°C than product processed to 68°C. Product cooked to 72°C was less ( $P<0.05$ ) yellow externally compared to those processed to 64 and 68°C. Purge color lightness increased ( $P<0.05$ ) in product thermally processed to 72 compared to 64°C. Purge was more red by 0.36 units ( $P<0.05$ ) on day 120 compared to day 10. Yellowness of purge color increased at 72°C compared to 64°C by 0.66 units. Purge was more yellow ( $P<0.05$ ) on d 120 compared to d 10 and 90. TBARS values decreased ( $P<0.05$ ) from 0.70 mg of malonaldehyde/100g on day 0 to 0.35 and 0.23 on d 90 and 120, respectively. Sensory panel scores showed that flavor intensity decreased ( $P<0.05$ ) as day of storage increased, and saltiness decreased from d 0 to d 90. Purge content increased ( $P<0.05$ ) from 1.45% to 1.90% in products cooked to 64 and 68°C, respectively. The amount of purge increased ( $P<0.05$ ) from 1.58% to 1.92% on day 10 and 90, respectively. While there were slight changes found in quality characteristics of smoked sausages during storage, many of these were minimal. Processors could reduce their internal endpoint temperature following USDA FSIS Appendix A guidelines with minimal effect on product quality. Vacuum packaged pre-cooked smoked sausages could be displayed under LED or FLS lighting with no effect on product quality.

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## **Acknowledgements**

I would like to thank my major advisor Dr. Elizabeth Boyle; her guidance, support and expertise has been essential throughout my graduate career. She has been extremely helpful in focusing my attention to detail, being a source of positive encouragement, and increasing my organizational abilities. As I prepare to enter the meat industry, I feel confident that she has prepared me for the challenges I will soon face. I appreciate the patience and dedication she has exhibited throughout my graduate experience.

I owe my deepest gratitude to Dr. James Higgins for his statistical expertise in setting up the study and analyzing the results. He has been vital to my understanding of the statistical design and data interpretation. I extend appreciation to my committee, Drs. Michael Dikeman, and Fadi Aramouni; their encouragement and guidance throughout my master's research have been essential. I deeply appreciate their time and the opportunity I got to learn from these great minds.

I would like to show my gratitude to Armour Eckrich for supplying the product and financial funding for this project as well as Adrian Mitchell and Janet Pippin for their commitment and trust to Kansas State University in carrying out this research. The time and effort these individuals spent organizing and specifying the project will greatly benefit the meat industry.

For all of the help I received from the graduate students and faculty I am truly grateful. Their assistance and expertise was imperative for the completion of this project. They cannot be commended enough for their time and effort. I am fortunate to be part of a superb set of individuals who go above and beyond their own duties in order to help others.

It is an honor to be a part of Kansas State University and the Department of Animal Sciences and Industry. The support and assistance in terms of facilities, equipment, and staff was beyond generous. Without this support, this research would not have been possible.

Finally, I would like to thank my family and friends as they have been an inexhaustible resource my entire life. Their reliable advice and encouragement have been of great assistance throughout graduate school. Their love and support has led me down the path of success and for that I am forever grateful.

# **Chapter 1 - Impact of Internal Endpoint Temperature on Smoked Sausage Displayed under LED and FLS Lighting**

Many meat processing establishments that produce ready-to-eat meat products follow the United States Department of Agriculture (USDA) Food Safety Inspection Services (FSIS) Appendix A (1999) to meet lethality performance standards for *Salmonella*. If quality attributes of products are not affected, energy and processing time can be altered by cooking products to a lower internal temperature as described in USDA FSIS Appendix A (1999). Two of the primary quality factors that should be evaluated in order to lower internal endpoint temperature are color deterioration and lipid oxidation during retail display.

Internal endpoint temperature has been shown to play a significant role in development of internal cooked color. In a study done in by Ryan, Seyfert, Hunt, & Mancini (2006) with ground beef patties, it was found that increasing endpoint temperature decreased interior  $a^*$  or redness regardless of cooking rate, but the extent of decrease in  $a^*$  was less ( $P < 0.05$ ) for patties that were cooked rapidly compared to those that were cooked slowly. Another study using low-fat pork and chicken batters found that  $L^*$  and  $a^*$  values generally increased in either species with an increase of final cooking temperature, particularly between 60°C and 70°C; however,  $b^*$  did not follow this same pattern (Jimenez-Colmenero, Fernandez, Carballo, & Fernandez-Martin, 1998). De Santos, Rojas, Lockhorn, & Brewer (2007) found that endpoint temperature had a significant effect on color measures in pork chops cooked to six internal endpoint temperatures. As endpoint temperature increased,  $L^*$  values increased,  $a^*$  values decreased, and hue angle increased showing a lighter, less red interior.

Internal endpoint temperature has also been shown to affect the rate of lipid oxidation in meat products. Cooking allows precursors to become readily available to oxygen and other free radical initiation compounds to drive the peroxidation of lipids (Spanier & Miller, 1996). Lipid oxidation during storage of cooked pork was lower ( $P < 0.01$ ) when the pork was cooked to an internal temperature of 72°C rather than 82°C. Also, when pork was cooked at a fast rate (2.0°C/min), oxidation was lower ( $P < 0.05$ ) compared to a slow rate (0.3°C/min) (Kingston, Monahan, Buckley, & Lynch, 1998). These findings agree with Mielche (1995) and Ang and Huang (1993) who reported that lipid oxidation increased as cooking temperature increased above 80-85°C. If meat is cooked quickly, the rapid coagulation of proteins, including iron-

containing proteins, may reduce the rate of iron release, making it less available for catalysis of lipid oxidation (Chen, Pearson, Gray, Fooladi, & Ku, 1984).

Lighting may also play a role in sausage color deterioration. Color is the most important characteristic in meat products because of consumer's reliance on appearance to determine quality (Sindelar, Cordray, Olson, Sebranek, & Love, 2007). Light and oxygen are the main causes of discoloration in cured cooked meat products due to the oxidation of nitrosylheme during storage (Pegg, Shahidi, & Fox, 1997). Research has shown that lighting source plays a major role in color shelf life. Ultraviolet light penetrates meat causing denaturation of the globin in myoglobin leading to discoloration (Lawrie, 1985). Andersen, Bertelsen, Boegh-Soerensen, Shek, & Skibsted (1988) found that illumination clearly affected surface color redness ( $a^*$ ) values in vacuum packaged ham samples stored in either a display cabinet with illumination, in the same display cabinet but protected from the light, or in a dark cold storage room. The samples that were protected from the light showed only minor color changes. Remarkable improvement in color stability of sliced, vacuum packaged ham was obtained by an initial dark storage period prior to display and exposure to light. The rationale for this obtained protection against discoloration was explained as an efficient depletion of oxygen in the product due to post-mortem processes and microbiological activity.

Light has also been shown to have a significant effect on the rate of lipid oxidation in meat products. Cured meats are less sensitive to photooxidation due to the addition of nitrite. Nitric oxide helps to inhibit lipid oxidation in meats (Kanner, Ben-Gera, & Berman, 1980). However, many studies have shown that nitric oxide is not enough to prevent oxidation during retail display. In a study looking at the quality characteristics of bologna sausages made with citrus fiber, thiobarbituric acid reactive substances (TBARS) values were higher ( $P < 0.05$ ) when stored under lighting conditions than those for samples stored in the dark (Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, & Perez-Alvarez, 2003). Lipid oxidation was strongly enhanced in samples displayed under lighting according to Andersen and Skibsted (1991) who reported that light is an important pro-oxidant in the process of lipid oxidation. This was in agreement with Rawls and Van Santen (1970) who stated exposure to light intensifies lipid rancidity.

There have not been many studies to determine how internal endpoint temperature or lighting affect cured meat products over time. Therefore, the objective of this study was to

determine if internal endpoint temperature or lighting type affects quality factors of smoked sausage when displayed refrigerated for up to 120 days.

## **Chapter 2 - Review of Literature**

### **Sausage History**

Sausage is one of the oldest forms of processed food and is known as the first processed meat product. How, where, or when it was first developed is unknown. Sausage making developed gradually over time. Preservation methods such as salting and drying were commonly used to keep meat for long periods of time. American Indians were the first to combine chopped dried meat with dried berries and fat pressed into a cake called “pemmican” used when food was scarce. The word “sausage” is relatively modern, derived from the Latin word “salsus” meaning salted or literally preserved meat (Rust, 1975).

Commercial development of sausage began in European countries when they began making their own unique sausages using a variety of spices, each naming their sausage from where it originated. Examples of these products include Frankfurters from Frankfurt Germany, Bologna from Bologna Italy, and Genoa Salami from Genoa. European sausage makers had to adapt their processing technologies to the climate of their country. Sausage producers in Italy and southern France developed dry sausage products. People of northern Europe enjoyed periods of cold weather and were able to specialize in smoked and cooked items such as summer sausage (Rust, 1975).

Fundamentally, sausage is comminuted meat. Products differ due to spices and processing methods. The earliest sausage makers used herbs and other condiments native to their locations for seasoning. Later, certain spices obtained from the Orient opened a new realm of unique flavor combinations. Spices that are common today were brought from the Orient by caravan in ancient or medieval times. Spices were so highly valued that cinnamon, cloves, and pepper were used as a tribute paid to Solomon by other Monarchs (Rust, 1975).

Processed meats in the U.S. have come full circle. Old world products were of high demand in the U.S., so processors duplicated products found in the European countries to try and meet this demand. In addition, new products were introduced to meet American tastes. Meat loaf products and sandwich meats starting being produced in America due to technological advancements in meat technology. The processed meat industry developed machinery to facilitate and mass produce meat products therefore making production of such products more

economical and profitable. This was accomplished by improving processing methods and increasing food safety training and awareness. The abundance of raw material (live animals), direct importation of spices from around the world, mechanical and cryogenic refrigeration, and modern packaging allowed American producers to manufacture any type of sausage at any time of the year. European producers began to look to America for advice on new technology and innovative products (Rust, 1975).

## **Factors Influencing Sausage Quality**

### ***Instrumental Color***

Product color can be instrumentally measured either through pigment extraction or reflectance color measurement. Reflectance color measurement is a more rapid approach that can be used repeatedly on the same samples (American Meat Science Association (AMSA), 2011). Currently, many options are available for instrumental color analysis and several types of colorimeters and spectrophotometers are available for use. Researchers can choose from several color systems (Hunter, CIE, and tristimulus); illuminants (A, C, D65, and Ultralume); observers ( $2^\circ$  and  $10^\circ$ ); and aperture sizes (0.64cm-3.2cm) (Mancini & Hunt, 2005). Reflectance data can be reported as CIE Lab-values, also known as  $L^*$  (lightness),  $a^*$  (red), and  $b^*$  (yellow). Hue angle ( $\tan^{-1}b^*/a^*$ ),  $a/b$  ( $a^*/b^*$ ), and saturation index  $((a^{*2}+b^{*2})^{(1/2)})$  are calculations of instrumental data used to monitor discoloration. Generally, the human eye is not able to perceive color differences until CIE values change by 1-2 units, which is why instrumental color measurement is widely used when measuring meat color. Lower values of  $a/b$  and saturation index and higher values of hue angle are indicators of discoloration (AMSA, 2011). In a study looking at the effect of lactate on beef bologna, Brewer, McKeith, Martin, Dallmier, & Wu (1992) found that  $a^*$  decreased ( $P<0.05$ ) and hue angles increased ( $P<0.05$ ) over time for all treatments. Research has shown that choosing the right instrument and illuminant may influence color measurements (Brewer, Zhu, Bidner, Meisinger, & McKeith, 2001). Other work has reported that there are no effects of illuminant and angle of observer on lightness measures when using CIE  $L^*a^*b^*$  and Hunter Lab systems (Garcia-Esteben, Ansorena, Gimeno, & Astiasaran, 2003). The Meat Color Measurement Guidelines from the AMSA (2011) report that instrumental color measurements are an objective color characterization that work well alone or in combination with visual color data. Many meat color studies include measures of lipid oxidation,

because myoglobin oxidation is often closely linked with lipid oxidation (AMSA, 2011). Aldehyde products of lipid oxidation initiate conformational changes in myoglobin causing increased heme oxidation and browning (Alderton, Faustman, Liebler, & Hill, 2003).

### ***Cured Meat Color***

Meat color stability is defined as the duration of an acceptable, saleable color (Kropf, 1993). Cured meat color is characteristically a pink color caused by the direct or indirect addition of curing ingredients (nitrite) into the product. Added nitrite binds to the heme moiety of deoxymyoglobin, with rapid reduction of the bound nitrite to nitric oxide, and simultaneous heme oxidation to the ferric form. Indication of this reaction can be determined by a visual rapid browning that occurs when nitrite is added to fresh meat. Under anaerobic conditions, brown nitric oxide metmyoglobin is then reduced to red nitric oxide myoglobin by added reductants such as erythorbate, or more slowly by endogenous reductants, in combination with metmyoglobin reductase enzymes. Denaturation of nitric oxide myoglobin and nitric oxide hemoglobin during cooking or fermentation exposes the centrally located porphyrin ring, resulting in cured meat color (nitric oxide hemochrome), due to the interaction between ferrous iron and nitric oxide. Cured pink color will fade to gray when exposed to light and oxygen (AMSA, 2011).

### ***Lipid Oxidation***

Many factors seem to affect lipid oxidation in animal tissues after slaughter, including species, anatomical location, diet, environmental temperature, sex and age, and phospholipid composition and content. During processing, several other factors influence the rate of oxidation such as: composition and freshness of raw meat components, cooking and heating, chopping, flaking, emulsification, deboning, and adding exogenous compounds such as salt, nitrite, spices, and antioxidants (Kanner, 1994). Brewer et al. (1992) found inconsistent differences in thiobarbituric acid (TBA) values of beef bologna; however, all samples remained below 0.65 over 10 wks. In a study evaluating mutton sausage, no treatment or storage differences ( $P>0.05$ ) were observed and all TBA numbers remained lower than 0.70. (Wu, Rule, Busboom, Field, & Ray, 1991). In contrast, Wang, Jiang, & Lin (1995) found that thiobarbituric acid reactive substances (TBARS) values of Chinese-style sausage for both vacuum packaged and modified atmosphere packaged (MAP) treatments increased ( $P<0.05$ ) with storage time. Vacuum

packaging resulted in a greater amount of oxidation than MAP. This agrees with Dransfield, Jones, & Mcfie (1981) who observed oxidation occurring in pork packaged under vacuum or CO<sub>2</sub> and stored at chilled temperatures for prolonged periods. However, when evaluating beef snack sausages made with varying amounts of partially defatted chopped beef (PDCB), TBA values were highest on d 0 and averaged 0.23 across all levels of PDCB. Average values for all other time periods were well below that level (Smith, Stalder, Keeton, & Papadopoulos, 1991).

Oxidative rancidity is the major cause of food deterioration (Gray, 1978). The important lipids susceptible to oxidation in food are primarily the unsaturated fatty acid moieties, particularly oleate, linoleate, and linolenate. The susceptibility and rate of oxidation of these fatty acids increase as degree of unsaturation increases. Unless mediated by other oxidants or enzyme systems, oxidation of unsaturated fatty acids proceeds through a free-radical chain mechanism involving initiation, propagation, and termination steps. Hydroperoxides are the major initial reaction products of fatty acids with oxygen. Subsequent reactions control the rate and products formed. These compounds are responsible for the development of off-flavors (Gray, 1978). To assess the extent of oxidation in a food product, a sensory panel is often used in conjunction with a chemical method. The 2-thiobarbituric acid test is probably the most extensively used chemical method for the semi-quantitative estimation of lipid oxidation in foods. This method is based on the spectrophotometric measurement of a red chromophore formed by the reaction of TBA with secondary products from lipid oxidation of unsaturated fatty acids and with malondaldehyde (MDA) being used as a calibration standard (Sorensen & Storgaard Jorgensen, 1996). The most common procedure described by Tarladgis, Watts, Younathan, & Dugan (1960) involves distillation of an acidified sample in order to separate the TBA-reactive substances (TBARS) from the food matrix. This distillation method works extremely well with meat products that contain greater than 14% fat, are cured, and contain artificial colorants (Sorensen & Storgaard Jorgensen, 1996).

### ***Packaging***

Packaging is vital to meat products. It provides protection from physical, chemical, and biological hazards as well as containing the product, communicating with consumers as a marketing tool, and providing ease of use and convenience (Yam, Takhistov, & Miltz, 2005). Cured meat products are commonly packaged in MAP that involves the removal of air or



substitution of air with a specific atmosphere encompassing the food item within sealed vapor-barrier materials (McMillin, Huang, Ho, & Smith, 1999). Vacuum-packaging, gas flushing, and naturally respiring products that use special permeable films and controlled atmosphere packaging are examples of MAP (Farber, 1991). There are many benefits that exist when using MAP ranging from increased shelf life to meat quality; however, these benefits have less potential with cured meats because the curing process already helps to extend shelf life (Church, 1993). Nevertheless, microbial spoilage and color deterioration are considered the main problems during the shelf life of meat products (Church, 1993). The application of MAP to processed meat has seen increased growth in recent years, but optimization of gas composition is critical to ensure product safety and quality (Moller, Jensen, Olsen, Skibsted, & Bertelsen, 2000). Carbon Dioxide (CO<sub>2</sub>) and Nitrogen (N<sub>2</sub>) are the most common gasses used in MAP. Due to its antimicrobial properties, CO<sub>2</sub> is widely used throughout the industry (Devlieghere, Debevere, & Van Impe, 1998), while N<sub>2</sub> is used as filler gas (Sorheim, Nissen, & Nesbakken, 1999). Color stability of cured meat packaged with MAP depends on a complex interaction between head space oxygen level, product to headspace volume ratio, and the level of luminance (Moller et al., 2003). One of the most common MAP strategies used for cured meats is vacuum packaging. Composite polymer films known as laminates have low water vapor and oxygen transmission rates, and are used for vacuum packaging. Films that are good barriers to water include polyethylene and oriented polypropylene. Those that are good barriers to oxygen include polyvinylidene chloride and ethylene vinyl alcohol. Combinations of these films are used to provide enclosures, form tightly to products, and provide efficient barriers to oxygen and moisture (Aberle, Forrest, Gerrard, & Mils, 2001). Vacuum packaging is desirable for cured meat products because it eliminates or significantly reduces the products contact with oxygen, which is known to fade cured meat color and increase oxidative rancidity (Sebranek & Fox, 1985). In a study done by Nam and Ahn (2002), vacuum-packaged meat was more resistant to lipid oxidation than aerobically packaged meat. Another study showed no differences between vacuum packaging, 100% N<sub>2</sub>, and 20% CO<sub>2</sub> and 80% N<sub>2</sub> for color, texture, and microbial quality for a long storage period of dry cured ham (Garcia-Esteban et al., 2003). Slices of ham packed in vacuum showed lower (P=0.001) TBARS values than hams packaged with N<sub>2</sub> and Argon (Ar) (Parra et al., 2012). In accordance, higher oxidative stability was observed for hams packed in

vacuum in comparison to those in modified atmospheres (Cilla, Martinez, Beltran, & Roncales, 2006).

### ***Purge***

Purge is the free water and associated soluble proteins that may exude from meat products causing a wet, unattractive retail package. This accumulation represents losses in palatability and nutritive value. The problem is minimal in meat products having high water binding capacity and in packaging that fits tightly around the meat, such as vacuum packaging, but can be a serious problem in vacuum packaged pork with low water holding capacity (Aberle et al., 2001). In a study looking at the quality changes of vacuum packaged fish sausages during storage, Cardoso, Mendes, Pedro, & Leonor Nunes (2008) found that purge loss remained low and constant over time, with no significant difference being detected. In contrast, when evaluating the storage stability of low-fat chicken sausages, 0% and 2% added fat formulations had a purge loss that remained constant, but the percentage of purge lost was significantly higher than for sausage formulated with 5% fat. However, after 45 d of storage, purge losses for the highest fat formulation increased (Andres, Garcia, Zaritzky, & Califano, 2006). Candogan and Kolsarici (2003) found that high-fat controls had the lowest ( $P < 0.05$ ) purge loss over refrigerated storage as compared to low-fat beef frankfurters. This increase in purge with storage time is in agreement with the findings of Blukas and Paneras (1993) and Hensley and Hand (1995) in low-fat frankfurters.

### ***pH***

The measurement of hydrogen ions in a meat product is defined as pH. This measurement is based on a scale from 0-14 with 7 being neutral, 14 as basic and 0 as acidic. The pH of sausage is extremely important for quality purposes because it is a major determinant of a meat product's ability to bind water, thus influencing texture, taste, and microbial deterioration. With elevated pH, water binding is enhanced, resulting in improved processing yields and juiciness. However, when pH is too high, increased protein denaturation occurs resulting in a soft texture. Products also become more suitable for microbial growth with an elevated pH (Aberle et al., 2001). In a study using dry cured ham, Guerrero, Gou, & Arnau (1999) found that high pH ( $> 6.2$ ) hams were softer, pastier, more crumbly, and more adhesive than the normal pH ( $< 5.8$ ) hams causing slicing difficulty. Too low of a pH results in loss of moisture, primarily from the surface, and

development of a hard exterior called case hardening in sausage manufacture (Aberle et al., 2001). Brewer et al. (1992) found that beef bologna that was vacuum packaged and stored at 4°C for up to 10 wks showed a drop in pH from 5.90 to 5.37. Typically, sausage products are slightly acidic giving them a distinctive flavor and texture, so it is critical to monitor pH in sausage products. This is most commonly done using a pH meter that measures the hydrogen cations surrounding a thin-walled glass bulb at its tip. These meters need to be calibrated frequently using at least two standard buffer solutions, typically pH 4 and 10 (Digital Analysis Corporation).

### ***Proximate Analysis***

When producing a sausage product, the percentage of moisture, fat and protein must be determined. According to the Code of Federal Regulations (CFR) 319.180 (2012), cooked sausage products shall not contain more than 30% fat and no more than 40% of a combination of fat and water. Companies are legally responsible for meeting these criteria. When meat, fat, water, and salt are mixed together and subjected to a high-speed cutting and shearing action, a batter is formed that is typical for sausage making. Formation of a typical meat batter consists of two related transformations: swelling of proteins and formations of a viscous matrix, which ultimately forms a heat-set gel upon cooking, and emulsification of dispersed fat droplets by soluble proteins (Aberle et al., 2001). Disruption of the fibrous structure of meat increases exposure of the proteins to extracellular and added water. The insoluble proteins exist as gel networks capable of absorbing this water. Formation of a matrix in sausage batters stabilizes the structure in finished products by immobilizing free water and preventing moisture loss during thermal processing (Aberle et al., 2001).

In a study determining the binding properties of bologna sausage made with varying fat and protein levels, it was found that protein and fat levels had an appreciable inverse effect on the amount of fluid released. The greater the amount of protein and fat, the lower the total expressible fluid (TEF). An increase in protein content generally causes an increase in the number of locations in the polypeptide chains capable of interacting during heating to form a more stable protein gel matrix and, therefore, permitting a smaller release of water and fat, producing lower TEF and purge loss (Carballo, Mota, Barreto, & Jimenez Colmenero, 1995). Carballo, Fernandez, Barreto, Solas & Jimenez Colmenero (1996) found that cooking loss was

significantly lower in high-fat bologna sausages than in low-fat formulations. Throughout storage, high-fat sausages exhibited less purge loss ( $P < 0.05$ ) than low-fat sausages. Also, the high-fat sausages were harder and chewier than the low-fat sausages. A decrease in fat content and an increase in water will lower the effective concentration of the protein acting to form the gel/emulsion matrix (Cavestany, Jimenez Colmenero, Solas, & Carballo, 1994). The most common methodology for measuring moisture, fat and protein are the methods of the Association of Official Analytical Chemists (AOAC).

### *Sensory Analysis*

Sensory evaluation is a common and very useful tool for quality assessment of processed meat products. It may help improve the quality of products by identifying quality defects using the senses to evaluate the general acceptability of the product. Sensory analysis allows panelists to evaluate the general appearance, odor, flavor, and texture of products, depending on the attribute to be assessed. When performing sensory analysis on a product, it is essential to have a testing room with appropriate lighting, temperature, and seating arrangements with individual testing compartments so as to keep distractions from other panelists at a minimum. On April 8, 2010, the Culinary Institute of America (CIA Consulting, 2010) evaluated two smoked sausage products (company A vs. 1 competitor). The products were tasted both boiled and grilled. The objective was to provide company A with a detailed analysis of their product by executing expert focus groups for sensory evaluation with at least 5 CIA chefs including Certified Master Chefs, and to deliver a comprehensive executive summary of the results including a full flavor profile and feedback on the product concept. The physical attributes they evaluated were texture, finish and mouth feel, presentation and eye appeal, and bite. The taste characteristics they assessed were flavor, moisture, smokiness and aroma. They had developed descriptor definitions for each of the attributes being examined. This not only gave company A expert insight as to how their product aligned with a competing product but the feedback gave them insight as to what could be improved upon in both smoked sausage products helping to increase sales and revenue.

Maca, Miller, Maca, & Acuff, (1997) evaluated beef top rounds that had been injected with salt and phosphate, cooked, and then vacuum packaged. They found that increased storage time, up to 84 d increased saltiness scores. In contrast, Fernandez-Fernandez, Vazquez-Oderiz, & Romero-Rodriguez (2002) found that increased storage time up to 29 wks of vacuum packaged

Galician chorizo sausage decreased saltiness. Brewer et al. (1992) found that beef flavor intensity decreased by the second week in their control bologna. In addition, off flavor scores increased by the second week of storage. Waldman, Westerberg, & Simon (1974) found that as refrigerated shelf storage time increased, overall panel ratings consistently declined for frankfurters formulated with different amounts of salt, sodium nitrite, and sodium isoascorbate. The control frankfurters prepared from meats preblended with different formulations but not stored were considered more desirable using a hedonic scale than any of the stored frankfurters.

### **Retail Display Lighting on Sausage Quality**

The meat industry is aware that lighting type and intensity can have a major role on the appearance of meat in retail display. Energy from lighting catalyzes the formation of metmyoglobin in fresh, frozen, and cured meats (Renner, 1990). Today, there are two common types of lighting used in meat display cases: light emitting diode (LED) and fluorescent (FLS) lighting, with FLS currently being the predominant type.

#### ***Lighting Effect on Color***

Color is the most important characteristic in meat products because of consumer's reliance on appearance to determine quality (Sindelar et al., 2007). Light and oxygen are the main causes of discoloration in cured cooked meat products due to the oxidation of nitrosylheme during storage (Pegg et al., 1997). It has been proven that a two-step reaction causes nitrosylheme to oxidize to ferric heme under illumination in the presence of oxygen. This process includes formation of ONOO<sup>-</sup> as an intermediate and transformation of Fe<sup>2+</sup> into Fe<sup>3+</sup> in the heme cavity (Munk, Huvaere, Van Bocxlaer, & Skibsted, 2010). Color is part of the electromagnetic spectrum ranging from 380-780 nm with violet having the shortest wavelength and red possessing the largest wavelength (Konica Minolta, 2007). Light sources with an emission spectrum mostly in the red section of 630-700 nm have been shown to be desirable for red meats (Kropf, 1980).

Research has shown that lighting source plays a major role in color shelf life. Ultraviolet light penetrates meat causing denaturation of the globin in myoglobin, which causes discoloration (Lawrie, 1985). According to Anderson et al. (1988), illumination clearly affected surface color redness (a\*) values in vacuum packaged ham samples stored in either a display cabinet with illumination, in the same display cabinet but protected from the light, or in a dark,

cold-storage room. The samples that were protected from the light showed only minor color changes. Remarkable improvement in color stability of sliced, vacuum packaged ham was obtained by an initial dark storage period prior to display and exposure to light. The rationale for this obtained protection against discoloration was explained as an efficient depletion of oxygen in the product due to post-mortem processes and microbiological activity (Andersen et al., 1988). Carballo, Cavestany, & Jimenez Colmenero (1990) showed that light caused an immediate drop in redness compared to batches of sliced bologna stored in the dark. In a study evaluating quality attributes of sliced, dry-cured Iberian ham stored under light vs. dark and different packaging systems, the influence of illumination was evident after 60 d of storage. Lightness ( $L^*$ ) and  $a^*$  values were lower ( $P<0.01$ ) in hams exposed to light than those kept in the dark (Parra et al., 2012). High and reduced-fat sliced bologna exposed to light showed an initial increase ( $P<0.05$ ) in  $L^*$  values but then decreased after d 6 of storage. This behavior was more apparent in reduced-fat samples. In addition, light caused an immediate decrease ( $P<0.05$ ) in  $a^*$  values, which was sharper in reduced-fat samples (Jimenez Colmenero, Carballo, Fernandez, Cofrades, & Cortes, 1997b). Haile, De Smet, Claeys, & Vossen (2011) confirmed that light has a detrimental effect on color stability of cooked ham. Products exposed to light showed higher ( $P<0.05$ )  $L^*$  and hue angle and lower ( $P<0.05$ )  $a^*$ ,  $a/b$  ratio, and chroma values compared to those stored in the dark.

### ***Lighting Effect on Lipid Oxidation***

Photooxidation of lipids in meat products can be a result of display lighting. Cured meats are less sensitive to photooxidation than fresh meats due to the addition of nitrite. Nitric oxide helps to inhibit lipid oxidation in meats (Kanner et al., 1980). However, many studies have shown that nitric oxide is not enough to prevent oxidation during retail display. In a study looking at the quality characteristics of bologna sausages made with citrus fiber, TBARS were higher ( $P<0.05$ ) when stored under lighting conditions than those for samples stored in the dark (Fernandez-Gines et al., 2003). Lipid oxidation was strongly enhanced in samples displayed under lighting according to Andersen et al. (1991) who reported that light is an important pro-oxidant in the process of lipid oxidation. This is in agreement with Rawls and Van Santen (1970) who stated exposure to light intensifies lipid rancidity. Moller and Skibsted (2004) found that without light exposure autoxidation caused by residual oxygen is less prominent. However, no

significant variations in autoxidative rancidity levels due to light exposure were observed in sliced pork bologna (Carballo et al., 1991). This could be due in part to the fact that photooxidation was more pronounced on the surface of the bologna slices, while TBARS are based on homogenate prepared by comminuting all of the slices in the package. In a study done at Kansas State University, LED lighting extended color shelf life of beef retail cuts by up to 1 d longer than FLS. However, LED lighting increased lipid oxidation in aerobically packaged pork loin chops (Steele, 2011). Haile et al. (2011) evaluated cooked ham stored in MAP, wrapped in foil and stored in the dark, and wrapped in foil and stored under light at 4°C. A decrease ( $P < 0.05$ ) in TBARS was observed as dark storage increased from d 0 to 21 and then stabilized over the remaining storage duration. There was no difference ( $P > 0.05$ ) between 21 and 35 d of dark storage. The most probable reason for this decline in TBARS value as dark storage duration increased could be the instability or transitory nature of MDA that reacts with TBA to generate a red color (Wang, Pace, Dessai, Bovell-Benjamin, & Phillips, 2002). In this study, they found higher TBARS values for ham products stored for a short duration in the dark compared to a long duration under light. They hypothesize that this may be due to either the prolonged storage time allowing the interaction of MDA and residual nitrite or other possible agents binding the MDA and causing an underestimation of the expected TBARS value.

### ***LED Lighting***

Technology for LED lighting began in the 1950's with commercial development starting in the late 60's (DOE, 2009). Currently, less than 1% of the refrigerated display cases are equipped with LED lighting (DOE, 2008). Over 1,463 companies around the world distribute LED lighting (LED Magazine, 2011).

Phosphor converted LEDs are more efficient than incandescent and compact fluorescent light bulbs leading to significant energy savings (Arik, 2009) The United States Department of Energy (DOE) realizes that the potential cost and energy savings associated with LED lighting is due solely to efficiency (DOE, 2009). Goals for the fiscal year of 2015 are to produce LED lighting systems costing less than \$2/klm with a color rendering index (CRI) greater than 80, correlated color temperature (CCT) less than 5000°K, and 126 lm/W luminaire that emits approximately 1000 lumens (DOE, 2009). Currently, warm light LED systems with CCT less than 3300°K possess 40-60 lm/W while compact FLS lighting possesses 35-60 lm/W. Both

systems possess similar efficacies; however, FLS technology is in its mature stages while LED systems hold the potential to improve two-fold in energy efficiency (DOE, 2009). LED lighting also provides longer operating life, lower maintenance and life cycle costs, minimal light loss, directional illumination, adjustable color, and uniform illumination (DOE, 2008). Since LED lighting provides lower energy costs, longer operating life and lower operating temperatures, research is needed to evaluate how LED lighting affects lipid oxidation rate and color stability of sausage.

### **Internal Endpoint Temperature on Sausage Quality**

Final internal processing temperature affects a number of properties of meat emulsions such as texture, juiciness, and color (Monagle, Toledo, & Saffle, 1974). Cooking conditions largely determine the kind of molecular associations (protein-protein) that occur during gelling processes (Camou, Sebranek, & Olson, 1989) and the way that certain fat properties (expansion and liquefaction) will behave (Whiting, 1988).

#### ***USDA FSIS Regulations Appendix A***

Many meat processing establishments that produce ready-to-eat meat products follow the United States Department of Agriculture (USDA) Food Safety Inspection Services (FSIS) Appendix A to meet the lethality performance standards for *Salmonella* to comply with inspection regulations (USDA FSIS, 1999). According to Appendix A, products cooked to an internal endpoint temperature of 64°C must be held for 107 sec to achieve a 6.5 log reduction, or 115 sec for a 7 log reduction. Products thermally processed to 68°C need to be held for 22 sec or 23 sec to achieve similar log reductions, respectively. After products reach an internal temperature of 70°C, *Salmonella* lethality is achieved instantly (USDA FSIS, 1999).

#### ***Effects of Internal Endpoint Temperature on Meat Color***

Internal endpoint temperature plays a significant role in development of internal cooked color. In a study done in 2006 with ground beef patties, Ryan et al. (2006) found that increasing endpoint temperature decreased interior a\* or redness regardless of the cooking rate, but the extent of decrease in a\* was less ( $P < 0.05$ ) for patties that were cooked rapidly compared to those that were cooked slowly. In a study looking at final internal endpoint temperature and its effects on ground pork to react with nitrite, they found that more pronounced reddening was observed



with lower internal endpoint temperatures (Seyfert, Kropf, & Hunt, 2004). Higher internal endpoint temperatures have been suggested to limit the occurrence of color problems that can arise when the fat level is reduced. In such cases, an internal temperature of 72-75° C is recommended (Wirth, 1988). However, in an experiment using high and low-fat bologna sausage, final internal endpoint temperature from 63°C to 78°C did not affect ( $P>0.05$ ) the surface color of the slices. Color did vary with fat level interactions ( $P<0.01$ ). In general,  $a^*$  values were higher in low-fat samples subjected to high final internal endpoint temperature than those cooked to only 63°C (Carballo et al., 1996). In a study using low-fat pork and chicken batters,  $L^*$  and  $a^*$  values generally increased in either species with an increase of final internal endpoint temperature. This was particularly evident between 60°C and 70°C;  $b^*$  did not follow this same pattern (Jimenez-Colmenero et al., 1998). Another study found that endpoint temperature had a significant effect on color measures in pork chops cooked to six internal endpoint temperatures. As endpoint temperature increased,  $L^*$  values increased,  $a^*$  values decreased, and hue angle increased resulting in a lighter, less red interior (De Santos et al., 2007).

### ***Effects of Internal Endpoint Temperature on Lipid Oxidation***

Internal endpoint temperature has been shown to affect the rate of lipid oxidation in meat products. In a study measuring the rate of lipid oxidation in roasts cooked to different internal endpoint temperatures, Spanier and Miller (1996) found that the higher the endpoint temperature, the greater the level of lipid oxidation. In addition, the inner part of the roast that was exposed to lower temperatures than the outer region showed lower levels of lipid oxidation. Noteworthy is the observation that these temperature dependent changes only appeared to become evident after a period of storage. Cooking to different endpoint temperatures did not produce any appreciable differences in TBARS level in freshly cooked meat. This observation suggests that while the structure and chemistry of the meat are immediately affected during the initial cooking process, the development of off-flavor volatiles occurs only after the meat is stored. Therefore, cooking allows precursors to become readily available to oxygen and other free radical initiation compounds to drive the peroxidation of lipids (Spanier & Miller, 1996). Lipid oxidation during storage of cooked pork was lower ( $P<0.01$ ) when the pork was cooked to an internal temperature of 72°C rather than 82°C. Also, when pork was cooked at a fast rate of 2.0°C/min, oxidation was

lower ( $P < 0.05$ ) compared to cooking at a slow rate of  $0.3^{\circ}\text{C}/\text{min}$  (Kingston et al., 1998). These findings agree with Mielche (1995) and Ang and Huang (1993) who reported that lipid oxidation increased as cooking temperature increased above  $80\text{-}85^{\circ}\text{C}$ . If meat is cooked quickly, the rapid coagulation of proteins, including iron-containing proteins, may reduce the rate of iron release, making it less available for catalysis of lipid oxidation (Chen, et al., 1984).

## **Chapter 3 - Shelf Life of Smoked Sausage Displayed Under Light Emitting Diode (LED) or Fluorescent (FLS) Lighting**

### **Abstract**

Quality attributes of vacuum packaged, skinless smoked sausage made with a combination of pork, turkey, and beef, cooked to 64, 68, or 72°C internal endpoint temperature following United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Appendix A, and displayed at 4° C for up to 120 d under light emitting diode (LED) and fluorescent (FLS) lighting were evaluated. External color, pH, thiobarbituric acid reactive substances (TBARS), proximate analysis, reheat yield, and sensory attributes were measured on d 0, 90, and 120 of display. Purge amount and color were measured on d 10, 90, and 120. Product was collected from a commercial processor on the d of production (d 0) and displayed in LED or FLS retail display cases set to the same operational and temperature profiles.

Lighting type had no effect ( $P>0.05$ ) on any of the attributes measured in this study. Average case temperature was 3.9°C and 3.5°C for the LED and FLS cases, respectively. Internal processing temperature and sampling day were the only factors contributing significant differences within measurements. External color was less ( $P<0.05$ ) red by 0.63 units in product thermally processed to 64°C than product processed to 68°C. Product cooked to 72°C was less ( $P<0.05$ ) yellow by 0.95 and 0.54 units respectively, compared to those processed to 64 and 68°C. Purge color lightness increased by 1.88 units ( $P<0.05$ ) in product thermally processed to 72 compared to 64°C. There was a temperature and d effect for purge color redness. As product endpoint temperature increased, purge color redness decreased ( $P<0.05$ ). Purge was more red by 0.36 units ( $P<0.05$ ) on d 120 compared to d 10. Yellowness of purge color increased at 72°C compared to 64°C by 0.66 units. Purge was more yellow ( $P<0.05$ ) on d 120 compared to d 10 and 90 by 0.36 and 0.49 units, respectively. TBARS values decreased ( $P<0.05$ ) from 0.70 mg of malonaldehyde/100g on d 0 to 0.35 and 0.23 on d 90 and 120, respectively. Fat content was 24.58% on d 0, and increased ( $P<0.05$ ) to 26.51% and 26.23% on d 90 and 120, respectively. Protein content was 10.72% in product cooked to 64°C while it was lower ( $P<0.05$ ) at 10.34% and 10.27% in products cooked to 68 and 72°C, respectively. There was a temperature and day effect on percent purge. Purge content increased ( $P<0.05$ ) from 1.45% to 1.90% in products

cooked to 64 and 68°C, respectively. In product processed to 72°C, there was 2.23% purge which was similar ( $P>0.05$ ) to product cooked to 68°C. While the amount of purge increased ( $P<0.05$ ) from 1.58% on d 10 to 1.92% on d 90, there was no additional increase ( $P>0.05$ ) in percent purge on d 120. Reheat yield was lower ( $P<0.05$ ) in 68°C than 64 or 72°C products. Sensory panel scores showed that flavor intensity decreased ( $P<0.05$ ) as day of storage increased, and saltiness decreased from d 0 to d 90. While there were slight changes found in quality characteristics of smoked sausages during storage, many of these were minimal. Processors could reduce their internal endpoint temperature following USDA FSIS Appendix A guidelines with minimal effect on product quality. Vacuum packaged precooked smoked sausages could be displayed under LED or FLS lighting with no effect on product quality.

## Introduction

Many meat processing establishments that produce ready-to-eat meat products follow USDA FSIS Appendix A (1999) to meet lethality performance standards for *Salmonella*. If quality attributes of the product are not affected, energy and processing time can be altered by cooking products to a lower internal temperature as described in USDA FSIS Appendix A (1999). Two of the primary quality factors that should be evaluated in order to lower internal endpoint temperature are color deterioration and lipid oxidation.

Internal endpoint temperature has been shown to play a significant role in development of internal cooked color. In a study done in by Ryan et al. (2006) with ground beef patties, it was found that increasing endpoint temperature decreased interior  $a^*$  or redness regardless of cooking rate, but the extent of decrease in  $a^*$  was less ( $P<0.05$ ) for patties that were cooked rapidly compared to those that were cooked slowly. Another study using low-fat pork and chicken batters found that  $L^*$  and  $a^*$  values generally increased in either species with an increase of final cooking temperature, particularly between 60°C and 70°C; however,  $b^*$  did not follow this same pattern (Jimenez-Colmenero et al., 1998). De Santos et al. (2007) found that endpoint temperature had a significant effect on color measures in pork chops cooked to six internal endpoint temperatures. As endpoint temperature increased  $L^*$  values increased,  $a^*$  values decreased, and hue angle increased showing a lighter, less red interior.

Internal endpoint temperature has also been shown to affect the rate of lipid oxidation in meat products. Cooking allows precursors to become readily available to oxygen and other free

radical initiation compounds to drive the peroxidation of lipids (Spanier et al., 1996). Lipid oxidation during storage of cooked pork was lower ( $P < 0.01$ ) when the pork was cooked to an internal temperature of  $72^{\circ}\text{C}$  rather than  $82^{\circ}\text{C}$ . Also, when pork was cooked at a fast rate ( $2.0^{\circ}\text{C}/\text{min}$ ), oxidation was lower ( $P < 0.05$ ) compared to a slow rate ( $0.3^{\circ}\text{C}/\text{min}$ ) (Kingston et al., 1998). These findings agree with Mielche (1995) and Ang and Huang (1993) who reported that lipid oxidation increased as cooking temperature increased above  $80\text{-}85^{\circ}\text{C}$ . If meat is cooked quickly the rapid coagulation of proteins, including iron-containing proteins, may reduce the rate of iron release, making less available for catalysis of lipid oxidation (Chen et al., 1984).

Lighting may also play a role in sausage color deterioration. Color is the most important characteristic in meat products because of consumer's reliance on appearance to determine quality (Sindelar et al., 2007). Light and oxygen are the main causes of discoloration in cured cooked meat products due to the oxidation of nitrosylheme during storage (Pegg et al., 1997). Research has shown that lighting source plays a major role in color shelf life. Ultraviolet light penetrates meat causing denaturation of the globin in myoglobin leading to discoloration (Lawrie, 1985). Illumination clearly affected surface color redness ( $a^*$ ) values in vacuum packaged ham samples stored in either a display cabinet with illumination, in the same display cabinet but protected from the light, or in a dark cold storage room. The samples that were protected from the light showed only minor color changes. Remarkable improvement in color stability of sliced, vacuum packaged ham was obtained by an initial dark storage period prior to display and exposure to light. The rationale for this obtained protection against discoloration was explained as an efficient depletion of oxygen in the product due to post-mortem processes and microbiological activity (Andersen et al., 1988).

Light has also been shown to have a significant effect on the rate of lipid oxidation in meat products. Cured meats are less sensitive to photooxidation due to the addition of nitrite. Nitric oxide helps to inhibit lipid oxidation in meats (Kanner et al., 1980). However, many studies have shown that nitric oxide is not enough to prevent oxidation during retail display. In a study looking at the quality characteristics of bologna sausages made with citrus fiber, thiobarbituric acid reactive substances (TBARS) values were higher ( $P < 0.05$ ) when stored under lighting conditions than those for samples stored in the dark (Fernandez-Gines et al., 2003). Lipid oxidation was strongly enhanced in samples displayed under lighting. Andersen et al. (1991) reported that light is an important pro-oxidant in the process of lipid oxidation. This was

in agreement with Rawls and Van Santen (1970) who stated exposure to light intensifies lipid rancidity.

The objective of this study was to determine if internal endpoint temperature or lighting type affects quality factors of smoked sausage when displayed refrigerated for up to 120 d.

## **Materials and Methods**

### ***Experimental Design***

Skinless smoked sausages cooked to an endpoint temperature of 64, 68, or 72°C were displayed refrigerated under LED or FLS lighting for a total of 6 temperature and display lighting combinations. Sausage packages were displayed for up to 120 days. Fat, moisture, protein content, pH, instrumental external color, TBARS, reheat yield, and descriptive sensory analysis were evaluated on d 0, 90, and 120 of display. On d 10, 90, and 120, percent purge and instrumental purge color were determined. A total of 36 packages per replication were displayed under each lighting type resulting in 72 packages evaluated per replication. Packages were randomly pulled for subsequent evaluation. This study was conducted in triplicate.

### ***Product Description***

Skinless smoked sausage formulated with, pork, beef, mechanically separated turkey water, corn syrup, 2% or less of dextrose, flavorings, autolyzed yeast, modified food starch, mechanically separated chicken, monosodium glutamate, potassium and sodium lactate salt, sodium diacetate, sodium phosphate and vitamin C (ascorbic acid) was obtained from a commercial supplier (Armour Eckrich, Junction City, KS). Sausages were cooked to three internal endpoint temperatures (64, 68, and 72°C) in a commercial facility following USDA FSIS Appendix A (1999). After thermal processing, product was immediately cooled in a proprietary brine solution containing salt, water, and citric acid until the internal temperature decreased to 4.4°C, following USDA FSIS Appendix B (1999). Sausages were then vacuum packaged, with 2 links per package, boxed, and immediately transported in coolers containing ice packs to Kansas State University. Products were coded and randomly assigned shelf locations in retail display cases under LED or FLS lighting.

### ***Retail Display Cases***

Two Hussmann Ingersoll 8 foot M5X (Bridgeton, MO) retail display cases were used in this study. One case was equipped with FLS lighting while the other contained LED. The cases were installed end-to-end with condenser units equipped with an on/off cycle counter and an hour meter in an adjacent room. Defrost cycles occurred spontaneously every 6 h. To minimize end-temperature fluctuations and simulate end-to-end placement, a 1.03 x 1.74 x 0.05 m piece of Owens Corning Formulator 150 insulation (Toledo, OH) was attached to the outside of each case.

Case temperatures were set to operate at 3°C with the case lighting off and similar condenser cycling. Temperatures were confirmed with 30 RD-Temp-XT Temperature Loggers (Omega Engineering, Stamford, CT) to be similar during 3 d of dark operation before d 0 of the study. Each display case had 4 adjustable shelves consisting of two sections and had a fixed bottom shelf. The top shelf width was 35.66 cm, shelf 2 was 40.64 cm, shelves 3 and 4 were 45.72 cm, and the bottom shelf was 72.39 cm wide. Shelves were arranged identically in both cases. As product was removed from a case for analysis, a 454 g plastic water bag was positioned in the empty location to simulate a full display case.
















### ***Display Lighting***

The sausages in both cases were illuminated 24 h/d. In the LED case, a canopy lighting fixture (Hussmann® EcoShine Model Nos. 4441720 and 4441721, Bridgeton, MO) positioned above the top shelf had a correlated color temperature (CCT) of 2867 K and a color rendering index (CRI) of 93. The bottom four shelves were illuminated with LED light bars (Hussmann® EcoShine Model No. 4441590, Bridgeton, MO) having a CCT of 3007 K and a CRI of 95.7. Lighting intensity in the LED case averaged 1627 lm. The FLS lighting (Sylvania Octron, F032/835/ECO, Danvers, MA) had a CCT of 3500K, a CRI of 82, and lighting intensity averaging 1712 lm.

### ***Case Temperatures***

Case temperatures were monitored throughout the study using I-button Thermochrons (DS1921 G Maxim Direct, Sunnyvale, CA). Three I-buttons were located on each shelf with one on the far right, far left, and center positions of each shelf for a total of 15 temperature loggers per case (Fig. 3-1). Temperatures were recorded every 4 h throughout the study.

**Figure 3-1. I-button temperature logger locations in fluorescent (FLS) and light emitting diode (LED) display cases.**

FLS and LED Cases			
Shelf			
1, Top			
2			
3			
4			
5, Bottom			

### *Instrumental External Color*

Sausages from one package representing each endpoint temperature and lighting type were analyzed for external color. A total of 6 packages were evaluated per replicate. Instrumental color measurements were taken in triplicate on the bottom of the package because the principle display panel covered most of the product. Color readings were taken through the clear vacuum packaging film for CIE lightness (\*L), redness (a\*), and yellowness (b\*) using Illuminant D65, an aperture of 13 mm and a 10° observer with the HunterLab MiniScan XE Plus™ (Model D/8-5; Hunter Associates Laboratory Inc., Reston, VA). Three color readings per package were taken on the two links ensuring that each link received at least one reading. Hue angle, saturation index and a/b ratios were calculated.

### *Percent Purge*

Percent purge was initially measured on d 10 as that is when purge started to accumulate in the packages. Two packages from each endpoint temperature and lighting type were sampled on d 10, 90 and 120 to measure percent purge and purge color. Percent purge was determined by first weighing the entire package; packages were then carefully cut open in the right bottom corner to extract the purge using a 15 cm Pasteur Pipette (Fisher Scientific, Fair Lawn, NJ). The purge was transferred into the small glass vials for purge color determination. The sausage was then removed from the package and blotted with a paper towel to ensure all purge had been removed. This same blotting procedure was followed with the empty package to dry the package. The sausage was then weighed separately from the empty package. The purge weight was



calculated using the formula: (entire package weight)-(sausage weight)-(empty package weight). Then, purge percentage was calculated using the formula: (purge weight/entire package weight) x 100.

### ***Instrumental Purge Color***

Instrumental purge color measurements were taken in triplicate analyzing the CIE lightness (\*L), redness (a\*), and yellowness (b\*) using Illuminant D65, an aperture of 13 mm and a 10° observer with the HunterLab MiniScan XE Plus™. Purge color was evaluated using the purge collected from packages used to determine percent purge. From each package, 3 ml of purge was pipetted into a small, flat bottom glass vial (Waring Commercial, New Hartford, CT), capped with a black screw top lid, and then covered with black electrical tape to ensure light could not interfere with the readings. A bench top stand was used hold the MiniScan allowing hands-free use and free rotation of the device. Vials were individually placed on the MiniScan to obtain readings. In addition, a piece of black cloth was placed over the vial when the readings were conducted. The vials were washed and dried between every sample.

### ***pH***

Sausage pH was determined using product from the same packages used to measure external color. Sausage links were cut in half crosswise and a pH probe (Hanna Instruments; H199163; Woonsocket, RI) attached to a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA) was inserted into the geometric center of each link.

### ***Proximate Analysis***

In order to prepare samples for proximate analysis and TBARS, sausages were manually chopped into fine pieces, and frozen (-80°C). Next, samples were pulverized using a table top blender and liquid nitrogen (model 33BL79; Waring Products, New Hartford, CT) and stored in 14.0 cm x 22.9 cm sterile plastic sampling bags (Fisher Scientific, Fair Lawn, NJ) at -80°C for up to one week for subsequent TBARS and proximate analysis determinations.

Moisture and crude fat content were measured using the SMART system 5 (CEM Corp., NC) procedure (AOAC Official Method PVM-1:2003 MEAT). Crude protein was measured using the LECO FP-2000 Protein/Nitrogen Analyzer (model 602-600; LECO Corp., MI) procedure (AOAC Official Method 990.03).

## ***TBARS***

Product oxidation was analyzed using a modified procedure of Tarladgis et al. (1960). From each sample 10 g  $\pm$  0.2 g was collected, and all samples were measured in duplicate. In a round bottom flask, 10 g sample, 97 ml distilled water, 2 ml hydrochloric acid (Fisher Scientific, Fair Lawn, NJ) solution, 1 ml sulfanilamide (Fisher Scientific, Fair Lawn, NJ) solution, and two boiling beads (Boileezers® Fisher Scientific, Fair Lawn, NJ) were combined. Samples were distilled until 50 ml was collected. Next, 5 ml of distillate was collected using a 5 ml pipette from the 50 ml sample and transferred to a 30 ml screw top test tube. Then, 5 ml of 2 thiobarbituric acid (MP Biomedicals, LLC Solon, OH) reagent was added. Test tubes containing the solutions were placed in a boiling water bath for 35 min, and then cooled in a cool tap water bath for 10 min. Absorbance was read on a Spectophic 21 spectrophotometer (Bausch & Lomb, Rochester, NY) at 532 nm, and mg of malonaldehyde was calculated from the absorbance reading.

## ***Reheat Yield***

Sausage links from one package representing each endpoint temperature and lighting type were weighed prior to reheating using a 27.9 cm electric skillet (The West Bend CO, West Bend WI, Model 9706 and 9801). Two cups of filtered distilled water were brought to a boil in the skillet set to 121°C. After the water began to boil, the sausage links were added and the thermostat was turned down until the water reached a simmer. All sausages were cooked until an internal temperature of 74°C was reached and then they were immediately weighed. Reheat yield was calculated using the formula: (re-heated weight/initial weight) x 100.

## ***Sensory***

Sensory analysis was performed using an experienced panel composed of graduate students and faculty from Kansas State University. All sensory panelists were screened using the American Society for Testing and Materials guidelines (1981). In addition, panelists were oriented with this product over several training sessions to familiarize themselves with the product and the descriptive 8-point scale. In between d 0 and 90, panelists were re-oriented with fresh product. Samples prepared for reheat yield determinations were used for sensory analysis. The attributes evaluated were bite, flavor intensity, saltiness, off flavor, and mouth feel/coating. Each of these attributes was ranked to the nearest 0.5 increment using an 8-point scale. The

scales used were: 8=extremely firm, extremely intense, extremely salty, extremely intense, and extremely heavy coating, 4=slightly soft, slightly bland, slightly unsalty, slight, and slight, and 1=extremely soft, extremely bland, not salty, none, and none. Two 1.3 cm pieces of each sample were randomly presented to panelists one at a time. In between samples, panelists were given distilled, filtered water, apple slices and unsalted saltine crackers to cleanse their palate.

### ***Statistical Analysis***

This was a randomized complete block design with a three way factorial treatment structure. Replication was used as a random effect. Data were analyzed using the PROC Mixed procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). The Satterthwaite adjustment was used for degrees of freedom error. Effects tested in the model included temperature (64, 68, and 72°C), lighting type (LED vs. FLS), d (0, 10, 90, and 120), temperature by lighting type, temperature by day, day by lighting type and temperature by lighting type by day. The least squares means procedure was used to separate treatment means ( $P < 0.05$ ).

### **Results and Discussion**

Main effects between day and temperature are summarized in Tables 1-3. Lighting type was not significant ( $P > 0.05$ ) for any of the attributes evaluated. In addition, there were no interactions ( $P > 0.05$ ) for temperature by lighting type, temperature by day, day by lighting type, and temperature by lighting type by day for any of the attributes evaluated (Appendix A). Mean pH was 6.10 and was similar ( $P > 0.05$ ) between treatments.

**Table 1. Probability values for temperature and day for external and purge L\*, a\*, b\*, a/b ratio, saturation index, and hue angle instrumental color.**

	<b>Temperature</b>	<b>Day</b>
<b>External Color</b>		
<b>L*</b>	0.1221	0.4789
<b>a*</b>	0.0186	0.576
<b>b*</b>	0.0035	0.1156
<b>a/b Ratio</b>	0.0404	0.1466
<b>Saturation Index</b>	0.0044	0.2641
<b>Hue Angle</b>	0.0292	0.1657
<b>Purge Color</b>		
<b>L*</b>	0.0068	0.8382
<b>a*</b>	<0.0001	0.0021
<b>b*</b>	0.0012	0.0272
<b>a/b Ratio</b>	<0.0001	0.1167
<b>Saturation Index</b>	0.1041	0.0081
<b>Hue Angle</b>	<0.0001	0.0374

**Table 2. Probability values for temperature and day for purge, pH, proximate analysis, thiobarbituric acid reactive substances (TBARS), and reheat yield.**

	<b>Temperature</b>	<b>Day</b>
<b>Purge (%)</b>	0.0005	0.0253
<b>pH</b>	0.2616	0.2634
<b>Moisture (%)</b>	0.3242	<0.0001
<b>Fat (%)</b>	0.7510	<0.0001
<b>Protein (%)</b>	<0.0001	0.0027
<b>TBARS</b>	0.7352	<0.0001
<b>Reheat Yield (%)</b>	0.0073	0.9291

**Table 3. Probability values for temperature and day for sensory analysis.**

<b>Sensory Attribute</b>	<b>Temperature</b>	<b>Day</b>
<b>Bite</b>	0.4557	<0.0001
<b>Flavor Intensity</b>	0.3953	<0.0001
<b>Saltiness</b>	0.0134	<0.0001
<b>Off Flavor</b>	0.7444	0.9131
<b>Mouthfeel</b>	0.6725	0.7246

### *External Color*

Mean external color measurements by temperature are shown in Table 4. Storage time did not affect ( $P<0.05$ ) external color in this study (Table 1). This is most likely due to the vacuum packing, storage temperature, and addition of nitrite to the product. These factors have been shown to prevent color deterioration over long periods of storage (Church, 1993). Generally, the human eye is not able to perceive color differences until CIE values change by 1-2 units, which is why instrumental color measurement is widely used when measuring meat color. There were no significant differences in lightness ( $L^*$ ) for temperature. This agrees with Ryan et al. (2006) who reported that  $L^*$  and  $b^*$  varied among internal endpoint temperature treatments with no clear, consistent trends. In contrast, Jimenez-Colmenero et al. (1998) found that  $L^*$  and  $a^*$  values generally increased with final cooking temperature in pork and chicken meat batters, particularly between 60-70°C. However, these batters were stored in flexible plastic jars and exposed to high pressure, which has been known to increase lightness (Jimenez Colmenero, Carballo, Fernandez, Barreto, & Solas, 1997a). External color was more ( $P<0.05$ ) red ( $a^*$ ) by 0.63 units in product thermally processed to 64°C than product processed to 68°C. However, no differences were seen in products cooked to 72°C. De Santos et al. (2007) found that in pork chops as the internal temperature increased,  $a^*$  values decreased. Ryan et al. (2006) found that, in general, increasing endpoint temperature decreased the interior  $a^*$  regardless of the cooking rate, but the extent of decrease for  $a^*$  was less ( $P<0.05$ ) for beef patties cooked rapidly than those cooked slowly. In a study looking at final internal endpoint temperature and the ability of ground pork to react with nitrite, more pronounced reddening was observed with lower internal endpoint temperatures (Seyfert et al., 2004). At lower temperatures, less myoglobin is denatured leaving

more undenatured myoglobin available to bind nitrite and produce nitric oxide (Seyfert et al., 2004). Carballo et al. (1996) found that increasing final cooking temperature from 63 to 78°C did not affect the color of meat emulsions used for bologna sausages. However, color did vary with fat level. They found that, in general, a\* values were higher in low-fat samples subjected to high final internal temperature than in those cooked only to 63°C.

Product cooked to 72°C was less (P<0.05) yellow (b\*) by 0.95 and 0.54 units respectively, compared to those processed to 64 and 68°C. Products that had the most discoloration as shown by the lowest a/b ratios were those cooked to an internal temperature of 64°C or 68°C. Sausages cooked to 64°C showed the most saturation indicating the most intense color. As the internal endpoint temperature increased from 68 to 72°C, hue angle decreased (P<0.05) by 1.02 units showing a less red, and a more well done cooked color.

**Table 4. Least squares means (Lsmeans) for instrumental exterior color attributes of smoked sausages cooked to three internal endpoint temperatures.**

	Temperature			SEM <sup>1</sup>
	64°C	68°C	72°C	
<b>L*</b>	49.60 <sup>a</sup>	49.58 <sup>a</sup>	49.10 <sup>a</sup>	0.90
<b>a*</b>	18.20 <sup>a</sup>	17.57 <sup>b</sup>	17.80 <sup>ab</sup>	0.22
<b>b*</b>	22.76 <sup>a</sup>	22.35 <sup>a</sup>	21.81 <sup>b</sup>	0.31
<b>a/b Ratio</b>	0.80 <sup>ab</sup>	0.79 <sup>b</sup>	0.82 <sup>a</sup>	0.02
<b>Saturation Index</b>	29.17 <sup>a</sup>	28.47 <sup>b</sup>	28.19 <sup>b</sup>	0.21
<b>Hue Angle</b>	51.32 <sup>ab</sup>	51.79 <sup>a</sup>	50.77 <sup>b</sup>	0.64

<sup>1</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### ***Purge Color***

Mean purge color measurements by temperature are shown in Table 5, and mean purge color measurements by day are shown in Table 6. Purge color lightness increased by 1.88 units (P<0.05) in product thermally processed to 72°C compared to 64°C. There was a slight increase in lightness (P>0.05) by 1.06 units from 68°C to 72°C. There was a temperature and day effect for purge color redness. As product endpoint temperature increased, purge color redness

decreased ( $P < 0.05$ ) from 64°C by 1.11 units and 0.23 units in product cooked to 68°C and 72°C, respectively. Yellowness of purge increased ( $P < 0.05$ ) by 0.35 units in product cooked to 68°C compared to 64°C and by 0.66 units when comparing 72°C to 64°C. There was a slight increase ( $P > 0.05$ ) in purge color yellowness in sausages cooked to 68°C compared to 72°C by 0.31 units. A larger a/b ratio indicates more redness and less discoloration (AMSA, 2011). The a/b ratio decreased ( $P < 0.05$ ) from 64°C to 68°C by 0.73 units; however, this was less apparent ( $P > 0.05$ ) when comparing product cooked to 68°C and 72°C since there was a decrease of only 0.11 units. The a/b ratio decreased ( $P < 0.05$ ) by 0.84 units in product cooked to 72°C compared to 64°C. The a/b ratio aligned with the a\* values indicating that, in general, when endpoint temperature increased, purge color became less red and less discolored. A larger saturation index or chroma indicates more intense hues in product color (AMSA, 2011). Saturation index was not affected ( $P > 0.05$ ) by internal endpoint temperature (Tables 1 & 5). Large hue angle values indicate less red and more metmyoglobin (brown) pigmentation thus indicating a more well-done cooked color (AMSA, 2011). Hue angle increased ( $P < 0.05$ ) from 64°C to 68°C by 33.82 units. This increase continued slightly ( $P > 0.05$ ) from 68°C to 72°C by 6.36 units.

Storage time did not affect ( $P > 0.05$ ) purge color lightness (Tables 1 & 6). Purge was more red by 0.34 and 0.36 units ( $P < 0.05$ ) on d 90 and 120 compared to d 10, respectively (Table 6). After d 90, storage time had no affect ( $P > 0.05$ ) on purge redness values. Purge was more yellow ( $P < 0.05$ ) on d 120 compared to d 10 and 90 by 0.36 and 0.49 units, respectively. The a/b ratio was not affected ( $P > 0.05$ ) by storage time. The saturation index was higher ( $P < 0.05$ ) on d 120 compared to d 10 and 90 by 0.48 and 0.50 units, respectively. Hue angle decreased from d 10 to d 90 by 7.66 units, but was not different ( $P > 0.05$ ) than d 120. Overall purge color became more red as days of storage increased up until d 90 and then leveled off; yellowness did not significantly increase until d 120, which showed the highest b\* value.

**Table 5. Least squares means (Lsmeans) for instrumental purge color attributes of smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Temperature			SEM <sup>1</sup>
	64°C	68°C	72°C	
<b>L*</b>	24.77 <sup>b</sup>	25.59 <sup>ab</sup>	26.65 <sup>a</sup>	0.44
<b>a*</b>	1.18 <sup>a</sup>	0.07 <sup>b</sup>	-0.16 <sup>c</sup>	0.10
<b>b*</b>	1.69 <sup>b</sup>	2.04 <sup>a</sup>	2.35 <sup>a</sup>	0.22
<b>a/b Ratio</b>	0.77 <sup>a</sup>	0.04 <sup>b</sup>	-0.07 <sup>b</sup>	0.08
<b>Saturation Index</b>	2.11 <sup>a</sup>	2.07 <sup>a</sup>	2.37 <sup>a</sup>	0.23
<b>Hue Angle</b>	54.18 <sup>b</sup>	88.00 <sup>a</sup>	94.36 <sup>a</sup>	2.67

<sup>1</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

**Table 6. Least squares means (Lsmeans)<sup>1</sup> for instrumental purge color attributes of refrigerated smoked sausage cooked to three internal endpoint temperatures and displayed under light emitting diode or fluorescent lighting for up to 120 days.**

	Days of Display		
	10	90	120
<b>L*</b>	25.76 <sup>a</sup> ±0.39	25.50 <sup>a</sup> ±0.39	25.75 <sup>a</sup> ±0.53
<b>a*</b>	0.13 <sup>b</sup> ±0.09	0.47 <sup>a</sup> ±0.09	0.49 <sup>a</sup> ±0.11
<b>b*</b>	1.95 <sup>b</sup> ±0.22	1.82 <sup>b</sup> ±0.22	2.31 <sup>a</sup> ±0.24
<b>a/b Ratio</b>	0.14 <sup>a</sup> ±0.07	0.31 <sup>a</sup> ±0.07	0.29 <sup>a</sup> ±0.09
<b>Saturation Index</b>	2.03 <sup>b</sup> ±0.23	2.01 <sup>b</sup> ±0.23	2.51 <sup>a</sup> ±0.25
<b>Hue Angle</b>	83.65 <sup>a</sup> ±2.37	75.99 <sup>b</sup> ±2.37	76.91 <sup>ab</sup> ±3.23

<sup>1</sup> ± standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### ***Percent Purge***

Mean percent purge measurements by temperature are shown in Table 7, and mean percent purge measurements by day are shown in Table 8. The amount of purge increased



( $P < 0.05$ ) from 1.45% to 1.90% in products cooked to 64 and 68°C, respectively. Cooking sausages to 72°C increased ( $P < 0.05$ ) purge percent by 0.78% compared to sausages cooked only to 64°C. In product processed to 72°C, there was 2.23% purge, which was similar ( $P > 0.05$ ) to product cooked to 68°C. Carballo et al. (1995, 1996) found that purge losses were not affected ( $P > 0.05$ ) by final internal temperature of bologna sausage with differing protein contents. He concluded that the variable that had the most effect on purge loss was protein content. The greater the protein present, the smaller was the amount of liquid that separated off during storage (Carballo et al., 1995). A relationship between purge and protein content was found in the current study. Purge content increased and protein content decreased with increasing internal endpoint temperatures. While the amount of purge increased ( $P < 0.05$ ) from 1.58% to 1.92% on d 10 and 90, respectively, there was no additional increase ( $P > 0.05$ ) in percent purge on d 120. Cardoso et al. (2008) found that purge loss in fish sausage remained low and almost constant over storage time, with no significant difference being detected. The observed purge loss in the fish sausages did not exceed 1.8%; however, those sausages were only stored up to 57 d. Andres et al. (2006) found that purge loss for chicken sausage with 0% and 2% added fat remained practically constant during storage (maximum value 9.71%). Purge loss values were lower ( $P < 0.05$ ) when 5% fat was added. When producing low-fat products water is usually added to make up for the decrease in fat. Increasing water beyond the traditional levels tends to diminish the stability of the meat batter. This partially explains the excess purge loss in low-fat sausages Andres et al. (2006).

**Table 7. Least squares means (Lsmeans) for percent purge by temperature from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Temperature			SEM <sup>1</sup>
	64°C	68°C	72°C	
<b>Purge (%)</b>	1.45 <sup>b</sup>	1.90 <sup>a</sup>	2.23 <sup>a</sup>	0.14

<sup>1</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ ( $P < 0.05$ ).

**Table 8. Least squares means (Lsmeans)<sup>1</sup> for percent purge by day from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Days of Display		
	10	90	120
<b>Purge (%)</b>	1.58 <sup>b</sup> ±0.12	1.92 <sup>a</sup> ±0.12	2.07 <sup>a</sup> ±0.17

<sup>1</sup> ± standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### *Proximate Analysis*

Mean proximate analysis measurements by temperature are shown in Table 9, and measurements by day are shown in Table 10. It is important to note that proximate analysis does not account for carbohydrates. Proximate totals for processed meat are lower than fresh meats due to the complexity of the ingredient formulation. No differences (P>0.05) were seen in percent moisture or fat as internal endpoint temperature increased. However, protein was 10.72% in product cooked to 64°C while it was lower (P<0.05) at 10.34% and 10.27% in products cooked to 68°C and 72°C, respectively. These results do not agree with those of Parrish, Olson, Miner, & Rust (1973) who found that moisture content decreased and fat content increased from 60°C to 80°C in beef rib steaks. This is most likely due to cookery method (dry heat vs. a controlled atmosphere smoke house), product type (whole muscle vs. emulsion type sausage), and ingredient formulation (phosphate in the sausage helps to bind water). Moisture content decreased from 51.50% on d 0 to 49.81% on d 90, while d 90 and 120 were similar (P>0.05). Fat content was 24.58% on d 0, and increased (P<0.05) to 26.51% and 26.23% on d 90 and 120, respectively. However, fat content on d 90 and 120 was similar (P>0.05). Protein content decreased (P<0.05) from 10.46% on d 90 to 10.24% on d 120. Day 0 and 90 were similar (P>0.05) for protein content. While, Mielnik, Aaby, Rolfsen, Ellekjaer, & Nilsson (2002) found that storage time had only minor affects on sausage composition, they did see an increase (P<0.01) in moisture from 69.70% at 6 wks to 70.20% at 18 wks and an increase (P<0.001) in fat content from 11.00% at 6 wks to 11.80% at 18 wks.

**Table 9. Least squares means (Lsmeans) for proximate analysis by temperature from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Temperature			SEM <sup>1</sup>
	64°C	68°C	72°C	
<b>Moisture (%)</b>	50.11 <sup>a</sup>	50.70 <sup>a</sup>	50.47 <sup>a</sup>	0.66
<b>Fat (%)</b>	25.70 <sup>a</sup>	25.65 <sup>a</sup>	25.96 <sup>a</sup>	0.77
<b>Protein (%)</b>	10.72 <sup>a</sup>	10.34 <sup>b</sup>	10.27 <sup>b</sup>	0.11

<sup>1</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

**Table 10. Least squares means (Lsmeans)<sup>1</sup> for proximate analysis by day from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Days of Display		
	0	90	120
<b>Moisture (%)</b>	51.50 <sup>a</sup> ±0.64	49.81 <sup>b</sup> ±0.64	49.97 <sup>b</sup> ±0.69
<b>Fat (%)</b>	24.58 <sup>b</sup> ±0.76	26.51 <sup>a</sup> ±0.76	26.23 <sup>a</sup> ±0.81
<b>Protein (%)</b>	10.62 <sup>a</sup> ±0.11	10.46 <sup>a</sup> ±0.11	10.24 <sup>b</sup> ±0.13

<sup>1</sup> ± standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### **TBARS**

Mean TBARS measurements by day are shown in Table 11. Internal endpoint temperature did not affect (P>0.05) TBARS values (Table 2). Although TBARS values remained low, on d 0 they were within the threshold range of consumer perceived oxidation of 0.5-1.0 according to Tarladgis et al. (1960). One possible explanation is that the incoming raw materials, such as the mechanically separated turkey, were already slightly oxidized. During storage residual nitrite might have stabilized the lipids and prevented oxidation. Another possible explanation is small residues of oxygen may have been present within the package and product, causing increased TBARS values initially (Olsen, Vogt, Veberg, Ekeberg, & Nilsson, 2005).

However, over time the available oxygen might have been consumed, rendering the product to be more stable (Olsen et al., 2005). Sensory results showed no significant differences in off flavor over storage (Table 14). TBARS values decreased ( $P < 0.05$ ) from 0.70 mg of malonaldehyde (MDA)/100g on d 0 to 0.35 and 0.23 on d 90 and 120, respectively. Cardoso et al. (2008) found that TBARS values for fish sausages were not significant over storage time. This agreed with their sensory results, since no panelist reported any rancid aroma or flavor, even in the control sausages after an 80 d storage time (Cardoso et al., 2008). Rancidity in cured meats is less of a problem than in fresh meat due to the addition of nitrite. Most cured meats are vacuum packaged, reducing the effects of oxygen and light exposure. Nitric oxide, the byproduct of nitrite helps to inhibit lipid oxidation (Kanner et al., 1980). In a study looking at early lipid oxidation in smoked, comminuted pork or poultry sausages with spices, Olsen et al. (2005) concluded that it is much more difficult to detect early lipid oxidation in complex matrixes than in simpler model systems. Even their sensory analysis turned out not to be straightforward. Lean poultry sausages developed less rancid odor and flavor during frozen storage for 11 months than fattier pork sausages with more polyunsaturated fatty acids. In a study evaluating packaging method and storage time on lipid oxidation of dry fermented sausage, Rubio, Martinez, Garcia-Cachan, Rovira, & Jaime (2008) found that peroxide index and thiobarbutric acid (TBA) values decreased ( $P < 0.05$ ) at the end of storage, which agrees with Ansorena and Astiasaran (2004) and Nassu, Guaraldo-Goncalves, Azebedo Pereira da Silva, & Becerra (2003) who found similar behavior with TBA values. It is well known that the initial step of lipid oxidation is the generation of transitory hydroperoxides, which degrade into malonaldehyde (MDA) and several other reactive compounds (Shahidi, 1994). On the other hand, Janero (1990) pointed out that a decrease in TBA values during storage could be attributed to MDA reaction with amino acids, sugars, and nitrite in complex formulations.

**Table 11. Least squares means (Lsmeans)<sup>1</sup> for TBARS( mg malonaldehyde/100g) by day from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Days of Display		
	0	90	120
<b>TBARS</b>	0.70 <sup>a</sup> ±0.08	0.35 <sup>b</sup> ±0.08	0.23 <sup>b</sup> ±0.10

<sup>1</sup> ± standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### ***Reheat Yield***

Mean reheat yield measurements by temperature are shown in Table 12. Storage time did not affect (P>0.05) reheat yield (Table 2). Reheat yield was lower (P<0.05) in product thermally processed to 68°C than 64°C or 72°C products (Table 12). All reheat yields were at or near 100%, which can be expected due to the moist heat cookery method that was used and the addition of phosphate and modified food starch in the formulation, which aids in water holding capacity and the products binding abilities.

**Table 12. Least squares means (Lsmeans) for reheat yield% by temperature from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

	Temperature			
	64°C	68°C	72°C	SEM <sup>1</sup>
<b>Reheat Yield (%)</b>	100.02 <sup>a</sup>	97.96 <sup>b</sup>	99.49 <sup>a</sup>	0.46

<sup>1</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

### ***Sensory Analysis***

Mean sensory analysis measurements by temperature are shown in Table 13, while mean sensory analysis measurements by day are shown in Table 14. Bite, flavor intensity, off flavor, and mouthfeel were not affected (P>0.05) by internal endpoint temperature. Saltiness was highest at 5.84 when product was cooked to 68°C. Bite decreased (P<0.05) by 0.42 and 0.48 units by d 120 compared to d 0 and 90, respectively. Sensory panel scores showed that flavor

intensity decreased ( $P<0.05$ ) as d of storage increased by 0.52 and 0.31 units, respectively. Olsen et al. (2005) found that meat flavor and acidic flavor sensory scores of vacuum-packaged pork sausages decreased ( $P<0.05$ ) after 1 mo of frozen storage. In contrast, poultry sausages decreased in acidic flavor ( $P<0.05$ ) after 6 mo of frozen storage (Olsen et al., 2005). Saltiness decreased ( $P<0.05$ ) from d 0 to d 90 by 0.23 units as well as from d 0 to d 120 by 0.33 units, while d 90 and 120 were similar ( $P>0.05$ ). These results agree with Fernandez-Fernandez et al. (2002) who found that increased storage time of vacuum packaged Galician chorizo sausage decreased saltiness. Off flavor and mouthfeel were not ( $P>0.05$ ) affected by day of storage.

**Table 13. Least square means (Lsmeans) for sensory analysis<sup>1</sup> by temperature from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

Sensory Attribute	Temperature			SEM <sup>2</sup>
	64°C	68°C	72°C	
<b>Bite</b>	3.79 <sup>a</sup>	3.82 <sup>a</sup>	3.73 <sup>a</sup>	0.12
<b>Flavor Intensity</b>	5.29 <sup>a</sup>	5.40 <sup>a</sup>	5.33 <sup>a</sup>	0.06
<b>Saltiness</b>	5.65 <sup>b</sup>	5.84 <sup>a</sup>	5.66 <sup>b</sup>	0.08
<b>Off Flavor</b>	1.26 <sup>a</sup>	1.28 <sup>a</sup>	1.34 <sup>a</sup>	0.12
<b>Mouthfeel</b>	3.58 <sup>a</sup>	3.69 <sup>a</sup>	3.61 <sup>a</sup>	0.10

<sup>1</sup> Scale: 8=extremely firm, extremely intense, extremely salty, extremely intense, and extremely heavy coating, 4=slightly soft, slightly bland, slightly unsalty, slight, and slight, and 1=extremely soft, extremely bland, not salty, none, and none

<sup>2</sup> SEM=standard error of the mean.

<sup>ab</sup> Lsmeans within row having different superscript letters differ ( $P<0.05$ ).

**Table 14. Least square means (Lsmeans)<sup>1</sup> for sensory analysis<sup>2</sup> by day from smoked sausages cooked to three internal endpoint temperatures and displayed under refrigeration for up to 120 days under light emitting diode or fluorescent lighting.**

Sensory Attribute	Days of Display		
	0	90	120
<b>Bite</b>	3.90 <sup>a</sup> ±0.12	3.96 <sup>a</sup> ±0.12	3.48 <sup>b</sup> ±0.13
<b>Flavor Intensity</b>	5.79 <sup>a</sup> ±0.05	5.27 <sup>b</sup> ±0.05	4.96 <sup>c</sup> ±0.07
<b>Saltiness</b>	5.90 <sup>a</sup> ±0.08	5.67 <sup>b</sup> ±0.08	5.57 <sup>b</sup> ±0.09
<b>Off Flavor</b>	1.27 <sup>a</sup> ±0.11	1.29 <sup>a</sup> ±0.11	1.32 <sup>a</sup> ±0.13
<b>Mouthfeel</b>	3.68 <sup>a</sup> ±0.09	3.59 <sup>a</sup> ±0.09	3.60 <sup>a</sup> ±0.12

<sup>1</sup> ± standard error of the mean.

<sup>2</sup> Scale: 8=extremely firm, extremely intense, extremely salty, extremely intense, and extremely heavy coating, 4=slightly soft, slightly bland, slightly unsalty, slight, and slight, and 1=extremely soft, extremely bland, not salty, none, and none

<sup>ab</sup> Lsmeans within row having different superscript letters differ (P<0.05).

## **Chapter 4 - Conclusions**

All vacuum packaged smoked sausages maintained an acceptable quality, not only immediately after production but also during storage at 4°C for up to 120 d. Lighting did not affect quality attributes of vacuum packaged precooked smoked sausages. Therefore, sausages could be displayed under LED or FLS lighting with no effect on overall quality. Although there were minimal quality differences due to internal endpoint temperature and storage time, these differences were not enough to have a detrimental effect on product quality. Internal endpoint temperature could be reduced following USDA FSIS Appendix A guidelines leading to changes in overall energy usage and processing time with insignificant effects on sausage quality.



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## Appendix A-Figures and Tables

### Figures and Tables Within Appendices

**Table A-1. Probability values for temperature by lighting type, temperature by day, day by lighting type, and temperature by lighting type by day for external and purge color L\*, a\*, b\*, a/b ratio, saturation index, and hue angle instrumental color.**

	Temperature by Light	Temperature by Day	Day by Light	Temperature by Light by Day
<b>External Color</b>				
<b>L*</b>	0.7283	0.3199	0.9439	0.8878
<b>a*</b>	0.5654	0.7712	0.9982	0.7604
<b>b*</b>	0.1414	0.6383	0.5567	0.7767
<b>a/b Ratio</b>	0.8021	0.8384	0.7493	0.9683
<b>Saturation Index</b>	0.1718	0.6139	0.5639	0.6898
<b>Hue Angle</b>	0.6627	0.8738	0.7635	0.9568
<b>Purge Color</b>				
<b>L*</b>	0.4249	0.6520	0.7074	0.6236
<b>a*</b>	0.8897	0.4221	0.6358	0.9556
<b>b*</b>	0.6106	0.8778	0.1988	0.7654
<b>a/b Ratio</b>	0.8845	0.8411	0.9748	0.9464
<b>Saturation Index</b>	0.5605	0.3258	0.1401	0.6110
<b>Hue Angle</b>	0.7313	0.9014	0.9780	0.9761



**Table A-2. Probability values for temperature by lighting type, temperature by day, day by lighting type, and temperature by lighting type by day for purge, pH, proximate analysis, thiobarbituric acid reactive substances (TBARS), and reheat yield.**

	<b>Temperature by Light</b>	<b>Temperature by Day</b>	<b>Day by Light</b>	<b>Temperature by Light by Day</b>
<b>Purge (%)</b>	0.8931	0.7539	0.9924	0.7199
<b>pH</b>	0.9674	0.1404	0.9815	0.8326
<b>Moisture (%)</b>	0.3185	0.7668	0.8499	0.1520
<b>Fat (%)</b>	0.1972	0.5602	0.9650	0.0738
<b>Protein (%)</b>	0.4885	0.7612	0.6301	0.5313
<b>TBARS</b>	0.7575	0.9931	0.7776	0.6966
<b>Reheat Yield (%)</b>	0.7353	0.9866	0.7253	0.0560

**Table A-3. Probability values for temperature by lighting type, temperature by day, day by lighting type, and temperature by lighting type by day for sensory analysis.**

<b>Sensory Attribute</b>	<b>Temperature by Light</b>	<b>Temperature by Day</b>	<b>Day by Light</b>	<b>Temperature by Light by Day</b>
<b>Bite</b>	0.8865	0.5042	0.2760	0.2503
<b>Flavor Intensity</b>	0.6243	0.9124	0.1888	0.9202
<b>Saltiness</b>	0.3499	0.4806	0.5355	0.6892
<b>Off Flavor</b>	0.2538	0.3454	0.2557	0.5616
<b>Mouthfeel</b>	0.1604	0.8269	0.5948	0.9297

## Appendix B-Statistical Codes

### Instrumental L\* External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model L = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model L = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

### Instrumental a\* External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model a = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model a = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental b\* External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model b = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model b = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental a/b Ratio External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model ab = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model ab = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental Saturation Index External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model SI = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model SI = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental Hue Angle External Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model HA = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model HA = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental L\* Purge Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp    ap    bp pab pSI pHA;  
    datalines;
```

```
proc mixed;  
class rep temp light day;  
model Lp = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model Lp = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental a\* Purge Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp    ap    bp pab pSI pHA;  
    datalines;
```

```
proc mixed;  
class rep temp light day;  
model ap = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model ap = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental b\* Purge Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp    ap    bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model bp = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model bp = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental a/b Ratio Purge Color

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp    ap    bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model pab = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model pab = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Instrumental Saturation Index Purge Color

**data;**

```
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;
    datalines;
```

**proc mixed;**

```
class rep temp light day;
model pSI = temp|light|day/ddfm = satterth;
random rep rep*temp*light*day;
lsmeans temp*light*day/slice = day;
lsmeans temp*light*day/pdiff;
run;
quit;
```

**proc mixed;**

```
class rep temp light day;
model pSI = temp|light|day/ddfm = satterth;
random rep rep*temp*light*day;
lsmeans temp day/pdiff;
run;
quit;
```

## Instrumental Hue Angle Purge Color

**data;**

```
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;
    datalines;
```

**proc mixed;**

```
class rep temp light day;
model pHA = temp|light|day/ddfm =
satterth;
random rep rep*temp*light*day;
lsmeans temp*light*day/slice = day;
lsmeans temp*light*day/pdiff;
run;
quit;
```

**proc mixed;**

```
class rep temp light day;
model pHA = temp|light|day/ddfm =
satterth;
random rep rep*temp*light*day;
lsmeans temp day/pdiff;
run;
quit;
```

## % Purge

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model Purgepct = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model Purgepct = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Product pH

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model pH = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model pH = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```



## Proximate Analysis % Moisture

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model Mpct = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model Mpct = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Proximate Analysis % Fat

```
data;  
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH  
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;  
      datalines;
```

```
proc mixed;  
class rep temp light day;  
model Fpct = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model Fpct = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day/pdiff;  
run;  
quit;
```

## Proximate Analysis % Protein

**data;**

```
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;
    datalines;
```

**proc mixed;**

```
class rep temp light day;
model Ppct = temp|light|day/ddfm = satterth;
random rep rep*temp*light*day;
lsmeans temp*light*day/slice = day;
lsmeans temp*light*day/pdiff;
run;
quit;
```

**proc mixed;**

```
class rep temp light day;
model Ppct = temp|light|day/ddfm = satterth;
random rep rep*temp*light*day;
lsmeans temp day/pdiff;
run;
quit;
```

## Lipid Oxidation-TBARS

**data;**

```
input Rep      Temp  Light$ Day  L      a      b ab SI HA  pH
TBARS      Mpct  Ppct  Fpct  Purgepct  Lp      ap      bp pab pSI pHA;
    datalines;
```

**proc mixed;**

```
class rep temp light day;
model TBARS = temp|light|day/ddfm =
satterth;
random rep rep*temp*light*day;
lsmeans temp*light*day/slice = day;
lsmeans temp*light*day/pdiff;
run;
quit;
```

**proc mixed;**

```
class rep temp light day;
model TBARS = temp|light|day/ddfm =
satterth;
random rep rep*temp*light*day;
lsmeans temp day/pdiff;
run;
quit;
```

## Reheat Yield

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

```
proc mixed;  
class rep temp light day;  
model RY = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model RY = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```

## Sensory Analysis Bite

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

```
proc mixed;  
class rep temp light day;  
model Bite = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

```
proc mixed;  
class rep temp light day;  
model Bite = temp|light|day/ddfm = satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```

## Sensory Analysis Flavor Intensity

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

### proc mixed;

```
class rep temp light day;  
model FlavI = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

### proc mixed;

```
class rep temp light day;  
model FlavI = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```

## Sensory Analysis Saltiness

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

### proc mixed;

```
class rep temp light day;  
model Saltiness = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

### proc mixed;

```
class rep temp light day;  
model Saltiness = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```

## Sensory Analysis Off Flavor

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

### **proc mixed;**

```
class rep temp light day;  
model OffFlavor = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

### **proc mixed;**

```
class rep temp light day;  
model OffFlavor = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```

## Sensory Analysis Mouthfeel

```
data;  
input Rep      Temp  Light$ Day  Bite  FlavI SaltinessOffFlavor  MouthFeel  RY;  
datalines;
```

### **proc mixed;**

```
class rep temp light day;  
model MouthFeel = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp*light*day/slice = day;  
lsmeans temp*light*day/pdiff;  
run;  
quit;
```

### **proc mixed;**

```
class rep temp light day;  
model MouthFeel = temp|light|day/ddfm =  
satterth;  
random rep rep*temp*light*day;  
lsmeans temp day temp*light*day /pdiff;  
run;  
quit;
```