Effects of naturally smoked sugar and frozen storage time on aerobically packaged bacon using a conventional and natural curing systems

by

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B.S., Kansas State University, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

Approved by:

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Abstract

Two studies were conducted to determine the effectiveness of naturally smoked sugar in a conventional and natural curing brines to inhibit lipid oxidation in frozen, aerobically packaged, layout style bacon. Commercial pork bellies were trimmed and cut in half creating anterior and posterior sections. Each section was randomly assigned to one of two treatments targeted 12% injection: control brine or a brine containing naturally smoked sugar (n =15/treatment). In the first study a conventional control brine consisted of 76.4% water, 11.8% salt, 8.00% sugar, 1.70% sodium phosphate, 1.60% sodium nitrite, and 0.450% sodium erythorbate. The treatment brine contained the same ingredients with the addition of 5.00% naturally smoked sugar. In the second study a natural control curing brine was utilized and contained 72.0% water, 13.4% sea salt, 8.00% cane sugar, and 6.67% celery juice. While treatment brine had the same ingredients as the natural control brine with the addition 5.00% smoked sugar. Bacon slices were randomly assigned to four sensory and GCMS frozen storage periods (0, 40, 80, and 120 day) or seven thiobarbituric acid reactive substances (TBARS) frozen storage times (0, 20, 40, 60, 80, 100, and 120 day). To measure lipid oxidation trained sensory evaluation, TBARS, and gas chromatography mass spectrometry (GCMS) was conducted after the assigned frozen storage periods. All bacon slices were stored aerobically at -18 ± 2 °C for their designated storage period.

The first study panelist scores for oxidized flavor of the conventional control bacon increased from day 0 to 120; whereas the naturally smoked sugar treatment had decreased panelist scores ($P > 0.16$) for oxidized flavor intensity compared to the control bacon. Also, TBARS results values for the conventional control bacon increased ($P < 0.01$) from day 20 to day 120; while the conventional naturally smoked sugar treatment remained constant ($P > 0.99$).

Hexanal content for conventional control increased ($P < 0.003$) during frozen storage; but naturally smoked sugar TBARS values were not different from day 0 of storage ($P > 0.734$). Concentration of heptanal in conventional control bacon was the highest ($P < 0.003$) at day 80 and 120 of frozen storage. Heptanal content in conventional bacon with naturally smoked sugar was not different from day 0 of frozen storage ($P > 0.846$). Conventional bacon formulated with naturally smoked sugar had greater concentrations of creosol and syringol than control bacon ($P < 0.003$).

In the second study, naturally cured bacon had increased panelist scores for oxidized flavor from day 0 to 120 of frozen storage ($P < 0.001$). Natural bacon with smoked sugar had oxidized flavor scores that remained constant during frozen storage ($P > 0.936$). Naturally cured bacon displayed increased TBARS values from day 20 to 120 of frozen storage ($P < 0.001$). Naturally cured bacon with smoked sugar exhibited static TBARS values throughout the frozen storage period ($P > 0.196$).

Thus, smoked sugar is an effective antioxidant in frozen sliced, aerobically packaged, conventionally cured and naturally cured bacon.

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Acknowledgements

This journey has been an unexpected rollercoaster of mistakes, life lessons, and friendships. I began this journey with the hopes of making an impact on students and the department, now as I look to my next chapter I realize that my life was impacted even more so. I have found myself surrounded by inspiring individuals that have become lifelong friends, and without them this journey would have made a much different path.

The individual who is responsible for encouraging me to pursue this path, Dr. Terry Houser. He has always challenged me to fulfill my potential, be the hardest working, and to strive for nothing less than my best. It can be easily said that without his guidance and ability to laugh at my mistakes, I would not be the person I am today. He constantly inspires students to reach their potential, and his passion for teaching is infectious. To just say "thank you" is not enough to express my gratitude for all that he has done for me. The accomplishments that we have shared over the last seven years will always remain the most special to me. Thank you for taking a chance on a short, fiery, stubborn kid to coach meat judging teams, allowing her to be a part of your family, and most of all supporting her year after year!

Thank you to my committee members for your guidance during the research and writing process. Dr. John Gonzalez, thank you for your endless supply of patience, always helpful advice, and sharing all your Star Wars knowledge. My master's journey would have been very dull without your involvement. Dr. Scott Smith, thank you for allowing me to take over your lab with bacon samples and teaching me to be a food chemist. I have learned so much from these gentlemen and I have earned a quality education at Kansas State because of them.

John Wolf, words cannot describe my gratitude for everything that you have given me the last seven years. From building my skills in the meat lab to helping me with research, you have

always been someone I can talk to when I needed. Thank you for letting me take over the meat lab at times and being a part of your family. I will always remember the laughs that we have shared. Sally Stroda, thank you for all our chats while cooking smelly bacon, and allowing MSA or classes to use the lab when needed. Dr. O'Quinn and Dr. Boyle, thank you so much for teaching me so much about statistics, sensory concepts, processing; I am truly grateful. I had a tremendous amount of help from Caleb Hurst and Trevor DeHaan, without your support I would still be mixing brine or running TBARS and GCMS. Thank you for putting up with me for so long. Also, Dr. Faris Karim, I would like to dedicate the GCMS portion to you. I am truly thankful for all you did in developing and collecting the GCMS research. I have gained so much from your teachings and thankful for our friendship.

I am very lucky to be a part of a legendary meat judging family. I am so appreciative of the opportunities to judge and even coach with these individuals. My journey to graduate school began with the 2013 meat judging team. I have gained so many lifelong friends from this team. Every member of that team has encouraged me to be my best and continued to challenge me. My coaches Aaron Tapian, Garret Dietz, and Bryce Gerlach you have taught be so much about motivating, teaching, and inspiring young meat judgers. You have inspired me to be the coach I was for the KSU meat judging team. Thank you for your friendship and mentorship. Ms. MaryAnn Matney; we surely have been an interesting pair; our friendship is one that is truly special to me. I will always love are adventures together and I am excited for the next ones to come.

The 2015, 2016, and 2017 meat judging teams that I was lucky enough to coach, I am grateful for the memories that were shared, and the amazing friendships I gained. I cannot thank you enough for all your hard work, dedication, and appreciation for the KSU meat judging

from the meat lab we would be here five years later? This is one crazy adventure and I'm so excited for our next chapter. Thank you for everything.

I am very proud to have been a part of such an amazing university, department, and meat science group whom I consider my family! I am excited for my next chapter; however, I am saddened to be leaving behind a great program, university, and family. I will always hold Kansas State close to my heart, and I will forever bleed purple.

Dedication

To my dad: Dave Hobson. Without your lessons of hard work, your endless encouragement, and love, this journey would not have been made.

Chapter 1 - Introduction

Bacon has become a staple in American cuisine, due to its versatility to become an ingredient in nearly any meal or to be a stand-alone item. Within the last 10 years, retail bacon prices have increased 57.8% (U.S. Bureau of Labor Statistics, 2017), showing little evidence of stabilizing. Food service bacon is a crucial component of the current high bacon demand. The challenges facing food service bacon include oxidative instability, due to its aerobic "layout" style packaging format.

It is widely accepted that length of frozen storage can increase a meat products susceptibility to lipid oxidation. It is also accepted that packaging environment can impact lipid stability. The aerobically packaged "lay-out" style is a very popular means of merchandising sliced bacon to food service establishments due to its ease of use, limited slice adhesion, and relatively low cost compared with vacuum or modified atmosphere packaging formats. Oxygen present in a meat system will generally increase lipid oxidation. For instance, Houser (2015) reported that food service lay-out style bacon had greater ($P < 0.05$) thiobarbituric acid reactive substances (TBARS) values than vacuum packaged bacon, indicating increased levels of lipid oxidation for aerobically packaged treatments. Additionally, aerobically packaged lay-out style bacon experienced dramatic increases ($P < 0.05$) in TBARS values post 28 days of frozen storage time (Houser, 2015). Increased rates of lipid oxidation in food systems usually manifests as increased levels of off-flavors and off-odors as detected by end users. It has been demonstrated by Lowe et al. (2014) that off-flavors and oxidized odors in bacon products increased ($P < 0.05$) as frozen storage time increased. However, limited trained sensory panel data is available that has tested the effects of frozen storage length on the sensory properties of lay-out style bacon.

The application of hard wood smoke by burning wood chips or sawdust is a common practice in U.S. bacon manufacturing. Although we most often think of the traditional smoking process as simple way to add more complex flavors, hardwood smoke is also a source of natural antioxidant activity. However, using only traditional hardwood smoke limits the penetration depth of naturally occurring antioxidants found in smoke to the surface of the pork belly. Liquid smoke produced by burning hardwood sources has been reported to contain multiple phenolic compounds that possess antioxidant activity such as syringol (Montazeri et al. 2012). Unlike traditional smoke, liquid smoke and smoke derivatives such as smoked sugar can be added directly to a curing brine moving the functional phenolic antioxidants found in hardwood smoke to the interior portions of the bacon slice. Yet, no data are available documenting the effect of adding smoke derived antioxidant compounds to raw pork bellies during the curing process to control lipid oxidation during extended frozen storage of bacon.

Chapter 2 - Literature Review

Factors Influencing Bacon Shelf-life

Controlling microbial spoilage and chemical deterioration in order to extend meat products' shelf life is a common goal among processors. While microbial spoilage is at the highest concern, prevention of chemical deterioration, such as lipid oxidation, is imperative to a quality eating experience for cured meat products. Extending cured meat shelf life is important for the food service sector of the meat industry because of fluctuating consumer demand and meat distribution systems. Maintaining a quality eating satisfaction within restaurants, hotels, and other institutions is of the utmost importance for continued success for the meat industry. The occurrence of lipid oxidation within a cured meat product leads to flavor, odor, and color changes; leading to the potential of an undesirable eating experience. Therefore, the role of lipid oxidation in a food service cured meat system needs to be fully understood to best protect product quality.

Oxidation of Meat Products

An oxidation reaction occurs when molecules lose electrons due to catalysts within the environment. In meat and meat products, oxidative reactions are a limiting factor for stability of quality characteristics. Oxidative reactions occur within protein complexes as well as within fat components. Products of oxidation reactions include meat color changes along with off-flavor development. Oxidative reactions occur throughout the production of meat products from feeding livestock to processing, storage, and cooking of meat products (Yun et al., 1987). Within the meat production system there are numerous catalysts for oxidation reactions. Understanding the mechanisms and promotors of oxidation reactions within the meat production system is important to properly apply inhibitors of oxidation.

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Mechanisms of Oxidation

Autoxidation can be broken down into three distinct stages: initiation, propagation, and termination as described by Morrissey et al. (1998). The autoxidation cycle begins with initiation where the hydrogens of an organic molecule (RH) are cleaved from the α-carbon creating free radicals (•). Alpha-carbons are those carbons that are adjacent to double bonds in an organic molecule. The reaction is illustrated below where HO• is the catalyst, yielding the free radical (R•) and water.

$$RH + HO• \rightarrow R• + H_2O$$
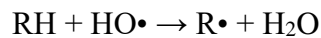
Propagation is the phase in which the free radical (R•) easily reacts with oxygen in the system to form a peroxyl radical (ROO•) (Morrissey et al., 1998).

$$R• + O_2 \rightarrow ROO•$$
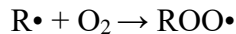
The peroxyl radical then captures a hydrogen from an organic molecule (RH′) to create an additional free radical (R•′) and volatile products (ROOH); self-propagating the reaction.

$$ROO• + RH′ \rightarrow ROOH + R•′$$
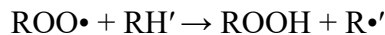
The newly created free radical (R•′) further reacts with oxygen to continue producing volatile products. The final stage of the oxidation reaction is termination and occurs when all free radicals have been utilized or an inhibitor has been applied to the system. The volatile products produced are in the forms of aldehydes, alcohols, and ketones (Morrissey et al., 1998). These oxidative products can be perceived as color, flavor, and odor changes. Simply, the formation of free radicals drives the oxidation reaction for meat and meat products. Free radical formation is regulated by factors that occur naturally within meat or introduced during further processing of meat products.

Promotors of Oxidation

Within a meat system there are multiple intrinsic and extrinsic components that encourage oxidation reactions, referred to as catalysts or pro-oxidants. Pro-oxidants include, but are not limited to oxygen, light, free metals, and salt (Morrissey et al., 1998). These catalysts exist normally within meat products such as the iron inside the myoglobin complex, or are inherent to the environment of the meat product such as oxygen present in a packaging system. The concentration of pro-oxidants, whether it is intrinsic or extrinsic will control the rate of autoxidation (Morrissey et al., 1998). The products created from autoxidation are responsible for warmed over flavor, off-flavors, off-odor, and other rancidity issues in meat products, ultimately leading to limit shelf-life of meat products (Tims et al., 1958, Tarladgis et al., 1959). Ensuring shelf-life and maintaining quality is extremely important for high fat products, due to unsaturated fatty acids' ability to easily and quickly breakdown through oxidation (Halliwell and Chirico, 1993).

Salt has an active role in the production of cured meat and is a pro-oxidant for lipid oxidation. The exact action that salt acts as a pro-oxidant is not known, yet researchers suggest sodium chloride can disrupt cell membranes, exposing iron from heme proteins, and inactivate antioxidant enzymes (Sebranek, 1999). The functionality of salt within a meat matrix is to add flavor, increase water holding capacity, extract proteins, and decrease microbial growth. Salt within a meat system exposes the iron in myoglobin during the extraction of proteins. Extraction of proteins allows iron within the heme complex to interact with pro-oxidants leading to oxidation reactions. Exposed iron molecules react with pro-oxidants to produce color changes in meat products, such as deoxymyoglobin, and oxymyoglobin undergoing an oxidation to transform into metmyoglobin (Mancini and Hunt, 2005). For example, a study conducted by Overholt et al. (2016) evaluated the effects of salt purity on lipid oxidation in ground pork

patties. Pork patties were mixed with 1.5% salts, then stored in refrigerated conditions for 11 days. All treatment combinations containing salt exhibited increased ($P < 0.05$) levels of TBARS values when compared to pork patties with no added salt. These results show that when processors include salt a meat system, an increase in oxidative products occurs. Salt is a crucial functional ingredient to a meat system; however, salt contains strong pro-oxidant properties.

Inhibitors of Oxidation

Antioxidants are compounds that inhibit or delay oxidation reactions within a food system (Morrissey et al., 1998). Antioxidants combat oxidative reactions by chain-breaking steps during autoxidation, chelation of metals, or the destruction of hydroperoxides (Frankel, 2005). Effective antioxidants can be broken into two classes, endogenous and exogenous. Endogenous antioxidants are those that are native to meat and meat products such as enzymes, while exogenous antioxidants are added ingredients to meat and meat products. There are many compounds that can inhibit lipid oxidation through multiple pathways. Frankel (2005) describes that antioxidant compounds work through chain-breaking reactions, chelating metals, or destroying hydroperoxide.

Phenolic antioxidants are known as chain-breaking compounds (Frankel, 2005). A phenolic structure is a six-carbon ring with alternating double bonds and attached hydroxyl group; this is the active component of antioxidants. Chain-breaking inhibitors affect the rate of lipid oxidation at the propagation stage where the phenolic portion (AH) interacts with the peroxyl free radical (ROO•) within the meat system. Instead of producing volatile compounds, products are stable such as hydroperoxide (ROOH) and the antioxidant radical (A•).

$$ROO• + AH \rightarrow ROOH + A•$$
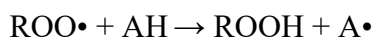
The antioxidant radical captures free hydrogens within the environment to continually limit peroxyl radicals transforming into volatile products. Therefore, antioxidant compounds continually break the three-stage cycle of autoxidation.

Processing techniques such as injection, cooking, and storage expose iron and other metals to the meat product's environment promoting oxidative reactions. To combat free metal oxidative reactions, compounds such as phosphates can be added to tightly bind with metals. The introduction of water into the meat system interacts with water soluble proteins (hemoglobin and myoglobin), releasing active iron molecules. The released iron needs to be chelated by phosphate or similar compounds to inhibit iron from undergoing oxidative reactions (Morrissey et al., 1998). Phosphates are capable of chelating metals within a meat system, inhibiting oxidation (Allen and Cornforth, 2009).

Secondary lipid oxidation products are volatile products that result in off-flavors. Primary oxidation products are hydroperoxides, these compounds are precursors to the formation of volatile products (Morrissey et al., 1998). The inhibition of hydroperoxide formation is done by reduction reactions (Frankel, 2005). Hydroperoxides are decomposed when metal ions and heme compounds are present through oxidation and reduction reactions, such as ferric iron transformed to ferrous iron (Skibsted et al., 1998). The end products of hydroperoxide decomposition are volatile compounds, aldehydes, ketones, and alcohols (Skibsted et al., 1998). After slaughter the hydroperoxide concentration decreases during storage, while the secondary oxidation products such as hexanal increase (Nielsen et al., 1997). Therefore, the identification of potential control points for oxidation along the meat production system will allow for proper oxidation management techniques. Potential control points in a meat production system may include the age of raw materials and incorporation of inhibitory ingredients.

Endogenous Antioxidants

Endogenous antioxidants are naturally present within meat and include enzymatic systems, and tocopherols. The functions of these inhibitors are to control pro-oxidant activity, capture free radicals, and limit reactive oxygen species (Decker and Mei., 1996). The effectiveness of endogenous antioxidants in cooked meat products vary due to the inactivation of enzymes during thermal processing (Decker and Mei, 1996).

In fresh meat, antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-PX), and superoxide dismutase (SD) work to catalyze reduction reactions for lipids (Decker and Mei, 1996). The antioxidant enzymes slow the rate of lipid oxidation by transforming hydrogen peroxides into water and molecular oxygen (Decker and Mei, 1996). Active within a high-water environment SD and CAT work to catalyze reactions that utilize oxygen anions and hydroxyl radicals that yield molecular water and oxygen. The function of GSH-PX is to catalyze the reduction of hydrogen or lipid peroxides resulting in fatty acid alcohols and water (Decker and Mei, 1996). Enzymatic activity can be influenced by species, muscle type, processing, and storage (Decker and Mei, 1996).

The activity of antioxidant enzymes can be inactivated during thermal processing (Mei et al., 1994). For example, the presence of CAT, GSH-PX and SD was quantified in ground turkey breast and thigh both raw and cooked (Lee et al., 1996). After cooking CAT and GSH-PX were significantly reduced ($P < 0.05$) in both turkey breast and thigh (Lee et al., 1996). Additionally, TBARS in cooked turkey breast and thigh were greater ($P < 0.05$) than raw turkey breast and thigh (Lee et al., 1996). Similar enzyme reduction in cooked turkey was also evaluated in pork and beef by Mei et al. (1994). As internal temperature increased for ground pork and beef, concentration of CAT, GSH-PX, and SOD decreased ($P < 0.05$; Mei et al., 1994). Also, Mei et al (1994) reported TBARS values for ground pork and beef increased as internal temperature

increased ($P < 0.05$). Thermal processing inactivates the antioxidants enzymes allowing for lipid oxidation to increase during storage.

In addition to thermal processing, salt concentration effects the functionality of antioxidant enzymes to inhibit lipid oxidation. Antioxidant enzymatic activity was quantified in ground pork patties that contained 0, 0.5, 1.0, and 2.0 percent salt in a study performed by Lee et al. (1997). During 70 days of frozen storage the enzymatic activity decreased in all pork patties, both unsalted and salted ($P < 0.05$). The amount of extracted enzymes in the pork patties decreased ($P < 0.05$) as the salt concentration increased. Therefore, as salt concentration and storage time increases the functionality of antioxidant enzymes to inhibit lipid oxidation in meat products decreases.

Tocopherols are compounds of vitamin E that contain a phenolic group to possess potential antioxidant properties. Vitamin E is native to many plants that are feed stuffs for livestock, which increases concentrations of tocopherols in the muscle (Morrissey et al., 1998). Pork chops from pigs fed α-tocopherol had lower ($P < 0.05$) TBARS values than pork chops from pigs not fed α-tocopherol (Monahan et al., 1992). Tocopherol is an effective antioxidant in fresh meat, however tocopherol concentration is highly dependent on the meat animal's diet.

Exogenous Antioxidants

Exogenous or extrinsic antioxidants are those ingredients added during processing of meat products to maintain oxidative and flavor stability through storage. The U.S. Department of Agriculture (USDA) classifies exogenous antioxidants as either being derived synthetically or from natural sources. Exogenous antioxidants are approved and controlled by the USDA Food Safety and Inspection Service (FSIS), as well as Food and Drug Administration (FDA). These government agencies approve antioxidant uses in meat products, in addition to regulating the

antioxidant ingredients as Generally Recognized As Safe (GRAS). The use of both synthetic and natural antioxidants has helped to prolong meat products' shelf life by inhibiting oxidative reactions.

Synthetic Antioxidants

Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been approved for use in meat products for many years (CFR 21.182). The antioxidant ability of BHA, BHT, or a combination of the two have been so effective in meat products that in many studies these compounds are the standard of control. The use of synthetic antioxidants in the production of high fat products such as bacon or sausage has inhibited lipid oxidation during storage, and maintained flavor.

Synthetic antioxidants come in multiple forms, BHA and BHT are fat soluble phenolic compounds. Pork sausage containing a combination of BHA and BHT stored frozen for 112 days displayed higher ($P < 0.05$) pork flavor scores compared to sausage processed without an added antioxidant (Sebranek et al., 2005). The antioxidant properties of synthetic ingredients are derived from the phenolic content, allowing for active free radical scavenging. In a study conducted by Ahn et al. (2002), cooked ground beef was subjected to refrigerated storage for three days. Ground beef patties treated with different antioxidants including BHA and BHT were evaluated for lipid oxidation by TBARS, hexanal content, and warmed-over flavor. Ahn et al. (2002) reported lower TBARS, hexanal content, and warmed-over flavor scores for ground beef patties treated with a combination of BHA and BHT compared to patties without added antioxidants ($P < 0.05$). Synthetic antioxidants have successfully helped processed meat products combat lipid oxidation and maintain flavor through storage. However, consumers have addressed

concerns about synthetic preservatives. The consumers' concerns have driven the industry to utilize natural antioxidants in meat and meat products.

Natural Antioxidants

Natural antioxidants can be incorporated into the meat system during the manufacturing process. Extracts of spices, fruits and smoke compounds are ingredients that have naturally high concentrations of phenolics (Shahidi et al., 1992). Natural antioxidants can be effective in inhibiting lipid oxidation, although the challenge is to not impact flavor or color of meat products.

Amongst the wide range of natural antioxidants used within the meat industry, rosemary is most the commonly used. The challenge for incorporating additional spices into a meat formulation is not altering flavor profiles, while inhibiting lipid oxidation. Sebranek et al. (2005) examined rosemary extract at 1,500 ppm and 2,500 ppm in raw and cooked pork sausage stored frozen for 112 days. Raw pork sausage treated with rosemary extract as an antioxidant had TBARS values that were lower ($P < 0.05$) than pork sausage without an antioxidant. However, in cooked pork sausage there were no differences in TBARS levels among rosemary treatments or sausage without added antioxidants ($P > 0.05$). The TBARS results from Sebranek et al. (2005) show that rosemary extracts are effective antioxidants in raw high fat processed meat products, although may not be as effective in cooked products.

The oxidative products measured with TBARS can be perceived by panelists as warmed-over flavor, off-flavors, and oxidized flavor. Trained sensory evaluation discovered that cooked pork sausage manufactured with rosemary extract at 2,500 ppm had lower ($P < 0.05$) warmed-over flavor scores than sausage without antioxidants (Sebranek et al., 2005). In addition, pork flavor scores were not altered for cooked pork sausage with and without added antioxidants ($P >$

0.05). While, raw pork sausage treated with rosemary extract at 2,500 ppm had higher ($P < 0.05$) pork flavor scores than pork sausage without added antioxidant. Sebranek et al. (2005) was able to demonstrate the antioxidant ability of rosemary extract in high fat meat products and did not hinder product flavor. Oregano, sage, and thyme are other effective spice extracts that contain antioxidant compounds and have shown to inhibit lipid oxidation (Abd El-Alim et al., 1999; Rojas and Brewer, 2007; 2008).

Fruit extracts are also a natural source of phenolic compounds (Zuo et al., 2002); plum, grape seed, and pomegranate are amongst the fruits that contain active antioxidants. Nunez de Gonzalez et al. (2008) showed that pre-cooked pork sausage formulated with 3% and 6% plum puree had lower ($P < 0.05$) TBARS levels than untreated precooked pork sausage. Cooked ground beef with grape seed extract was stored at 4 °C and hexanal content was measured to determine lipid oxidation. Hexanal content for cooked ground beef with grape seed extract was lower ($P < 0.05$) than an untreated control (Ahn et al., 2002). Also, Ahn et al. (2007) reported that grape seed extract was successful at inhibiting lipid oxidation as evidenced by lower ($P < 0.05$) TBARS values in cooked ground beef compared to untreated control. Pomegranate rind powder was included in cooked chicken patties and stored for 15 days at 4 °C (Naveena et al., 2008). The cooked chicken patties with pomegranate rind powder exhibited lower ($P < 0.05$) TBARS values compared to chicken patties without antioxidants. Moreover, pomegranate rind and juice powder added to cooked chicken patties exhibited no differences in off-odor, sweet flavor, or chicken flavor for trained sensory scores compared to the control chicken patties ($P > 0.05$).

Hardwood smoke and smoke derived products are also considered strong antioxidants. Hardwood smoke's main function during the manufacturing process is to impart flavor, odor,

and color to bacon. Applying smoke to a meat product is also a method of preservation, due to high concentrations of phenolic compounds (Kjallstrand and Petersson, 2001).

Woods have three major components, cellulose, hemicellulose, and lignin. The components of wood smoke breakdown during burning also referred to as pyrolysis (Rozum, 2009). Pyrolysis of wood results in flavors or functional compounds from cellulose, hemicellulose, and lignin. The breakdown of cellulose the largest component of wood, results in acids and aldehydes that are responsible for the tartness of smoke flavor and antimicrobial activity (Shafizadeh, 1984). Hemicellulose is the first to breakdown during burning, producing aldehydes responsible for browning and furans that contribute to overall smoke flavor (Rozum, 2009). Lignin pyrolysis yields phenolic compounds that provide unique flavor attributes and antioxidant capabilities. Syringol, eugenol, isoeugenol, guaiacol, and creosol are the major phenolic compounds in wood smoke, each with unique flavor and odor characteristics (Rozum, 2009).

The incorporation of liquid smoke products to the curing of bacon as an antioxidant is not well studied. Wendorff (1981) demonstrated that liquid smoke applied to pork fat had lower ($P < 0.05$) peroxide values than an untreated control. The inhibition of lipid oxidation by liquid smoke is exhibited in precooked beef patties in a study conducted by Estada-Muñoz et al. (1998). Pre-cooked beef patties treated with liquid smoke had decreased ($P < 0.05$) TBARS values when compared to patties without liquid smoke throughout 90 days of frozen storage. Hardwood smoke and its' derivatives have been utilized in the meat industry to improve flavor and other sensory attributes, however the antioxidant capabilities of smoke have created new opportunities for applications in meat products.

Meat Curing Systems

Curing meat products is an ancient method of preservation. With the discovery of salt in the early 3,000 B.C. preservation of meat was created (Romans et al., 1995). Meat that was treated with salt peter or potassium nitrate produced the desirable reddish pink color and flavor of cured meats (Sebranek and Fox, 1985). Sodium chloride and forms of nitrite are responsible for desirable cured meat characteristics.

The evolution of the meat industry from a slaughter based system to producing further processed products has led to utilizing curing agents to extend meat products' shelf life. However, potential human health issues surrounding the use of sodium nitrite in meat products have developed over the years (Sebranek and Bacus, 2007). The issues concerning cured meats include potential development of carcinogenic compounds and sodium levels in the human diet (Sindelar et al., 2012).

The hazards surrounding nitrite toxicity include the direct poisoning of nitrite and the formation of *N*-nitroso compounds (Swann, 1975). Methemoglobinemia is the result of nitrite oxidizing hemoglobin to methemoglobin (Swann, 1975). The concentration of methemoglobin in blood is controlled by the enzyme methemoglobin reductase and the cofactor NADH (Swann, 1975). Sensitivity to methemoglobinemia may be determined by the ratio of methemoglobin reductase and NADH. Nitrate and nitrite also present a toxicity issue because of the formation of *N*-nitroso compounds. *N*-nitroso compounds include multiple chemical structures of nitrosamines (Swann, 1975). Nitrosamines are stable molecules, however when metabolized nitrosamines form active toxic, mutagenic, and carcinogenic metabolites (Magee and Barnes, 1967). Nitrosamines' carcinogenic activity may induce cancers in specific organs, most commonly the liver (Swann, 1975). Cancers in the liver, kidney, and lung were observed in rats and mice that

were subjected to nitrosamine compounds (Lee et al., 1964). The tumors found in different organs of rats and mice appeared to be caused from nitrosamines metabolized by the specific organs' tissues (Lee et al., 1964). Nitrite poisoning and *N*-nitroso compounds are health hazardous issues that the USDA-FSIS hopes to prevent by limiting ingoing nitrite levels in meat products.

Cured meat products contain residual nitrite after processing. If residual nitrite is heated too quickly and to a high temperature (pan frying) carcinogenic nitrosamines can be formed (Pegg and Shahidi, 2000). Salt is typically incorporated into processed meat formulations at 1% to 2%, resulting in a source of sodium in the consumer's diet. If sodium levels are excessive in the diet leads to hypertension and other heart related issues (Ruusunen and Puolanne, 2005). These concerns have driven the meat industry to investigate alternative curing methods. There are distinct curing systems utilized in the meat processing industry, a sodium nitrite based system, naturally sourced nitrite based system, or dry curing process.

Conventional Curing Systems

Sodium nitrite is a highly water-soluble ingredient, and as a raw ingredient is highly toxic to consume. Due to the toxicity of nitrite the USDA regulates the amount of ingoing nitrite to limit the residual nitrite in the finished product. According to USDA-FSIS (1995) the maximum ingoing sodium nitrite for comminuted products is 156 ppm and for immersion or injected products 200 ppm is allowed. Bacon processing is the exception to 200 ppm for injected products, instead the allowable ingoing sodium nitrite for bacon is 120 ppm (USDA-FSIS, 1995). Bacon requires a curing accelerator at 550 ppm added with 120 ppm of sodium nitrite to minimize residual nitrite in cooked bacon (USDA-FSIS, 1995).

The functionality of nitrite in a meat matrix is to form nitric oxide which is responsible for cured meat color and flavor. Sodium nitrite ($NaNO_2$) is dissolved in water or directly added into the meat matrix, which causes $NaNO_2$ to dissociate into nitrite ions (NO^-_2) to react with hydrogen ions (Pegg and Shahidi, 2000). The nitrous acid pathway is one of three reactions to yield nitrous acid (HNO_2).

$$NaNO_2 \rightarrow Na^+ + NO^-_2$$

$$NO^-_2 + H^+ \leftrightarrow HNO_2$$
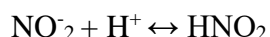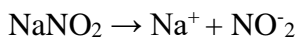
There are three pathways to produce NO. The first is the nitrous acid pathway, the second is the use of added reductants (sodium ascorbate and erythorbate), and the third is endogenous reductants. The nitrous acid pathway is mainly governed through the decrease of pH (Sebranek and Fox, 1985).

Nitrous acid is used to produce dinitrogen trioxide (N_2O_3), then is reduced to nitric oxide (NO) and nitrogen dioxide (NO_2).

$$4HNO_2 \leftrightarrow 2N_2O_3 + H_2O$$

$$2N_2O_3 \leftrightarrow 2NO + 2NO_2$$

When nitric oxide is exposed to oxidized protein, the nitric oxide will bind with the iron molecule inside the heme complex. The binding of nitric oxide to the iron transforms myoglobin into nitrosylmetmyoglobin that is brown in color (Killday et al., 1988).

$$NO + myoglobin\ (Fe^{3+}) \rightarrow Nitrosylmetmyoglobin$$
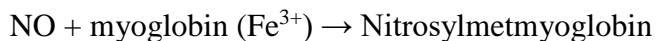
Once the nitric oxide has bound to myoglobin, heat is applied to the product that will result in the stable pink cured meat pigment, nitrosylhemochrome (Killday et al., 1988).

$$Nitrosylmetmyoglobin + heat \rightarrow Nitrosylhemochrome$$

Nitrite has antioxidant properties due the binding with the iron molecule in the heme proteins, resulting in occupied hydrogen radicals (Pegg and Shahidi, 2000). As little as 50 ppm of nitrite in beef, pork, or chicken can inhibit lipid oxidation during storage (Morrissey and Techivangana, 1985).

Natural Curing Systems

Sodium nitrite has proven to be a highly functional ingredient by creating specific color, flavor, and antimicrobial characteristics. Finding a single ingredient to replace sodium nitrite with all its functions is unlikely. Natural sources of nitrate can be found in high concentration in vegetables such as celery. Nitrate serves as the precursor to nitrite, thus products that have slow curing times may use nitrate as a curing agent (Sebranek and Bacus, 2007). Vegetable juice and powders contain nonreactive forms of nitrate, therefore a nitrate reducing bacteria is necessary for nitrite to be formed (Sebranek and Bacus, 2007). Incubation time for products containing vegetable juice or powder and starter culture is important for the complete conversion of nitrate to nitrite.

Sindelar et al. (2007) researched the effects of celery juice powder, nitrate reducing bacteria, and incubation time in frankfurters. Frankfurters were manufactured with *Staphylococcus carnosus* starter culture as well as 0.2% and 0.4% celery juice powder. Frankfurters were incubated for 30 or 120 minutes at 38 °C. Nitrite levels in frankfurters incubated for 120 minutes were greater ($P < 0.05$) than frankfurters incubated 30 minutes (Sindelar et al., 2007). Sindelar et al. (2007) showed that longer incubation time for nitrate cured products will provide a more complete nitrate to nitrite conversion. Residual nitrite was measured and compared to sodium nitrite cured frankfurters. After thermal processing frankfurters cured with 0.4% celery juice powder and incubated for 120 minutes revealed the

greatest ($P < 0.05$) amount of residual nitrite compared to other treatment combinations or a sodium nitrite cured control (Sindelar et al., 2007).

The addition of incubation time to the manufacturing of commercial naturally cured meat products may not be cost effective. Ingredient development has discovered that vegetable juice and powder can be pre-converted from nitrate to nitrite. The pre-converted vegetable juice and powder has more concentrated and consistent levels of nitrite, allowing for more uniformly cured meat products. Krause et al. (2011) tested the curing properties of non-converted and pre-converted vegetable juice powders in ground ham. There were no differences in TBARS values between non- and pre-converted vegetable juice powders. Additionally, a* and b* values were not different in non- and pre-converted vegetable juice powder, as well as sodium nitrite cured ham ($P > 0.05$). These results indicate that pre-converted vegetable juice powder is an effective natural curing agent.

In addition to alternatives for curing agents, other brine components such as cure accelerators may need to be substituted. Cherry powders are high in ascorbic acid that function as natural nitrite reductants (Sebranek and Bacus, 2007). The inclusion of natural reductants drives nitrite to reduce into nitric oxide, allowing for cured meat color and flavor to develop (Terns et al., 2011).

Packaging Styles of Bacon

Lipid oxidation is the major reason for deterioration of cured meats, due to the high concentration of salt and thermal processing (Yun et al., 1987). The exposure to pro-oxidants has led to the development of technologies to limit free radical formation such as packaging systems and antioxidant usage. Aerobic and anaerobic packaging systems are impactful to cured meats oxidative stability.

Aerobically Packaged Bacon

Aerobic packaging systems utilize large loosely packed unsealed plastic bags that are placed in cardboard boxes, and leaves the product exposed to oxygen. Food service or Hotel, Restaurant, and Institutional bacon is commonly packaged aerobically with bacon slices laid flat on bacon paper then stacked in the plastic lined box. The food service industry utilizes aerobic packaging due to the economic advantages compared to modified atmosphere and vacuum packaging, limited product adhesion, as well as ease of use. Oxygen is a strong pro-oxidant in cured meats, oxidative stability can be controlled with limited exposure to oxygen. Houser (2015) reported increased ($P < 0.05$) TBARS levels after 28 days for bacon stored frozen and exposed to oxygen. Additionally, Ahn et al. (2000) had presented increased ($P < 0.05$) TBARS values for pork patties stored for three weeks. The presence of oxygen in a cured meat packaging environment promotes lipid oxidation and the development of rancid flavors. Lowe et al. (2014) reported the development of off-flavor and off-odor in food service bacon packaged aerobically and stored at -33 °C for 90 days. Trained sensory panelists had increased off-flavor and off-odor scores for food service bacon from day 0 to 90 of frozen storage ($P < 0.05$; Lowe et al., 2014).

Anaerobic Packaging of Bacon

Anaerobic packaging systems eliminate atmospheric gases from the meat products' environment. Anaerobic packaging style is heavily used with retail cured meat products and fresh primal cuts. Anaerobic packaging maintains product freshness and cured products withstand autoxidation, making anaerobic systems the preferred method for product distribution. Houser (2015) illustrated with bacon and Ahn et al. (2000) showed with pork patties that TBARS values for vacuumed packaged products remained the same throughout frozen or refrigerated storage ($P > 0.05$). However, vacuumed packaged retail bacon still developed off-flavors and odors as detected by trained sensory panelists during 120 days of storage (Lowe et al., 2014). As

a result, lipid oxidation in vacuumed packaged bacon may still be present during long term storage.

Ease of handling product in a fast pace restaurant is important for food service bacon. Thinly sliced bacon that are vacuumed packaged, such as retail style bacon, tend to adhere together, and make the bacon slices difficult to separate. Therefore, the advantage of anaerobic packaging in delaying lipid oxidation may not outweigh the challenges of handling procedures for sliced cured products.

Frozen Storage of Raw Bellies

The purpose of freezing, thermal processing, curing, and other methods of preservation is to restrict or completely inhibit microbial, chemical, enzymatic, and physical deterioration of a meat product (Skibsted et al., 1998). Freezing is an excellent method in storing meat products due to less qualitative changes. Freezing bacon has some disadvantages for qualitative properties, such as pigment changes and lipid oxidation.

Maintaining quality of raw pork bellies is dependent on the metmyoglobin reducing activity. Pigment changes during extended frozen storage of raw meat results in lipid oxidation pro-oxidants. Decreases in pH governs the consumption of NADH, which uses enzymes to react with oxygen to form superoxide and hydrogen oxide (Skibsted et al., 1998). The pigment change of oxymyoglobin to deoxymyoglobin requires the oxidation of NADH by metmyoglobin reductase (Mancini and Hunt, 2005). The reduction of oxymyoglobin occurs under low-oxygen partial pressures as well as through enzymatic activity of the electron transport chain. Lactate dehydrogenase converts lactate to pyruvate and NADH, allowing for the replenishment of NADH (Mancini et al., 2004). As frozen storage increases, depletion of NADH occurs leading to lipid oxidation within phospholipid membranes (Satoh and Shikama, 1981). The extended

storage of raw bellies can be susceptible to lipid oxidation due to the increasing levels of hydrogen ions by the reduction of NADH.

The relationship of hydrogen ions, iron, and oxygen lead to lipid oxidation occurring during frozen storage. Lipid oxidation was evaluated by Herrick et al. (2016) with TBARS in food service bacon. Herrick et al. (2016) showed increased ($P < 0.05$) TBARS values for food service style bacon stored frozen at -33 °C for 12 weeks. Lowe et al. (2014) stored food service bacon at -33 °C for 90 days displayed increased off-flavor and off-odor trained sensory scores over the frozen storage period ($P < 0.05$). Herrick et al. (2016) and Lowe et al. (2014) demonstrated that in low freezing temperatures lipid oxidation will occur. Bacon is a unique product that freezing for an extended period of time does not limit the development of off-flavors and oxidative products.

Fatty Acid Composition

Fatty acid composition in fresh pork bellies used for bacon production can influence bacon quality characteristics. Saturated, monounsaturated, and polyunsaturated fatty acids are those that contribute to fatty acid composition in animal fats. Unsaturated fatty acids are those that are prone to lipid oxidation, due to the presence of at least one double carbon bond. Pork fatty acid composition can be easy influenced by the animals' diet components, such as dried distillers grains. Dried distillers grains (DDG) and dried distillers grains with solubles (DDGS) are by-products of ethanol production. These by-product feed ingredients have high concentration of fat, which contain high levels of unsaturated fatty acids (Sauders et al., 2009).

Pigs do not synthesize polyunsaturated fatty acids, therefore polyunsaturated fatty acids found in the fat deposits of pigs are gained from the diet (Babatunde et al., 1968). The fatty acid make up of pig diets dictates the composition of pork fat. With large variations in pork fat

quality, the meat industry has historically used iodine value to measure the amount of unsaturated fatty acids within pork fat. Whitney et al. (2006) found that when pigs were fed increasing amounts of DDGS (0, 10, 20, and 30%) within a 5-phase feeding system, they exhibited different iodine values. Increased DDGS in the diet resulted in increased ($P < 0.05$) iodine values. Whitney et al. (2006) reported that DDGS in pig diets increased the amount of unsaturated fatty acids within pork fat.

Increasing the abundance of unsaturated fatty acids in fresh pork bellies leads to a greater potential for lipid oxidation (Morrissey et al., 1998). However, there are few studies that investigated lipid oxidation in food service bacon produced from bellies with different iodine values. Houser (2015) is one of the few that separated fresh pork bellies into three different iodine value categories (High = 76.9, Intermediate = 70.9, and Low = 67.7) and processed the bellies into food service bacon. Although, in disagreement with others hypothesis, amongst the three iodine value categories there were no differences ($P > 0.05$) in TBARS values. The differences in iodine value from Houser (2015) did not have an impact on lipid oxidation, but more research needs to be conducted with greater differences in iodine value.

Additional bacon quality characteristics that can be influenced by fatty acid composition include belly firmness, slicing yield, and cook loss. Bellies that were selected for the high iodine value category were softer ($P < 0.05$) than bellies in the low iodine value category (Houser, 2015). In support, Moreno et al. (2008) reported that increasing total unsaturated fatty acid levels yields softer bellies. Soft raw pork bellies may produce low slicing yields in bacon (Apple et al., 2007). Even though lipid oxidation is minimally impacted by fatty acid composition in food service bacon, other bacon quality attributes may be directly influenced.

Assays to Determine Lipid Oxidation in Cured Meat Products

Sensory Evaluation

Evaluating lipid oxidation objectively with instrumental assays is necessary, however to accurately determine thresholds for lipid oxidation in meat products sensory testing should be conducted. Original research by Tims et al. 1958 discovered the undesirable flavor that was produced from aged meat products and was defined as warmed-over flavor (WOF). The development of WOF is a product of oxidation in meat products (Tims et al., 1959). As sensory evaluation techniques have become more advanced, flavors produced from lipid oxidation have become well defined. Specifically, for bacon Gatlin et al. (2006) defined oxidized flavor as foods that exhibit cardboard, painty, and reheated characteristics. Quantification of well-defined flavors should be evaluated by trained sensory panelists that thoroughly understand the specific attributes and specific meat products to be evaluated. Trained sensory evaluation is a sensitive method in evaluating attributes such as lipid oxidation and can be related to objective measurements of those attributes (Spanier et al., 1998).

Ultimately, the abundance of secondary oxidation products in a cooked pork product have a linear relationship with the WOF scores from panelists (Shahidi et al., 1987). The relationship between secondary oxidative products and off-flavor development allows for objective measurements such as TBARS to be linked to subjective measurements of lipid oxidation such as sensory evaluation. Trained sensory evaluation may determine if oxidative products are perceived in food service bacon, while TBARS and GCMS measures the abundance of oxidative products.

According to the American Meat Science Association Guidelines for Bacon (Olson et al., 1985), there are many properties of visual, aroma, flavor, and texture that are considered

important for consumer satisfaction of bacon. Some examples of characteristics that have been

deemed important for bacon are internal and external color, smoke aroma, smoke flavor,

saltiness, along with brittleness, and chewiness (Olson et al., 1985). Flavor attributes such as

saltiness, smoke intensity, cured lean and fat, as well as sweetness all contribute to overall bacon

flavor (Gatlin et al., 2006). Utilizing trained sensory evaluation may detect changes in flavor

components of bacon that could have increased lipid oxidation products. To help panelists

thoroughly understand bacon flavor, a commonly produced commercial bacon may be used as a

reference (Gatlin et al., 2006). Utilizing a common commercial bacon as a reference for panelists

limit bias amongst trained panelists and between panel sessions. Measuring multiple important

flavor components of bacon along with potential off-flavor development provide a detailed

evaluation of possible changes in overall bacon flavor.

Thiobarbituric Acid Reactive Substances

Thiobarbituric Acid Reactive Substances (TBARS) is an analytical method to determine lipid oxidation in meat products. Tarladgis et al. (1960) determined the distillation method of TBARS to concentrate and quantify oxidation products. The TBARS method quantifies malonaldehyde within a meat sample by a colorimetric measurement of red chromogen. Malonaldehyde is an oxidative product that is derived from a three-carbon fragment that contains at least one double bond. A reaction between two molecules of thiobarbituric acid (TBA) reagent and one molecule of malonaldehyde create the red pigment. Then, the optical density of the red pigment is read at 532 nm to determine malonaldehyde concentration (Ulu, 2004). The distillation method of TBARS allows for clear distillate of malonaldehyde to be obtained, along with a less intensive heat treatment for maximum color development (Taraladgis et al., 1960). However, when cured meat is evaluated with TBARS, the residual nitrite present in the product makes malonaldehyde nonreactive (Ulu, 2004). Therefore, the addition of sulfanilamide reacts with the residual nitrite, allowing malonaldehyde and TBA reagent to form the red pigment (Ulu, 2004).

The most common method to analytically determine lipid oxidation is variations of TBARS (Melton, 1983). A TBARS value is reported in mg of malonaldehyde per kg of sample, and has been shown to be associated with increased off-flavors perceived by sensory panelists. Tarladgis et al. (1960) reported that TBARS are correlated (0.89) to off-flavors in cooked pork and when TBARS values reach 1.0, sensory panelists will detect off-flavors and off-odors. Although, the TBARS value of 1.0 and its relationship to off-flavors is difficult to apply to all meat products due to the lack of studies conducted on a variety of meat products.

Gas Chromatography Mass Spectrometry

The products of lipid oxidation from food products include ketones, alcohols, and aldehydes; these compounds are responsible for off-flavors and off-odors. The volatiles that are produced during lipid oxidation can be separated with gas chromatography (GC) and the ions identified by mass spectrometry (MS; Cadwallader and Macleod, 1998). Gas chromatography isolates flavor volatiles from headspace sampling or solvent extraction (Cadwallader and Macleod, 1998). All flavor compounds are volatile, headspace sampling utilizes this property to collect and analyze flavor volatiles. A Solid Phase Microextraction (SPME) fiber is one method to collect volatiles from the headspace of a food product. The SPME is a polymer-coated fiber that absorbs the volatiles as the sample is heated and the compounds are released from the test product. Ruiz et al. 1998 explained the importance of temperature (40 or 60 °C) at which volatiles are released from a meat sample, along with the time (20, 40, or 60 minutes) the SPME fiber is exposed to the headspace. For a high fat cured meat sample, the most effective temperature and time combination to release flavor volatiles is 60 °C and between 20 to 40 minutes of SPME exposure (Ruiz et al., 1998). The absorbed volatiles in the SPME fiber are then thermally desorbed in the GC port. Helium gas is utilized to carry the flavor volatiles through the separation column of the GC. The MS is a very effective method to work in tandem with GC to identify the individual ions that make up the flavor volatiles (Cadwallader et al., 1998). High resolution, selected ion monitoring (SIM), chemical ionization, and negative ion chemical ionization are methods for identification of individual ions. The SIM monitoring system uses pre-selected ions to detect the compound of interest. Compounds are recognized by the SIM method, can be quantified with high sensitivity in a complex matrix such as meat products (Garcia-Regureiro and Diaz, 1989).

Many aldehydes have been identified as compounds of off-flavors, with hexanal as the most abundant oxidative product (Mandić et al., 2014). The aldehydes produced during lipid oxidation, such as hexanal (0.93) and nonanal (0.74) are highly correlated to TBARS values (Ahn et al., 1999). The GCMS assay is an accurate and simple method for an objective measurement of lipid oxidation in meat products and identification of compounds responsible for off-flavor development.

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Chapter 3 - Effects of naturally smoked sugar and frozen storage time on foodservice packaged bacon.

Abstract

The purpose of this study was to determine the effectiveness of naturally smoked sugar as an antioxidant in foodservice aerobically packaged bacon. Pork bellies (Institutional Meat Purchasing Specifications, IMPS #409) were trimmed and cut in half yielding an anterior and posterior section. Each section was randomly assigned to one of two treatments targeted 12% injection: control brine or a brine with naturally hardwood smoked sugar ($n = 15$/treatment). Once fully processed into finished product, bacon slices were randomly assigned to four frozen storage periods for sensory and gas chromatography analysis (0, 40, 80, and 120 days) or seven frozen storage times (0, 20, 40, 60, 80, 100, and 120 days) for thiobarbituric acid reactive substances (TBARS) analysis. All boxed bacon slices were stored aerobically at -18 ± 2 °C for their designated storage period. To measure oxidative products and flavor stability, trained sensory evaluation, TBARS, and gas chromatography were conducted after the appropriate frozen storage period. There were significant ($P < 0.001$) Treatment × Day interactions for TBARS, hexanal and heptanal concentration, and oxidized flavor intensity. Control bacon presented increased ($P < 0.001$) panelist scores for oxidized flavor intensity from day 0 to 120; whereas the naturally smoked sugar treatment had lower panelist scores ($P > 0.16$) for oxidized flavor intensity compared to the control bacon. The TBARS values for the control treatment increased ($P < 0.01$) from day 20 to day 120; while the naturally smoked sugar treatment remained constant ($P > 0.99$). Hexanal content increased ($P < 0.003$) during frozen storage for the control bacon; but were not different from day 0 of frozen storage for naturally smoked sugar ($P > 0.734$). Heptanal in control bacon was the highest ($P < 0.003$) at day 80 of frozen storage, while

heptanal content in naturally smoked sugar bacon was not different ($P > 0.846$) over the 120 days of frozen storage. The ability of naturally smoked sugar to function as an effective antioxidant in frozen sliced foodservice bacon was confirmed by the inhibition of lipid oxidative products.

Keywords: Aerobic shelf-life, bacon, lipid oxidation, naturally smoked sugar, sensory

Introduction

Bacon has become a staple in American cuisine, due to its ability to serve as an ingredient in nearly any meal or be a stand-alone item. Within the last 10 years, retail bacon prices have increased 57.8% (U.S. Bureau of Labor Statistics, 2017), showing little evidence of stabilizing. Foodservice bacon is a crucial component of the current high bacon demand. The challenges facing foodservice bacon include oxidative instability, due to its aerobic "layout" style packaging format.

It is widely accepted that length of frozen storage can increase a meat product's susceptibility to lipid oxidation (Skibsted et al., 1998). It is also accepted that the aerobic packaging environment can impact lipid stability (Houser, 2015). The aerobically packaged "layout" style is popular means of merchandising sliced bacon to foodservice establishments due to its ease of use, limited slice adhesion, and relatively low packaging cost compared with vacuum or modified atmosphere packaging formats. Oxygen presence will generally increase rates of lipid oxidation in food systems, which manifests as increased levels of off-flavors and off-odors as detected by end users. Lowe et al. (2014) demonstrated off-flavors and oxidized odors in bacon products increased as frozen storage time increased. To date, limited trained sensory panel data is available that has tested the effects of frozen storage length on the sensory properties of layout style bacon. Oxygen presence in a meat system will generally increase lipid

oxidative products. Houser (2015) reported that foodservice layout style bacon had greater

TBARS values than vacuum packaged bacon, indicating increased levels of lipid oxidation for

aerobically packaged bacon. Additionally, aerobically packaged layout style bacon experienced

dramatic increases in TBARS values post 28 days of frozen storage time (Houser, 2015).

The application of hard wood smoke by burning wood chips or sawdust is a common

practice in U.S. bacon manufacturing. Although we most often think of the traditional smoking

process as simple way to add more complex flavors, hardwood smoke is a source of natural

antioxidant activity (Rozum, 2009). However, using only traditional hardwood smoke limits the

penetration depth of naturally occurring antioxidants found in smoke to the surface of the pork

belly. Liquid smoke produced by burning hardwood sources has been reported to contain

multiple phenolic compounds that possess antioxidant activity such as syringol (Montazeri et al.

2012). Unlike traditional smoke, liquid smoke and smoke derivatives such as smoked sugar can

be added directly to a curing brine moving the functional phenolic antioxidants found in

hardwood smoke to the interior portions of the bacon slice. Yet, no data are available

documenting the effect of adding smoke derived antioxidant compounds to raw pork bellies

during the curing process to control lipid oxidation during extended frozen storage of bacon.

Therefore, the objective of this study was to determine the effectiveness of adding naturally

smoked sugar to a curing brine to prevent lipid oxidation in frozen, sliced, aerobically packaged

foodservice bacon.

Materials and Methods

Belly Selection

Twenty-four hours postmortem, fresh pork bellies (IMPS #409) were collected at a

Midwest commercial processing facility and transported under refrigerated conditions (3 ± 1 °C)

to the Kansas State University Meat Laboratory (Manhattan, KS, U.S.A.). Bellies were stored for

three d at 2 ± 1 °C before processing. Immediately prior to processing, five bellies were trimmed

and cut in half yielding an anterior and posterior belly section weighing approximately 2.8 kg

each. Each half was randomly assigned to one of two treatments: a control curing brine or a

curing brine with added naturally hardwood smoked sugar (RA12032, Red Arrow Products,

Manitowoc, WI, U.S.A.).

Bacon Processing

Control and treatment brines were formulated for a 12% injection rate. The control curing

brine consisted of 76.4% water, 11.8% salt, 8.00% sugar, 1.70% sodium phosphate (Brifisol 450

Super, ICL Performance Products, St. Louis, MO, U.S.A.), 1.60% modern cure (6.25% Nitrite,

Excalibur, Pekin, IL, U.S.A.) and 0.450% sodium erythorbate (0700139-V, Excalibur, Pekin, IL,

U.S.A.). The treatment brine contained 72.4% water, 11.8% salt, 7.00% sugar, 1.70% sodium

phosphate (Brifisol 450 Super, ICL Performance Products, St. Louis, MO, U.S.A.), 1.60%

modern cure (6.25% Nitrite, Excalibur, Pekin, IL, U.S.A.), 0.450% sodium erythorbate

(0700139-V, Excalibur, Pekin, IL, U.S.A.) and 5.00% smoked sugar (RA12032, Red Arrow

Products, Manitowoc, WI, U.S.A.).

Green weights were collected for each belly half and are exhibited in Table 3.1. The half

belly was then placed in a multineedle injector (Model N30 Wolftec Inc., Werther, Germany),

and injected to 20% of green weight. All injected bellies were hung on a smokehouse truck for

two hours to equilibrate prior to thermal processing. A drip weight was collected to insure 12%

of brine was retained. After drip weights were collected, bellies were put into a single truck

smokehouse (D7752 Mauer Inc., Reichenau, Germany) for smoking/cooking. A standard thermal

processing schedule was utilized and included: stage one with smokehouse setting of 57°C dry

bulb and 30°C wet bulb for 30 minutes; stage two consisted of 54°C dry bulb, 44°C wet bulb, and natural smoke applied for 30 minutes; stage three was 54°C dry bulb and 35°C wet bulb for 150 minutes; stage four was 57°C dry bulb and 30°C for wet bulb for 130 minutes to reach an internal belly temperature of at least 54°C. Cooked bellies were placed into a chiller (2 ± 1°C) for 12 hrs. After cooling, chilled weights were collected to calculated cook. Each half belly was sliced 1.5 mm thick with a horizontal slicer (Model Puma 700 F, Treif, Oberlahr, Germany) from the anterior to posterior end.

Six slices were selected randomly throughout each half belly to form a composite sample for proximate and fatty acid analysis. The remaining slices were laid out randomly on non-coated bacon sheet paper (28 cm × 43 cm, Formax, Mokena, IL, U.S.A.). Bacon slices were randomly assigned to four sensory and gas chromatography mass spectrometry (**GCMS**) storage periods (0, 40, 80, 120 days) or seven TBARS storage times (0, 20, 40, 60, 80, 100, or 120 days). All d 0 sensory, GCMS, and TBARS bacon slices were collected the day of slicing and vacuumed packaged. Once vacuumed packaged, all samples were stored in -80°C freezer until analysis to prevent further lipid oxidation. The remaining sensory, TBARS, and GCMS sheets were stacked with day 20 on top and day 120 on the bottom, and placed in a poly-liner (clear 3 mm thick bag, Cargill, Wichita, KS, U.S.A) and a corrugated cardboard box with fitted lid (43.8 cm × 28.6 cm × 10.2 cm, Uline, Pleasant Prairie, WI, U.S.A). All boxed slices were stored aerobically at a temperature of −17.8 ± 2°C for the appropriate storage time. The experiment was replicated three times, with all replications being manufactured and cooked separately from one another.

Fat, Moisture, and Protein Analysis

Composite samples of bacon slices were cut into small pieces, frozen in liquid nitrogen, pulverized in a blender (Model 33B179, Waring Products, New Hartford, CT, U.S.A.), and

stored frozen. Proximate samples were removed from the pulverized composite samples. Fat and moisture percentages were analyzed using the Association of Official Analytical Chemists (AOAC) PVM-1 (2003) method. Additionally, protein composition was determined by utilizing the AOAC 992.15 (1994) method. Fat, moisture, and protein values were presented as a percent of total composition.

Fatty Acid Analysis

Fatty acid analysis was performed with some modifications from Sukhija and Palmquist, (1988). One gram of pulverized composite bacon sample was weighed into screw-cap tubes with Teflon-lined caps for fatty acid analysis. Each bacon sample was mixed with three mL of methanolic-HCL and two mL of an internal standard consisting of two mg/mL of methyl tridecanoic acid in benzene. Tubes with bacon, methanolic-HCL and internal standard were flushed with nitrogen and capped tightly. Samples were then heated in a water bath for 120 minutes at 70 °C. After heating, two mL of benzene and three mL of K_2CO_3 were added to samples. Samples were centrifuged for five minutes at $1,000 \times g$. The top layer of solvents and fat were removed, then placed in two mL glass vial and Teflon-lined cap was crimped tightly closed. Gas chromatography was used to analyze fatty acid composition. Fatty acid composition percentages are reported as a percentage of total fatty acids. Iodine value was determined by the following equation: C16:1(0.95) + C18:1(0.86) + C18:2(1.732) + C18:3 (2.616) + C20:1 (0.785) + C22:1(0.723), (AOAC, 1997).

Trained Sensory Evaluation

Attributes and reference sample were chosen with guidance from Gatlin et al. (2006), in addition to a descriptive panel performed by the Kansas State University Meat Science Department before training sessions. Panelists participated in eight panel training sessions to familiarize themselves with scale anchors and reference samples. Panelists evaluated bacon

samples on continuous 100-point line scale for saltiness, smoke intensity, bacon flavor, oxidized flavor, and other off-flavors. Zero would indicate extremely not salty, not smoky, and bland, along with no oxidized or other off-flavors. A 100 on the continuous line scale was be extremely salty, smoky, along with intense bacon flavor, oxidized flavor, and other off-flavors. Final scale anchors consisted of the following solutions: 0.5% salt in deionized water indicated a 60 on the saltiness scale, in addition to 0.125% smoked sugar and deionized water exhibited an 80 on the smoke intensity scale. An oxidized flavor anchor was determined at a score of 100 on the scale with foodservice bacon that was packaged aerobically and stored for two years in a -29 °C freezer.

In order to reduce the variation of panelists scores, a reference bacon was chosen during the descriptive panel, similar to Gatlin et al. (2006). A commonly available commercial brand of bacon was used as the reference sample due to the inclusion of liquid smoke as an ingredient and similarities to the research bacon. The reference bacon had the following sensory values: saltiness (40-50), smoke intensity (30-40), bacon flavor intensity (40-55), and no oxidized or other off-flavors detected.

After all frozen storage times (0, 40, 80, 120 day) were completed and collected, all bacon samples from the same belly were randomly assigned to a trained sensory panel. This random sample assignment allowed for the control and naturally smoked sugar treatment to be evaluated in one panel. Sensory samples were placed on wire cooking racks in a Blodgett dual-flow, forced-air oven (DFD-201, G.S. Blodgett Co., Inc., Burlington, VT, U.S.A.) to cook at 176.7 °C for six minutes while rotating the pans 180° halfway through the cooking process. After cooking, slices were blotted with paper towels to remove excess grease as described by Lowe et al. (2014). At least eight panelists were used for each panel of sensory evaluation.

Panelists were stationed in individual booths under a combination of red and green light. Apples, crackers, and water were provided to cleanse the palates between samples (Olson et al., 1985). The salt and smoke solutions were given before warm-up samples were presented for reference. The reference bacon was offered as a warm-up sample prior to every sensory panel. After the warm-up sample and discussion, samples from the same belly (control and naturally smoked sugar treatment) at all frozen storage times were evaluated in random order by the trained panelists.

Thiobarbituric Acid Reactive Substances

Lipid oxidation was determined by a distillation method of TBARS as described by Sebranek et al. (2001). Eight slices per storage time were pulverized as described in the fat, moisture, and protein analysis section. Ten grams of pulverized sample mixed with deionized water (97 mL), one mL of sulfanilamide (0.5% sulfanilamide, 20% HCL, and deionized water), two mL of HCL (50% HCL and deionized water), and five drops of antifoam in a round bottom flask. The round sample mixture was heated to boil and condensation from the boiling mixture was collected and cooled through a glass condenser, until 50 milliliters of distillate was reached. Five milliliters of distillate were combined with thiobarbituric acid (TBA; 0.28% and deionized water) in a 15-milliliter plastic tube and capped tightly; this step was repeated to create duplicates. Distillate and TBA tubes were placed in a boiling water bath for 35 minutes, then transferred to cold water bath for 10 minutes. Once cooled, two milliliters of distillate and TBA mixture was transferred into a spectrophotometer cuvette and absorbance was measured on a spectrophotometer (Eon, BioTek Instruments, Winooski, VT, U.S.A.) at 532 nm, compared to a blank cuvette containing one milliliter deionized water and one milliliter TBA reagent.

Thiobarbituric acid reactive substances are expressed as milligrams of malonaldehyde per kg of sample.

Gas Chromatography Mass Spectrometry (GCMS)

The production of volatiles was analyzed with gas chromatography mass spectrometry (GCMS) and solid phase microextraction (SPME), following the procedures of Yu et al. (2008). The gas chromatography used was a 5890 Series II Plus (Agilent Technologies, Santa Clara, CA, U.S.A.) fitted with a split/split-less injection port containing a 0.75 mm I.D. liner connected to 5972 Series mass spectrometer (Agilent Technologies, Santa Clara, CA, U.S.A.). Chemstation software (G1701BA Version B.01.00) was used for data acquisition, volatiles were separated with HP-5ms column (5% phenyl-methylpolysiloxane; 60 m × 2.5 mm × 2.5 µm, Agilent Technologies, Santa Clara, CA, U.S.A.). The SPME fiber (75µm thickness, fused silica, CAR/PDMS; Supelco, Bellefonte, PA, U.S.A.) was placed in a manual SPME holder and utilized for headspace extraction. Prior to sample injection the SPME fiber was subjected to 300°C for 30 minutes in the GCMS port for preconditioning.

Three grams of pulverized bacon with two milliliters of water and internal standard (1 ppm 2-Cholorphenol) were placed into a 20-milliliter vial and capped tightly with Teflon silica septum (VWR International, LLC; Radnor, PA). Closed vials were vortexed for one minute and then transferred to a heating block for 15 minutes at 60°C to equilibrate. The SPME fiber was exposed to the head-space of the sample for 30 minutes at 60°C (Ruiz et al., 1998). Samples were injected in split-less mode with the injector temperature set to 250 °C and purge-off time set to one minute. Oven temperatures were set to the following: 40 °C held for one minute, ramped to 185 °C at 10 °C min^{-1} then held for 2.5 minutes. The total run time was 18 minutes. The carrier gas was ultra-high purity helium with a constant flow rate of one mL/min.

The mass spectrometer (MS) was programmed to select individual ions (SIM) that are specific to the aldehydes and phenolic compounds. The MS system was routinely calibrated using the auto-tune calibration process of the mass selective detector. The aldehydes selected to determine lipid oxidation were based on correlated values to TBARS from Ahn et al. (1999). The aldehydes and their specific ion makeup chosen were hexanal (56, 57, 72, 82 m/z), heptanal (55, 70, 81, 96 m/z), and nonanal (56, 57, 70, 98 m/z). Phenolic compounds selected were creosol (95, 123, 138, 139 m/z) and syringol (93, 111, 139, 154 m/z). A standard curve was generated for each compound to calculate concentrations in ppm. The standard curves are as follows: hexanal was prepared at 0.1 ppm, 0.25 ppm, 0.5 ppm, and 0.75 ppm; heptanal was arranged at 0.1 ppm, 0.25 ppm, 0.5 ppm, and 0.75 ppm; nonanal was prepared at 0.05 ppm, 0.1 ppm, 0.2 ppm, and 0.5 ppm; creosol was prepared at concentrations of 0.25 ppm, 0.5 ppm, 1 ppm, and 1.5 ppm; syringol concentrations were 20 ppm, 50 ppm, 100 ppm, and 200 ppm. A stock solution of 1,000 ppm of each standard was prepared in ethanol and then diluted to specific working standard concentrations in water for all compounds. The quantified aldehydes and phenolic compounds from frozen foodservice bacon was reported in ppm.

Statistical Analysis

Data was analyzed using the PROC MIXED procedure of SAS Version 9.3 (SAS Institute Inc., Cary, NC, U.S.A.). Proximate analysis and fatty acid composition utilized an incomplete block design with fixed effects of belly half and treatment with random effects of replication and belly number. The TBARS, GCMS, and sensory analysis were analyzed as a split plot with repeated measures, whole plot factor treatment with the sub-plot factor of storage day. Fixed effects included Treatment, Day, and interaction, while random effects included Replication and Belly within Replication. The covariance structure for the repeated measures

was heterogeneous compound symmetry. The results from GCMS were log transformed in order to have equal variance to detect difference in means. The log transformed data and the standard errors were back transformed using the delta method to report values as ppm. A paired T-test was utilized to separate mean differences between treatments with a Tukey-Kramer adjustment for all pairwise comparisons and the denominator degrees of freedom were adjusted with Kenward-Roger adjustment. Means were determined to be significantly different at an α-level of $P < 0.05$.

Results

Fat, Moisture, and Protein Analysis

There were no Belly half × Treatment interactions or Treatment main effect for fat, moisture, or protein analysis ($P > 0.10$). The mean proximate composition of anterior belly sections and posterior belly sections are described in Table 3.2. Anterior belly sections were fatter, had less moisture, and contained less protein compared with posterior belly sections ($P < 0.001$).

Fatty Acid Analysis

There were no Belly half × Treatment interactions or Treatment main effect for all individual fatty acids found within anterior and posterior belly sections ($P > 0.16$). The mean percentages of individual fatty acids are represented in Table 3.3. There were no differences between anterior belly sections and posterior belly sections for all individual fatty acids ($P > 0.06$); except Linoleic acid (C18:2n6t) where the posterior belly sections had a greater ($P = 0.01$) concentration than the anterior belly section. Also, α-Linolenic acid (C18:3n6) had a greater ($P = 0.02$) concentration in the anterior belly sections than the posterior section. Additionally, total saturated, monounsaturated, and polyunsaturated fatty acid percentages were not different

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between anterior and posterior belly section ($P > 0.21$). Finally, the calculated Iodine Value for the anterior and posterior belly sections were not different ($P = 0.13$).

Trained Sensory Evaluation

Mean values for oxidized flavor intensity for control and bacon produced with the addition of naturally smoked sugar are presented in Figure 3.1. A Treatment $\times$ Day interaction was found for oxidized flavor intensity ($P < 0.001$). Control and naturally smoked sugar treated bacon had oxidized flavor intensity scores that were not different ($P = 0.47$) on day 0 of frozen storage. While, on day 40, 80, and 120 of frozen storage the control bacon exhibited increased oxidized flavor intensity scores compared to bacon manufactured with naturally smoked sugar ($P < 0.001$).

There were no Treatment $\times$ Day interactions or Day main effect for panelist scores of saltiness, smoke intensity, bacon flavor intensity, and other off-flavors ($P > 0.08$). Main effect mean values for panelist scores including the attributes of saltiness, smoke intensity, bacon flavor intensity, and other off-flavors are presented in Figure 3.2. Trained panelists scored bacon samples manufactured with naturally smoked sugar greater for saltiness, smoke intensity, as well as bacon flavor intensity compared with control samples ($P < 0.001$). There was no difference ($P = 0.95$) in the intensity of other off-flavors present between the control treatment and bacon formulated with naturally smoked sugar.

Thiobarbituric Acid Reactive Substances

Mean values for TBARS analysis for the control and bacon manufactured with the addition of naturally smoked sugar are presented in Figure 3.3. There was a Treatment $\times$ Day ($P < 0.01$) interaction found for bacon samples formulated with and without added naturally smoked sugar for TBARS values. Control and naturally smoked sugar treated bacon were not different

for TBARS values on day 0 and 20 of frozen storage ($P = 0.24$). After 20 days of frozen storage, control bacon displayed greater TBARS values than bacon processed with naturally smoked sugar ($P < 0.0004$).

Gas Chromatography Mass Spectrometry

There were Treatment × Day interactions for hexanal (Figure 3.4) and heptanal (Figure 3.5) concentrations in control and the naturally smoked sugar treatment ($P < 0.004$). Control and bacon manufactured with naturally smoked sugar exhibited no differences ($P = 0.57$) in hexanal concentration on day 0 of frozen storage. Although, on days 40, 80, and 120 of frozen storage the bacon with naturally smoked sugar had lower concentrations of hexanal compared to the control bacon ($P < 0.01$). On days 0, 40, and 120 of frozen storage heptanal concentrations for bacon processed without and with naturally smoked sugar were not different ($P > 0.29$). Heptanal concentration on day 80 of frozen storage was greater ($P = 0.0016$) for control bacon compared to the naturally smoked sugar treatment.

Mean values for nonanal concentration for frozen foodservice bacon stored aerobically for 120 days are represented in Table 3.4. There was no Treatment × Day interaction or Treatment main effect for nonanal concentration for control and naturally smoked sugar treated bacon ($P > 0.16$). There was a Day main effect for foodservice bacon stored aerobically and frozen for 120 days ($P = 0.05$). Frozen foodservice bacon had greater ($P = 0.05$) concentration of nonanal at day 120 compared to day 0 of aerobic storage.

Finally, there was no Treatment × Day interaction or Day main effect for creosol and syringol content for control and naturally smoked sugar treated bacon ($P > 0.07$). There was a Treatment main effect ($P < 0.003$), mean values for concentrations of creosol and syringol are presented in Table 3.5 for control bacon and bacon manufactured with naturally smoked sugar.

Bacon processed with naturally smoked sugar exhibited greater concentrations of creosol and syringol than the control bacon ($P < 0.003$).

Discussion

To fully understand the capability of naturally smoked sugar, bellies were selected to represent a high-quality population of raw bellies that are low in unsaturated fatty acids. Calculated iodine values for the collected bellies were 68.85 for anterior sections and 67.03 for posterior belly sections. Iodine values in an experiment conducted by Houser (2015), segmented raw bellies into three iodine value categories (High = 77.9; Intermediate = 72.6; Low = 66.5). Iodine values from bellies collected in this study are similar to the Low iodine value category in Houser (2015). The fatty acid composition of the bellies selected for this study are comparable to the fatty acid percentages reported by Houser (2015). This comparison of iodine values concludes the raw bellies selected to challenge naturally smoked sugar would limit the effects of fatty acid composition on lipid oxidation in foodservice bacon.

Trained sensory evaluation showed that oxidized flavor increased throughout extended frozen storage. This confirms results from Lowe et al. (2014). Lowe et al. (2014) found that foodservice bacon stored frozen for 90 days at -33 °C exhibited increased off-flavor scores from trained panelists. In addition to increased off-flavor scores, Lowe et al. (2014) also presented increased off odor scores from trained panelists for foodservice bacon throughout the 90 days frozen storage. However, Soladoye et al. (2017) reported no differences in off-flavor intensity scores for vacuumed packaged bacon stored for 28 days in refrigerated temperatures (4 °C). The lack of off-flavors in the Soldaoye et al. (2017) study could be due to the short storage time in an anaerobic environment as well as the refrigerated temperatures, compared to the 120 days of frozen storage in aerobic conditions where prooxidants are highly active. The challenge for

foodservice bacon packaged in an aerobic environment is lipid oxidation, which produces compounds that panelists can detect throughout frozen storage even at colder temperatures (-33 °C) such as Lowe et al. (2014). Therefore, limiting lipid oxidation in foodservice bacon that is subjected to extended frozen storage should be a priority to prevent oxidized flavor development. Younathan and Watts (1959) discovered that a TBARS value of 1.0 is highly correlated with off-flavors, which was further supported in cooked pork by Tarladgis et al. (1959). However, evaluating lipid oxidation in bacon from Houser (2015), Lowe et al. (2014), as well as this study show no consistent day where TBARS values reach a value of 1.0. Therefore, further sensory studies need to be conducted to determine a threshold of TBARS values at which consumers perceive bacon to be unacceptable.

The TBARS values for bacon formulated without an added antioxidant closely resemble previous work from Houser (2015) which documented a significant increase in TBARS values over an extended frozen storage with aerobically packaged foodservice bacon. Additionally, Sebranek et al. (2005) reported increasing TBARS levels for raw frozen pork sausage formulated without an antioxidant throughout 112 days of storage. These studies along with this experiment support that bacon stored frozen in an aerobic environment for an extended period of time will oxidize.

The hexanal concentration of foodservice bacon formulated without naturally smoked sugar increased as storage time increased. Hexanal has been shown to be an effective indicator of lipid oxidation (Shahidi et al., 1987). The concentration of hexanal was highly correlated with TBARS as well as sensory acceptability scores in cooked ground pork shown by Shahidi et al. (1987). As hexanal content increased, so did TBARS values (Shahidi et al., 1987), these results agree with the hexanal content and TBARS values in this study. Additionally, Ahn et al. (1999)

also reported hexanal concentration as highly correlated to TBARS values in cooked sausage. Hexanal concentration can be an indicator of lipid oxidation, this study shows that hexanal will increase throughout frozen storage resulting in lipid oxidation.

In addition to increased hexanal concentration, heptanal content increased during the frozen storage of foodservice bacon. Heptanal is not as heavily researched as hexanal, however, Yu et al. (2008) was able to identify heptanal as a product of lipid oxidation in bacon. Furthermore, Ruiz et al. (1998) used headspace solid phase microextraction to identify heptanal in dry-cured ham. This is the first study to illustrate heptanal concentration will increase during frozen storage of foodservice bacon, resulting in increased lipid oxidation over time.

The GCMS method is an accurate and sensitive objective measurement of lipid oxidation (Garcia-Regureiro and Diaz, 1989). Future research can utilize GCMS to accurately identify and quantify specific aldehydes that are highly correlated to oxidized flavor. The GCMS method may be an alternative to TBARS as an objective measure of lipid oxidation due to the identification of oxidative products and less intensive sample preparation.

The inclusion of naturally smoked sugar in the brine formulation decreased the presence of oxidized flavor indicating that lipid oxidation was prevented during the extended frozen storage. It is well known that smoking meat products is a way of preservation. The lignin component of hardwoods is responsible for the production of phenolic compounds, which contributes to the antioxidant properties of smoke products (Rozum, 2009). There have been multiple studies on the phenol composition of liquid smokes (Knowles et al., 1975; Simon et al., 2005; Montazeri et al., 2013) as well as the antioxidant potential of smoke products (Wendorff, 1981). Few studies have examined trained sensory evaluation for smoke products as a potential antioxidant for foodservice bacon. However, it is known the inclusion of phenolic antioxidant

into meat products will inhibit the formation of off-flavors. For example, cooked frozen pork sausage treated with 2,500 ppm of rosemary extract exhibited lower trained sensory scores for warmed over flavor compared to cooked sausage formulated with less than 1,500 ppm of rosemary extract (Sebranek et al., 2005). These sensory results from Sebranek et al. (2005) confirm these data collected from trained sensory evaluation that naturally smoked sugar can limit the development of oxidative products, leading to a more desirable eating experience.

The trained sensory data show the addition of naturally smoked sugar into the brine formulation served as an effective antioxidant without hindering other flavor attributes typically found in bacon. Saltiness, smoke intensity, and bacon flavor were greater for bacon processed with naturally smoked sugar. Smoke and smoke derived products are known for imparting flavor onto meat products. This is evidenced by these data collected from trained panelists where scores for saltiness, smoke intensity, and bacon flavor intensity were greater from bacon containing naturally smoked sugar. Cellulose, hemicellulose, and lignin contribute to smoke flavor; however, phenols are responsible for the common pungent flavor of smoke. Other phenolic descriptors include sharp, dry or charred wood, and sweet/fruity (Rozum, 2009). Further, these results indicate that the addition of naturally smoked sugar to a curing brine may present additional benefits to flavor development in sliced bacon.

The antioxidant ability of naturally smoked sugar to effectively inhibit lipid oxidation is seen by lower TBARS values compared to the control in this study, due to high concentrations of phenolic compounds. Phenolic compounds have been quantified by many researchers (Guillén and Manzanos, 1999; and Montazeri et al., 2013) in smoke and liquid smoke products. In raw frozen pork sausage with added antioxidants TBARS values remained lower than pork sausage that did not contain an antioxidant (Sebrank et al. 2005). However, TBARS levels for cooked

frozen pork sausage were not different from cooked sausage formulated without antioxidants. This suggests that bacon that is partially cooked and stored frozen must contain an added antioxidant such as naturally smoked sugar to limit lipid oxidation during storage. The inhibition of lipid oxidation by liquid smoke is exhibited in precooked beef patties in a study conducted by Estrada-Muñoz et al. (1998). Similar to TBARS values of naturally smoked sugar, precooked beef patties treated with liquid smoke had decreased TBARS values when compared to patties that did not include liquid smoke throughout 90 days of frozen storage.

Montazeri et al. (2013) identified and quantified volatile as well as semi-volatile compounds within liquid smoke, the greatest concentrations of compounds were classified as phenolic. Phenolic compounds are active in scavenging for free radicals thus, inhibiting lipid oxidation. Estrada-Muñoz et al. (1998) confirmed liquid smoke products contain sufficient phenolic compounds to act as an effective antioxidant in cooked meat products.

For bacon treated with naturally smoked sugar, hexanal and heptanal concentrations remained unchanged through 120 days of frozen storage time. Hexanal is highly correlated to TBARS values and off-flavor development (Shadihi et al., 1987), leading to the conclusion that naturally smoked sugar limits lipid oxidation during frozen storage. Ahn et al. (2002) reported hexanal content was lower for cooked ground beef patties with antioxidants than those without added antioxidants. Naturally smoked sugar is an effective antioxidant because of lower concentrations of hexanal and heptanal during frozen storage of foodservice bacon. The hexanal and heptanal concentrations support the TBARS data collected for bacon treated with naturally smoked sugar.

Nonanal concentrations for frozen foodservice bacon stored aerobically were the greatest on day 120. Ahn et al. (1999) reported in cooked sausage nonanal is highly correlated with

TBARS values. Also, multiple researchers were able to identify nonanal as an oxidized product using GCMS in bacon and dry-cured ham (Ruiz et al., 1998; and Bosse et al., 2017). However, this is among the first studies to illustrate concentrations of nonanal increasing during aerobic frozen storage of bacon. The range of concentration for nonanal over the 120- day frozen storage period of foodservice bacon is small. It is not surprising that as frozen storage time increased lipid oxidization became more prevalent.

Including naturally smoked sugar into the brine of foodservice bacon is an effective antioxidant because of the phenolic compounds present. Many have been able to identify phenolic compounds in smoke as well as smoke derived products (Knowles et al., 1975; Simon et al., 2005; Montazeri et al., 2013). The GCMS was able to identify and quantify creosol and syringol, phenolic compounds commonly found in liquid smoke products, in bacon manufactured with naturally smoked sugar (Montazeri et al., 2013). With greater concentrations of creosol and syringol found in naturally smoked sugar treatment, supports the phenolic compounds are responsible in limiting lipid oxidation. Also, the placement of the phenolic compounds could impact the antioxidant ability of naturally smoked sugar. The GCMS detected lower levels of creosol and syringol in control bacon, these phenolics are sourced from traditional hardwood smoke applied to the surface of the belly. The incorporation of naturally smoked sugar into the brine allows for the phenolic compounds to actively inhibit lipid oxidation within the interior of bacon slices.

In addition, phenolic compounds are a source for smoke flavor in meat products. The greater concentration of smoke phenolic compounds will contribute the pungent smoke flavor in foodservice bacon (Rozum, 2009). Thus, naturally smoked sugar contributes both antioxidant properties and flavor to foodservice bacon.

Conclusion

In summary, the inclusion of naturally smoked sugar into bacon formulations successfully inhibited lipid oxidation when products where stored aerobically and subjected to extended periods of frozen storage. The ability of naturally smoked sugar to function as an antioxidant in sliced bacon was confirmed with both subjective sensory evaluations along with objective measurements of lipid degradation products. Also, aerobically packaged frozen bacon formulated without the addition of phenolic antioxidants such as those present in naturally smoked sugar had significant challenges in lipid stability as frozen storage length increased. Meat processors manufacturing sliced bacon intended for aerobic, frozen storage should seriously consider the use of technologies aimed at improving lipid stability in their products or risk decreased purchasing of bacon products by consumers at foodservice establishments.

Tables and Figures

Table 3.1 Bacon Processing Parameters

	Brine Characteristics	
	Control	Treatment
Green Weight (kg)	2.84	2.77
Percent Pump (%)	12.3	11.5
Cook Loss (%)	2.97	1.22
Brine pH	5.76	4.80

[1] Cook Loss = [(Chilled Weight – Green Weight) / Green Weight] $\times$ 100

Table 3.2 Proximate composition of anterior and posterior half bellies

Proximate Component (%)	Belly Half		SEM	P – value
	Anterior	Posterior		
Fat	40.3[a]	33.5[b]	1.63	$P < 0.0001$
Moisture	41.1[b]	47.6[a]	1.32	$P < 0.0001$
Protein	12.7[b]	15.2[a]	0.426	$P < 0.0001$

[a,b] Means with different superscripts within the same row are different ($P < 0.05$)

Table 3.3 Fatty acid composition of anterior and posterior half bellies

Fatty Acid (%)	Belly Half			
	Anterior	Posterior	SEM	P - value
Capric acid (C10:0)	0.25	0.83	0.69	$P = 0.42$
Myristic acid (C14:0)	0.95	0.85	0.10	$P = 0.33$
Pentadecylic acid (C15:0)	0.29	0.22	0.08	$P = 0.42$
Palmitic acid (C16:0)	16.44	16.31	1.01	$P = 0.90$
Palmitoleic acid (C16:1)	1.53	1.65	0.18	$P = 0.50$
Margaric acid (C17:0)	1.32	2.08	0.90	$P = 0.41$
Stearic acid (C18:0)	12.56	13.18	1.18	$P = 0.61$
Oleic acid (C18:1n9c)	38.37	37.01	2.45	$P = 0.59$
Vaccenic acid (C18:1n7)	0.89	0.80	0.54	$P = 0.86$
Linoleic acid (C18:2n6t)	0.02[b]	0.44[a]	0.01	$P < 0.01$
Linoleic acid (C18:2n6)	18.09	15.88	1.08	$P = 0.06$
α-Linolenic acid (C18:3n6)	0.27[a]	0.06[b]	0.08	$P = 0.02$
α-Linolenic acid (C18:3n3)	0.44	1.39	1.11	$P = 0.42$
Eicosadienoic acid (C20:2)	0.41	0.32	0.14	$P = 0.52$
Arachidonic acid (C20:4n6)	0.81	0.88	0.23	$P = 0.75$
Total SFA[1]	32.16	33.56	1.80	$P = 0.45$
Total MUFA[2]	41.72	40.95	1.42	$P = 0.60$
Total PUFA[3]	20.12	20.31	0.71	$P = 0.21$
Iodine Value, g/100g[4]	68.85	67.03	1.13	$P = 0.13$

[a,b] Means with different superscripts within the same row are different ($P < 0.05$).

[1] Total saturated fatty acids = {(C10:0) + (C12:0) + (C14:0) + (C15:0) + (C16:0) + (C17:0) + (C18:0) + (C20:0) + (C22:0)} where the parentheses indicate concentration.

[2] Total monounsaturated fatty acids = {(C14:1) + (C16:1) + (C17:1) + (C18:1n9c) + (C18:1n7) + (C20:1) + (C22:1n9)} where the parentheses indicate concentration.

[3] Total polyunsaturated fatty acids = {(C18:2n6t) + (C18:2n6c) + (C18:3n6) + (C18:3n3) + (C20:2) + (C20:3n3) + (C20:4n6)} where the parentheses indicate concentration.

[4] Calculated as IV = {(C16:1) × 0.95 + (C18:1) × 0.86 + (C18:2) × 1.732 + (C18:3) × 2.616 + (C20:1) × 0.785 + (C22:1) × 0.723} where the parentheses indicate concentration, (AOCS, 1997).

Table 3.4 Mean nonanal[2] concentrations for foodservice bacon stored frozen for 120 days

	Frozen Storage Time (Days)					
	0	40	80	120	SEM	P - value
Nonanal (ppm)	0.087374[b]	0.087590[ab]	0.089168[ab]	0.089185[a]	0.0010308	$P = 0.05$

[1] Means with different superscripts are significantly different ($P < 0.05$).
[2] Product of lipid oxidation.

Table 3.5 Mean creosol[2] and syringol[2] concentrations (ppm) for control[3] and treatment[4] foodservice bacon

Phenolic Compound (ppm)	Treatment Combination			
	Control	Treatment	SEM	*P* - value
Creosol	1.52[b]	2.14[a]	0.223	$P < 0.01$
Syringol	47.2[b]	67.8[a]	6.35	$P < 0.01$

[1] Means within the same row with different superscripts are significantly different ($P < 0.05$).
[2] Phenolic smoke compounds.
[3] Control Bacon = no smoked sugar added
[4] Treatment Bacon = naturally smoked sugar added

Figure 3.1 Mean panelist scores for oxidized[2] flavor intensity for control[3] and treatment[4] foodservice bacon stored frozen for 0-120 days

[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment $\times$ Day was significant at ($P < 0.05$).

[2] Product of lipid oxidation; Evaluated on a continuous line scale; 0 = no oxidized flavor and 100 = extremely intense oxidized flavor.

[3] Control Bacon = no smoked sugar added
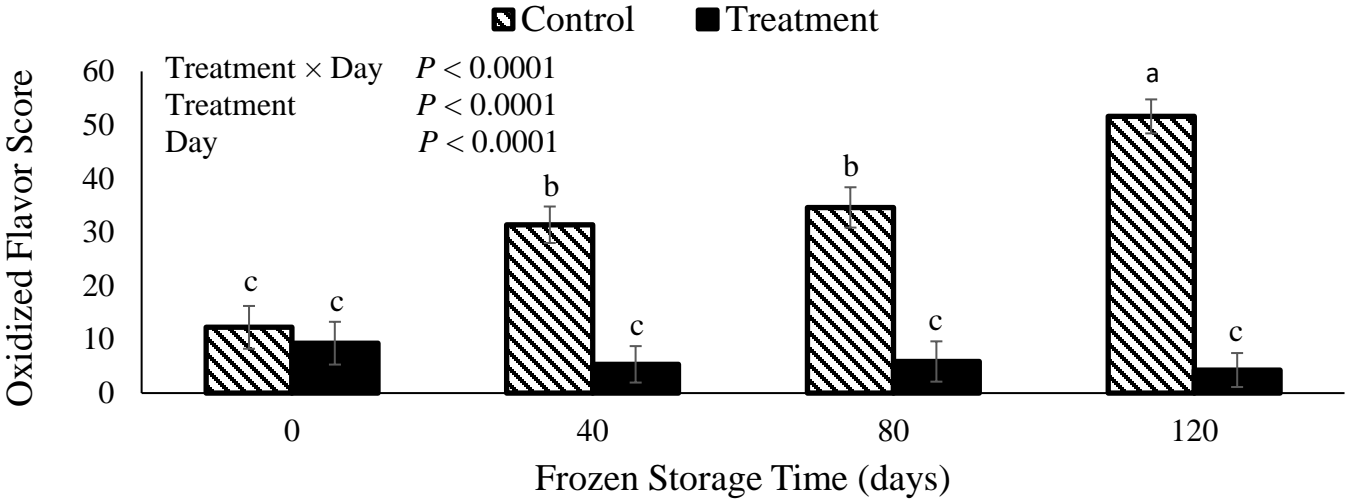
[4] Treatment Bacon = naturally smoked sugar added

Figure 3.2 Mean panelist scores for saltiness[2], smoke intensity[3], bacon flavor intensity[4], and other off-flavors[5] for control[6] and treatment[7] foodservice bacon

[1] Means within the same attribute with different superscripts are significantly different ($P < 0.05$).
[2] Evaluated on a continuous line scale; 0 = extremely not salty and 100 = extremely salty.
[3] Evaluated on a continuous line scale; 0 = extremely not smoky and 100 = extremely smoky.
[4] Evaluated on a continuous line scale; 0 = extremely bland and 100 = extremely intense.
[5] Evaluated on a continuous line scale; 0 = none present and 100 = extremely intense.
[6] Control Bacon = no smoked sugar added
[7] Treatment Bacon = naturally smoked sugar added

Figure 3.3 Mean thiobarbituric acid reactive substances (TBARS)[2] values for control[3] and treatment[4] foodservice bacon stored frozen for 120 days

······Control ——Treatment

Treatment × Day $P < 0.0001$
Treatment $P < 0.0001$
Day $P < 0.0001$

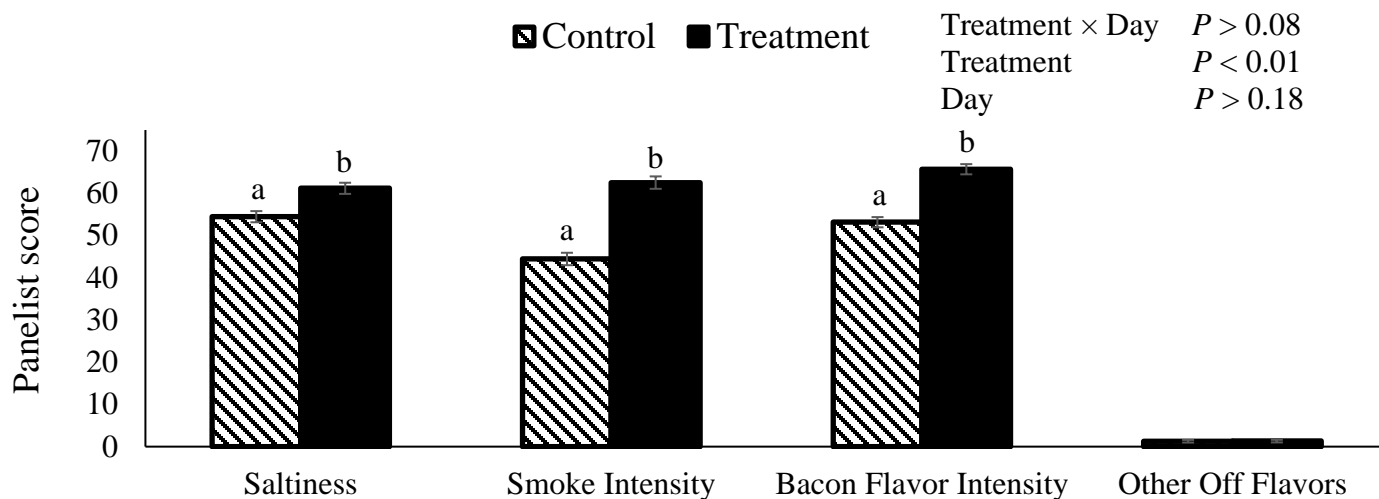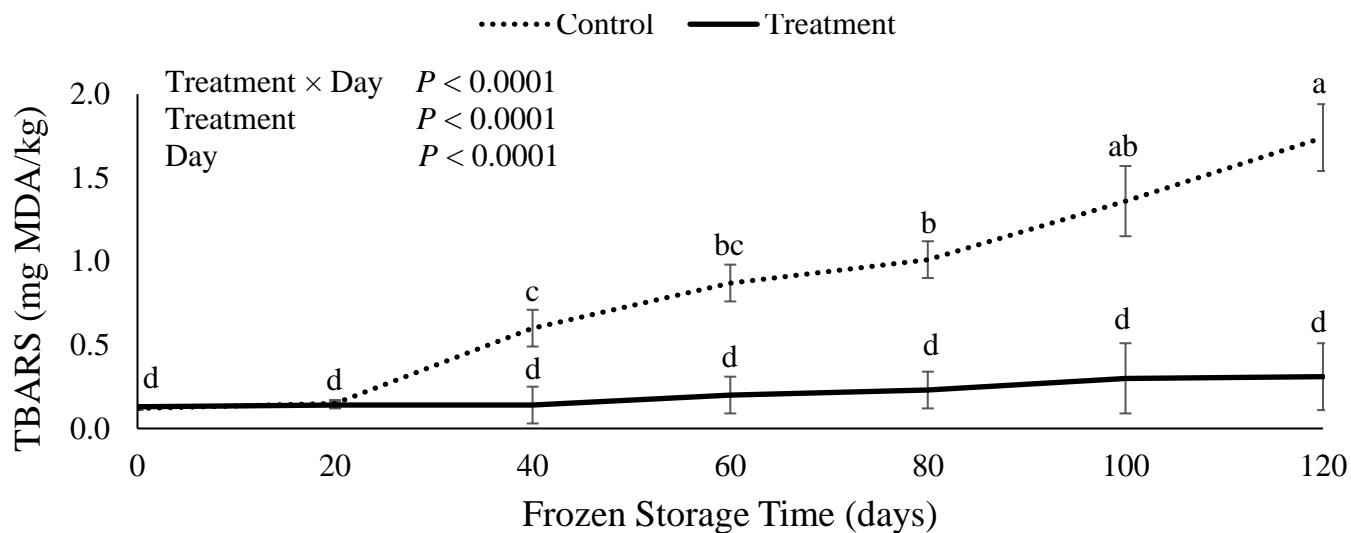[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment × Day was significant at ($P < 0.05$).
[2] Measure of lipid oxidation
[3] Control Bacon = no smoked sugar added
[4] Treatment Bacon = naturally smoked sugar added

Figure 3.4 Mean hexanal[2] content for control[3] and treatment[4] foodservice bacon stored frozen for 120 days
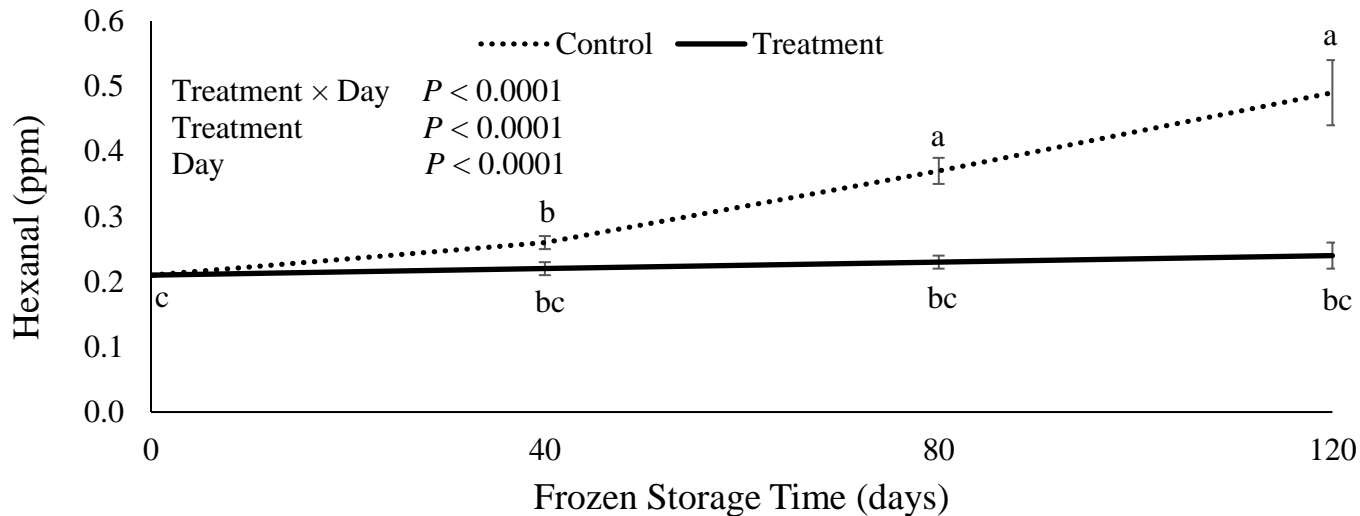
[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment ×
    Day was significant at ($P < 0.05$).
[2] Product of lipid oxidation
[3] Control Bacon = no smoked sugar added
[4] Treatment Bacon = naturally smoked sugar added

Figure 3.5 Mean heptanal[2] concentrations for control[3] and treatment[4] foodservice bacon stored frozen for 120 days

[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment × Day was significant at ($P < 0.05$).

[2]Product of lipid oxidation

[3] Control Bacon = no smoked sugar added

[4] Treatment Bacon = naturally smoked sugar added

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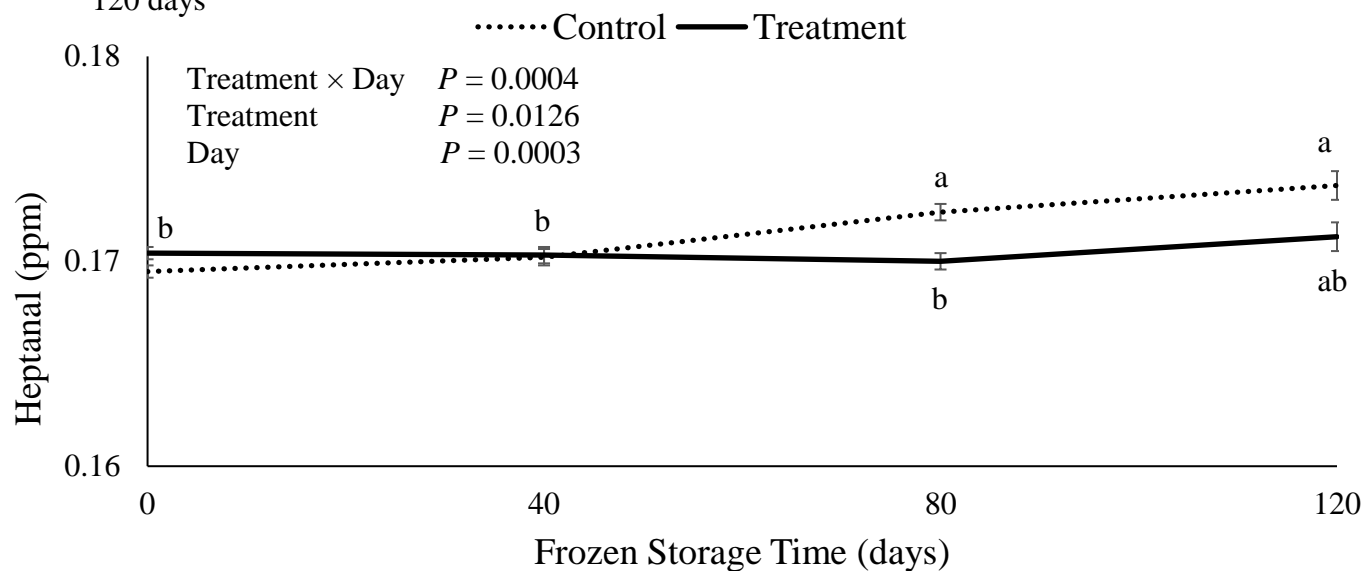Ahn, J., Olson, D.G., Jo, C., Love, J., and Jin, S.K. (1999). Volatiles production and lipid oxidation in irradiated cooked sausage as related to packaging and storage. *J. Food Sci*. 64, 226-229.

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Chapter 4 - Effects of naturally smoked sugar and frozen storage time on foodservice packaged bacon using a natural curing system

Abstract

The objective of this study was to determine the effectiveness of smoked sugar in a natural curing brine to prevent lipid oxidation of frozen, aerobically packaged, lay-out style bacon. Pork bellies (Institutional Meat Purchase Specifications, IMPS #409) were trimmed and cut in half yielding an anterior and posterior section. Each section was randomly assigned to one of two brines were injected to 12% of green weights: a control brine with water, salt, sugar, and cultured celery juice; or a curing brine with added smoked sugar ($n = 15$/treatment). After thermal processing and slicing, bacon slices were randomly assigned to four sensory frozen storage periods (0, 40, 80, and 120 day) or seven thiobarbituric acid reactive substances (TBARS) frozen storage times (0, 20, 40, 60, 80, 100, and 120 day). All bacon samples were stored aerobically at -18 ± 2 °C for their designated storage period. To measure flavor and oxidative stability, trained sensory evaluation and TBARS was conducted. Control bacon had increased panelist scores for oxidized flavor from day 0 to 120 ($P < 0.001$). Bacon with smoked sugar had oxidized flavor scores that remained constant during frozen storage ($P > 0.936$). Panelists scored bacon manufactured with smoked sugar higher for saltiness, smoke intensity, and bacon flavor compared to control ($P < 0.001$). Control bacon displayed increased TBARS values from day 20 to 120 ($P < 0.001$). Bacon with smoked sugar exhibited static TBARS values throughout the frozen storage period ($P > 0.196$). Thus, smoked sugar is an effective antioxidant in frozen sliced, foodservice naturally cured bacon.

Keywords: frozen storage, lipid oxidation, naturally cured bacon, sensory, smoked sugar

Introduction

        Within the 2016 year, organic and natural food sales have reached an all-time high of $47 billion in sales, the meat sector was responsible for 17%, equaling $991 million in sales (Organic Trade Association, 2017). This growth in natural and organic foods are due to consumer preferences of eliminating antibiotics, pesticides, hormones, and genetic modifications. Consumer concerns are also driving processors to reduce the amount of ingredients as well as replacing ingredients with easily recognizable components in processed products, referred to as clean labeling. The definition of natural is broad and may be interpreted in many ways, the term "minimally processed" serves as the largest hurdle. The USDA (2005), Food Standards and Labeling Policy Book describes minimally processed as the traditional processes in which to make the product edible, safe for consumption, or to preserve the product. It can be challenging for processed meats such as bacon to fit into the natural category, because of requirements to exclude added nitrates or nitrites, artificial flavoring, or chemical preservatives, (9 CFR 319.2). It is also required products along with its ingredients must be minimally processed (USDA, 2005).

        Sodium nitrite is a well-known antioxidant in cured meat products. Sindelar et al. (2007) investigated five different brands of bacon for quality and sensory attributes. Bacon brands studied by Sindelar et al. (2007) consisted of one uncured bacon, one sodium nitrite cured bacon, and three naturally cured bacon brands. Sindelar et al. (2007) found sodium nitrite cured bacon exhibited the lowest TBARS values compared to all other alternatively cured bacon brands, while uncured bacon exhibited the greatest TBARS values compared to all other bacon brands. Furthermore, in consumer sensory evaluation, the uncured bacon brand had the lowest flavor scores compared to bacon brands containing nitrite.

Foodservice bacon is susceptible to lipid oxidation because of the aerobic packaging format. Inhibiting lipid oxidation in bacon is important to sustain a quality eating experience for consumers. There are many factors that contribute to lipid oxidation, the most well-known is the presence of oxygen within the packaging system (Lowe et al., 2014). Increased rates of lipid oxidation in food systems usually manifests as increased levels of off-flavors and off-odors as detected by end users. Lowe et al. (2014) demonstrated off-flavors and oxidized odors in foodservice style frozen bacon increased as frozen storage time increased; however, there is limited research of trained sensory evaluation of foodservice bacon, and even less for naturally cured bacon products. Aerobically packaged "layout" style is a very popular means of merchandising sliced bacon to foodservice establishments due to its ease of use, limited slice adhesion, and relatively low cost compared with vacuum or modified atmosphere packaging formats. Houser (2015) reported frozen foodservice layout style bacon had greater TBARS values than frozen vacuum packaged bacon, indicating increased levels of lipid oxidation for aerobically packaged treatments.

Although consumers most often think of the traditional smoking process as simply a way to add more complex flavors, hardwood smoke is a source of natural antioxidant activity. However, using only hardwood smoke limits the penetration depth of naturally occurring antioxidants found in smoke to the surface of the product. Liquid smoke produced by burning hardwood sources has been reported to contain multiple phenolic compounds that possess antioxidant activity such as syringol (Montazeri et al. 2013). Unlike traditional smoke, liquid smoke and smoke derivatives such as smoked sugar can be added directly to a curing brine moving the functional phenolic antioxidants found in hardwood smoke to the interior portions of the bacon slice. Smoked sugar qualifies for a natural label and contains an abundance of

phenolic compounds that are required for an effective antioxidant. The study conducted in chapter three indicates naturally smoked sugar is an effective antioxidant in a conventional curing brine, yet smoked sugar has not been challenged by a natural curing system to which lipid oxidation could potentially be more abundant. Therefore, the objective of this study was to determine the effectiveness of smoked sugar in a natural curing brine to prevent lipid oxidation in frozen, aerobically packaged, foodservice bacon.

Materials and Methods

Belly Selection

Twenty-four hours postmortem, fresh pork bellies (IMPS #409) were collected at a Midwest commercial processing facility and transported under refrigerated conditions (3 ± 1 °C) to the Kansas State University Meat Laboratory (Manhattan, KS, U.S.A.). Bellies were stored for three d at 2 ± 1 °C before processing. Immediately prior to processing, five bellies were trimmed and cut in half yielding an anterior and posterior belly section weighing approximately 2.8 kg each. Each half was randomly assigned to one of two treatments: a control curing brine or a curing brine with added naturally hardwood smoked sugar (RA12032, Red Arrow Products, Manitowoc, WI, U.S.A.).

Bacon Processing

Control and treatment brines were formulated for a 12% injection rate. The control natural curing brine consisted of 72.0% water, 13.4% sea salt (Morton Refined Sea Salt, Hood Packaging Corp., Monticello, AR, U.S.A.), 8.00% cane sugar (Golden Gran Evap Cane Juice, Kerry Ingredients, Beloit, WI, U.S.A.), and 6.67% celery juice (Accel 2000H, Kerry Ingredients, Beloit, WI, U.S.A.). The treatment brine contained 68.0% water, 13.4% sea salt (Hood Packaging Corp., Monticello, AR, U.S.A.), 7.00% cane sugar (Golden Gran Evap Cane Juice,

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Kerry Ingredients, Beloit, WI, U.S.A.), 6.67% celery juice (Accel 2000H, Kerry Ingredients, Beloit, WI, U.S.A.) and 5.00% smoked sugar (RA12032, Red Arrow Products, Manitowoc, WI, U.S.A.).

 Green weights were collected for each belly half, exhibited in Table 4.1. The half belly was then placed in a multineedle injector (Model N30 Wolftec Inc., Werther, Germany), and injected to 20% of green weight. All injected bellies were hung on a smokehouse truck for two hours to equilibrate prior to thermal processing. A drip weight was collected to insure 12% of brine was retained. After drip weights were collected bellies were put into a single truck smokehouse (D7752 Mauer Inc., Reichenau, Germany) for smoking/cooking. A standard thermal processing schedule was utilized and included: stage one with smokehouse setting of 57°C dry bulb and 30°C wet bulb for 30 minutes; stage two consisted of 54°C dry bulb, 44°C wet bulb, and natural smoke applied for 30 minutes; stage three was 54°C dry bulb and 35°C wet bulb for 150 minutes; stage four was 57°C dry bulb and 30°C for wet bulb for 130 minutes to reach an internal belly temperature of at least 54°C. Cooked bellies were placed into a chiller (2 ± 1°C) for 12 hrs. After cooling, chilled weights were collected to calculated cook. Each half belly was sliced 1.5 mm thick with a horizontal slicer (Model Puma 700 F, Treif, Oberlahr, Germany) from the anterior to posterior end.

 Six slices were selected randomly throughout each half belly to form a composite sample for proximate and fatty acid analysis. The remaining slices were laid out randomly on non-coated bacon sheet paper (28 cm × 43 cm, Formax, Mokena, IL, U.S.A.). Bacon slices were randomly assigned to four sensory storage periods (0, 40, 80, 120 days) or seven TBARS storage times (0, 20, 40, 60, 80, 100, or 120 days). All d 0 sensory and TBARS bacon slices were collected the day of slicing and vacuumed packaged. Once vacuumed packaged, all samples were stored in

-80°C freezer until analysis to prevent further lipid oxidation. The remaining sensory, TBARS, and GCMS sheets were stacked with day 20 on top and day 120 on the bottom, and placed in a poly-liner (clear 3 mm thick bag, Cargill, Wichita, KS, U.S.A) and a corrugated cardboard box with fitted lid (43.8 cm × 28.6 cm × 10.2 cm, Uline, Pleasant Prairie, WI, U.S.A). All boxed slices were stored aerobically at a temperature of −17.8 ± 2°C for the appropriate storage time. The experiment was replicated three times, with all replications being manufactured and cooked separately from one another.

Fat, Moisture, and Protein Analysis

Composite samples of bacon slices were cut into small pieces, frozen in liquid nitrogen, pulverized in a blender (Model 33B179, Waring Products, New Hartford, CT, U.S.A.), and stored frozen. Proximate samples were removed from the pulverized composite samples. Fat and moisture percentages were analyzed using the Association of Official Analytical Chemists (AOAC) PVM-1 (2003) method. Additionally, protein composition was determined by utilizing the AOAC 992.15 (1994) method. Fat, moisture, and protein values were presented as a percent of total composition.

Fatty Acid Analysis

Fatty acid analysis was performed with some modifications from Sukhija and Palmquist, (1988). One gram of pulverized composite bacon sample was weighed into screw-cap tubes with Teflon-lined caps for fatty acid analysis. Each bacon sample was mixed with three mL of methanolic-HCL and two mL of an internal standard consisting of two mg/mL of methyl tridecanoic acid in benzene. Tubes with bacon, methanolic-HCL and internal standard were flushed with nitrogen and capped tightly. Samples were then heated in a water bath for 120 minutes at 70 °C. After heating, two mL of benzene and three mL of K_2CO_3 were added to

samples. Samples were centrifuged for five minutes at $1,000 \times g$. The top layer of solvents and fat were removed, then placed in two mL glass vial and Teflon-lined cap was crimped tightly closed. Gas chromatography was used to analyze fatty acid composition. Fatty acid composition percentages are reported as a percentage of total fatty acids. Iodine value was determined by the following equation: C16:1(0.95) + C18:1(0.86) + C18:2(1.732) + C18:3 (2.616) + C20:1 (0.785) + C22:1(0.723), (AOAC, 1997).

Trained Sensory Evaluation

Attributes and reference sample were chosen with guidance from Gatlin et al. (2006), in addition to a descriptive panel performed by the Kansas State University Meat Science Department before training sessions. Panelists participated in eight panel training sessions to familiarize themselves with scale anchors and reference samples. Panelists evaluated bacon samples on continuous 100-point line scale for saltiness, smoke intensity, bacon flavor, oxidized flavor, and other off-flavors. Zero would indicate extremely not salty, not smoky, and bland, along with no oxidized or other off-flavors. A 100 on the continuous line scale was be extremely salty, smoky, along with intense bacon flavor, oxidized flavor, and other off-flavors. Final scale anchors consisted of the following solutions: 0.5% salt in deionized water indicated a 60 on the saltiness scale, in addition to 0.125% smoked sugar and deionized water exhibited an 80 on the smoke intensity scale. An oxidized flavor anchor was determined at a score of 100 on the scale with foodservice bacon that was packaged aerobically and stored for two years in a -29 °C freezer.

In order to reduce the variation of panelists scores, a reference bacon was chosen during the descriptive panel, similar to Gatlin et al. (2006). A commonly available commercial brand of bacon was used as the reference sample due to the inclusion of liquid smoke as an ingredient and similarities to the research bacon. The reference bacon had the following sensory values:

saltiness (40-50), smoke intensity (30-40), bacon flavor intensity (40-55), and no oxidized or other off-flavors detected.

After all frozen storage times (0, 40, 80, 120 day) were completed and collected, all bacon samples from the same belly were randomly assigned to a trained sensory panel. This random sample assignment allowed for the control and naturally smoked sugar treatment to be evaluated in one panel. Sensory samples were placed on wire cooking racks in a Blodgett dual-flow, forced-air oven (DFD-201, G.S. Blodgett Co., Inc., Burlington, VT, U.S.A.) to cook at 176.7 °C for six minutes while rotating the pans 180° halfway through the cooking process. After cooking, slices were blotted with paper towels to remove excess grease as described by Lowe et al. (2014). At least eight panelists were used for each panel of sensory evaluation. Panelists were stationed in individual booths under a combination of red and green light. Apples, crackers, and water were provided to cleanse the palates between samples (Olson et al., 1985). The salt and smoke solutions were given before warm-up samples were presented for reference. The reference bacon was offered as a warm-up sample prior to every sensory panel. After the warm-up sample and discussion, samples from the same belly (control and naturally smoked sugar treatment) at all frozen storage times were evaluated in random order by the trained panelists.

Thiobarbituric Acid Reactive Substances

Lipid oxidation was determined by a distillation method of TBARS as described by Sebranek et al. (2001). Eight slices per storage time were pulverized as described in the fat, moisture, and protein analysis section. Ten grams of pulverized sample mixed with deionized water (97 mL), one mL of sulfanilamide (0.5% sulfanilamide, 20% HCL, and deionized water), two mL of HCL (50% HCL and deionized water), and five drops of antifoam in a round bottom

flask. The round sample mixture was heated to boil and condensation from the boiling mixture was collected and cooled through a glass condenser, until 50 milliliters of distillate was reached. Five milliliters of distillate were combined with thiobarbituric acid (TBA; 0.28% and deionized water) in a 15-milliliter plastic tube and capped tightly; this step was repeated to create duplicates. Distillate and TBA tubes were placed in a boiling water bath for 35 minutes, then transferred to cold water bath for 10 minutes. Once cooled, two milliliters of distillate and TBA mixture was transferred into a spectrophotometer cuvette and absorbance was measured on a spectrophotometer (Eon, BioTek Instruments, Winooski, VT, U.S.A.) at 532 nm, compared to a blank cuvette containing one milliliter deionized water and one milliliter TBA reagent. Thiobarbituric acid reactive substances are expressed as milligrams of malonaldehyde per kg of sample.

Statistical Analysis

Data was analyzed using the PROC MIXED procedure of SAS Version 9.3 (SAS Institute Inc., Cary, NC, U.S.A.). Proximate analysis and fatty acid composition utilized an incomplete block design with fixed effects of belly half and treatment with random effects of replication and belly number. The TBARS and sensory analysis were analyzed as a split plot with repeated measures, whole plot factor treatment with the sub-plot factor of storage day. Fixed effects included Treatment, Day, and interaction, while random effects included Replication and Belly within Replication. The covariance structure for the repeated measures was heterogeneous compound symmetry. A paired T-test was utilized to separate mean differences between treatments with a Tukey-Kramer adjustment for all pairwise comparisons and the denominator degrees of freedom were adjusted with Kenward-Roger adjustment. Means were determined to be significantly different at an α-level of $P < 0.05$.

Results

Fat, Moisture, and Protein Analysis

There were no Belly Half × Treatment interactions or Treatment main effect for fat, moisture, or protein composition ($P > 0.16$). Table 4.2 contains the mean values for anterior and posterior belly sections. Anterior belly sections had a greater percentage of fat, less moisture, and contained less protein ($P < 0.001$) compared to posterior belly sections.

Fatty Acid Analysis

There were no Belly half × Treatment interactions or Treatment main effect for all individual fatty acids found within anterior and posterior belly sections ($P > 0.06$). The mean percentages of individual fatty acids are represented in Table 4.3. Anterior belly sections had greater percentages of Oleic Acid (C18:1n9c), Linolenic Acid (C18:2n6t), as well as a higher calculated iodine value than the posterior counterparts ($P < 0.03$). Posterior belly sections contained greater percentages of Lauric Acid (C12:0) and Arachidonic Acid (C20:4n6) than the anterior belly sections ($P < 0.04$). There were no differences between anterior and posterior belly sections among remaining fatty acids ($P > 0.06$).

Trained Sensory Evaluation

Mean values for oxidized flavor intensity for the control treatment and smoked sugar treatment are presented in Figure 4.1. A Treatment × Day interaction was found for oxidized flavor intensity ($P < 0.001$). Control and smoked sugar treatment were no different on day 0 of frozen storage for oxidized flavor intensity ($P = 0.92$). Days 40, 80, and 120 of frozen storage control bacon was scored higher by trained panelists for oxidized flavor intensity compared to naturally cured bacon processed with smoked sugar ($P < 0.001$).

There were no Treatment × Day interactions for panelist scores of saltiness, smoke intensity, bacon flavor intensity, and other off-flavors ($P > 0.05$). Treatment means for panelist scores including the attributes of saltiness, smoke intensity, bacon flavor intensity, and other off-flavors are presented in Figure 4.2. Trained panelists scored bacon samples manufactured with smoked sugar higher ($P < 0.001$) for saltiness, smoke intensity, as well as bacon flavor intensity compared with control samples. There was no difference ($P = 0.77$) in the intensity of other off-flavors between the control and bacon formulated with smoked sugar. Lastly, there was a main effect of Day in trained panelist scores for bacon flavor intensity ($P = 0.005$; data not shown). For all bacon samples, day 40 had the highest ($P = 0.01$) trained panelist scores for bacon flavor intensity, while day 120 exhibited the lowest ($P = 0.03$) trained panelist scores.

Thiobarbituric Acid Reactive Substances

Control and smoked sugar treatment mean values for TBARS are reported in Figure 4.3. There was a Treatment × Day interaction found for TBARS values in samples formulated without and with smoked sugar ($P < 0.001$). Naturally cured control and smoked sugar treatment bacon displayed no difference in TBARS values on day 0 and 20 of frozen storage ($P > 0.34$). Whereas, the naturally cured control bacon had increased TBARS values compared to the smoked sugar treatment from day 40 to 120 of frozen storage ($P < 0.0001$).

Discussion

The purpose of fatty acid analysis was to determine the quality of the raw bellies selected to challenge smoked sugar as an antioxidant in this study. Houser (2015) evaluated raw belly quality by separating raw bellies into three iodine value categories (High = 77.9; Intermediate = 72.6; Low = 66.5). With the mean iodine values of 68.06 for anterior belly sections and 64.59 for the posterior belly sections, the bellies selected for this study could be considered as high quality

raw bellies. Additionally, the fatty acid percentages are comparable to the percentages found in the Low category of iodine value by Houser (2015).

This is among the first studies to demonstrate that a high fat naturally cured product will oxidize during frozen storage. Although, Sindelar et al. (2007) discovered that naturally cured bacon will oxidize similar to bacon cured with sodium nitrite. The lack of evidence that illustrates uncured products oxidizing in frozen storage may be due to the absence of oxygen in the environment. Sindelar et al. (2007) evaluated bacon under vacuum packaging, which would limit lipid oxidation over time.

When meat products oxidize, volatile products are created, which result in off-flavors perceived by consumers. Trained sensory evaluation showed increased oxidized flavor intensity scores for naturally cured bacon with no added antioxidant throughout frozen storage and confirms results from Lowe et al. (2014). Foodservice bacon stored in an aerobic frozen (-33 °C) environment had increased panelist scores for off-flavor and off-odor. The increases of off-flavors and oxidized flavor intensity in foodservice bacon are indicative of lipid oxidation during aerobic frozen storage. The challenge for foodservice bacon is the exposure to oxygen for long storage periods, allowing pro-oxidants to form volatile compounds. Lowe et al. (2014) showed that these volatile compounds are produced at colder temperatures (-33 °C), supporting that aerobic storage is a desirable environment for lipid oxidation. Thus, controlling lipid oxidation during extended aerobic frozen storage for naturally cured foodservice bacon is essential to preventing the creation of off-flavors.

It is well known that aerobically stored, frozen meat products will oxidize over time. Lipid oxidation was seen in naturally cured bacon by increased TBARS levels during aerobic frozen storage, these data agree with Sebranek et al. (2005) and Houser (2015). Sebranek et al.

(2005) reported increased TBARS levels for raw frozen pork sausage formulated without an antioxidant throughout 112 days of storage. Houser (2015) investigated sodium nitrite cured layout style bacon that was stored aerobically and frozen for 154 days. Layout style bacon with no added antioxidants exhibited increased TBARS values after day 28 of frozen storage. Increased TBARS levels during frozen storage as reported by Sebranek et al., (2005) and Houser (2015), as well as, this study confirm that naturally cured bacon exposed to extended aerobic frozen storage will produce lipid oxidation products. Therefore, the need to include an antioxidant is necessary to maintain oxidative stability in naturally cured foodservice bacon.

Early studies evaluating TBARS and sensory analyses suggest that a TBARS value of 1.0 for cured meat is highly correlated to off-flavors (Tarladgis, 1959; Younathan and Watts 1959). However, evaluating lipid oxidation in bacon from Houser (2015), Lowe et al. (2014), as well as this study show no consistent day where TBARS values reach a value of 1.0. Therefore, further sensory research needs to be conducted to determine the appropriate day of frozen storage and TBARS value that would result in unacceptable oxidized foodservice bacon.

The incorporation of smoked sugar in the brine formulation inhibited the presence of oxidized flavors indicating that lipid oxidation was prevented during extended frozen storage. There have been multiple studies on the phenol composition of liquid smokes (Knowles et al., 1975; Simon et al., 2005; Montazeri et al., 2013) as well as the antioxidant potential of smoke products (Wendorff, 1981). However, there have been few studies that examine smoke products as an antioxidant with trained sensory evaluation at limiting oxidized flavor development especially in naturally cured bacon. The inclusion of phenolic antioxidants into meat products has been shown to inhibit the formation of oxidative products which ultimately result in off-flavors. Sebranek et al. (2005) reported that cooked pork sausage stored frozen and formulated

with an antioxidant had lower trained sensory scores for warmed-over flavor. Our data would agree with previous authors and support that smoked sugar can limit the development of oxidative products in naturally cured frozen bacon.

The trained sensory data show that the inclusion of naturally smoked sugar into the brine formulation served as an effective antioxidant without hindering other flavor attributes commonly found in bacon. Saltiness, smoke intensity, and bacon flavor were greater for bacon processed with naturally smoked sugar compared with control bacon. This is not surprising as smoke and smoke derived products are utilized in the meat industry to enhance the flavor of meat products. All components of smoke including cellulose, hemicellulose, and lignin contribute to smoke flavor; however, phenols are responsible for the notable pungent flavor of smoke (Rozum, 2009). Other phenolic descriptors include sharp, dry or charred wood, and sweet/ fruity (Rozum, 2009) flavors. Further, these results indicate that the addition of smoked sugar to a curing brine may present additional benefits to flavor development in sliced naturally cured bacon.

Finally, bacon flavor decreased slightly for all naturally cured bacon samples during frozen storage. These results may not reveal a large enough difference for consumers to detect, yet the concept of decreasing bacon flavor over time should be further explored for naturally cured foodservice bacon. It is known that nitrite is an antioxidant and maintains cured meat flavor throughout storage. Yun et al. (1987) discovered that nitrite cured, and naturally cured pork sausages had differences in flavor acceptability scores from sensory evaluation. Naturally cured pork sausages had lower acceptability scores than nitrite cured sausage. Flavor stability of cured meat products is dependent on multiple factors, storage conditions and ingoing nitrite levels. Natural curing systems may have varying levels of ingoing nitrite due to the sources of nitrite or nitrate (Sindelar et al., 2007).

The antioxidant ability of smoked sugar to effectively inhibit lipid oxidation was shown by lower TBARS values and oxidized flavor intensity scores compared to the control bacon. Phenolic compounds found in smoked sugar are the active components of antioxidants that scavenge for free radicals that limit the rate and extent of lipid oxidation. Concentrations of phenolic compounds have been quantified as the most abundant portion of smoke and liquid smoke products (Guillén and Manzanos, 1999; and Montazeri et al., 2013). The use of phenolic antioxidant compounds in raw frozen pork sausage exhibited lower TBARS values than pork sausage formulated without added phenolic antioxidants (Sebrank et al., 2005). In agreeance with TBARS values of smoked sugar treated bacon, precooked beef patties treated with liquid smoke had decreased TBARS values when compared to patties that did not include liquid smoke throughout 90 days of frozen storage (Estrada-Muñoz et al., 1998). Estrada-Muñoz et al. (1998) confirm that liquid smoke products contain sufficient phenolic compounds to act as an effective antioxidant in cooked meat products.

Conclusion

The addition of smoked sugar into naturally cured bacon formulations successfully inhibited lipid oxidation stored aerobically and subjected to extended periods of frozen storage. The ability of smoked sugar to function as an antioxidant in sliced bacon was confirmed with both subjective sensory evaluations along with objective measurements of lipid degradation products. Also, aerobically packaged frozen naturally cured bacon formulated without smoked sugar had significant challenges in lipid stability, as well as flavor stability as frozen storage length increased. Meat processors manufacturing sliced, naturally cured bacon intended for aerobic, frozen storage should seriously consider the use of technologies aimed at improving

lipid stability in their products or risk decreased purchasing of bacon products by consumers at foodservice establishments.

Tables and Figures

Table 4.1 Bacon Processing Parameters

	Brine Characteristics	
	Control	Treatment
Green Weight (kg)	2.77	2.84
Percent Pump (%)	13.2	12.7
Cook Loss (%)	2.56	1.12
Brine pH	8.08	4.48

[1] Cook Loss = [(Chilled Weight – Green Weight) / Green Weight] $\times$ 100

Table 4.2 Proximate composition of anterior and posterior half bellies

Proximate Component (%)	Belly Half			
	Anterior	Posterior	SEM	*P*-value
Fat	40.4^a	30.6^b	1.42	$P < 0.0001$
Moisture	41.3^b	49.7^a	1.32	$P < 0.0001$
Protein	12.6^b	15.5^a	0.539	$P = 0.0001$

[a,b] Means with different superscripts within the same row are different ($P < 0.05$)

Table 4.3 Fatty acid composition of anterior and posterior half bellies

Fatty Acid (%)	Belly Half		SEM	*P*-value
	Anterior	Posterior		
Capric acid (C10:0)	0.27	0.46	0.10	*P* = 0.10
Lauric acid (C12:0)	0.12[b]	0.23[a]	0.04	*P* = 0.04
Myristic acid (C14:0)	0.77	0.80	0.11	*P* = 0.86
Pentadecylic acid (C15:0)	0.42	0.52	0.12	*P* = 0.37
Palmitic acid (C16:0)	15.91	15.16	0.59	*P* = 0.39
Palmitoleic acid (C16:1)	1.21	1.30	0.22	*P* = 0.75
Margaric acid (C17:0)	1.58	2.05	0.38	*P* = 0.30
Heptadecenoic acid (C17:1)	0.18	0.27	0.07	*P* = 0.28
Stearic acid (C18:0)	12.20	11.49	0.52	*P* = 0.19
Oleic acid (C18:1n9c)	37.66[a]	34.39[b]	1.32	*P* = 0.02
Vaccenic acid (C18:1n7)	0.73	0.58	0.33	*P* = 0.72
Linoleic acid (C18:2n6t)	0.06	0.02	0.04	*P* = 0.30
Linoleic acid (C18:2n6c)	18.54[a]	17.06[b]	0.40	*P* < 0.01
α-Linolenic acid (C18:3n3)	0.30	1.03	0.92	*P* = 0.45
Gadoleic acid (C20:1)	0.55	0.56	0.08	*P* = 0.95
Eicosadienoic acid (C20:2)	0.52	1.79	1.42	*P* = 0.38
Arachidonic acid (C20:4n6)	1.12[b]	2.08[a]	0.33	*P* = 0.03
Total SFA[1]	31.49	30.50	1.35	*P* = 0.73
Total MUFA[2]	40.54	37.27	1.47	*P* = 0.07
Total PUFA[3]	20.81	22.33	2.41	*P* = 0.54
Iodine Value, (g/100g)[4]	68.06[a]	64.59[b]	1.17	*P* = 0.04

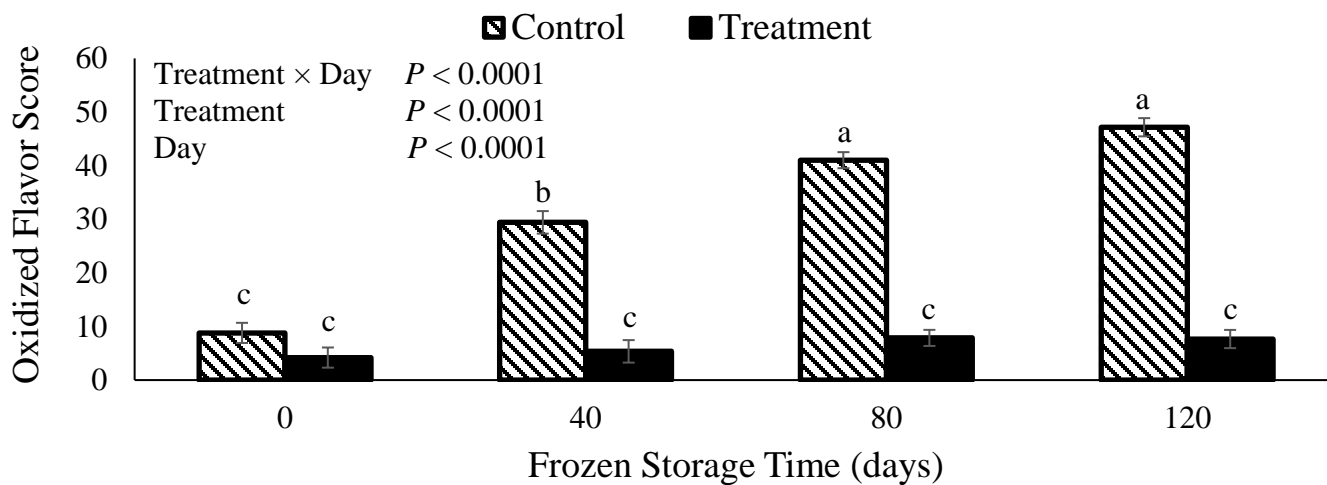[a,b] Means with different superscripts within the same row are different ($P < 0.05$).

[1] Total saturated fatty acids = {(C10:0) + (C12:0) + (C14:0) + (C15:0) + (C16:0) + (C17:0) + (C18:0) + (C20:0) + (C22:0)} where the parentheses indicate concentration.

[2] Total monounsaturated fatty acids = {(C14:1) + (C16:1) + (C17:1) + (C18:1n9c) + (C18:1n7) + (C20:1) + (C22:1n9)} where the parentheses indicate concentration.

[3] Total polyunsaturated fatty acids = {(C18:2n6t) + (C18:2n6c) + (C18:3n6) + (C18:3n3) + (C20:2) + (C20:3n3) + (C20:4n6)} where the parentheses indicate concentration.

[4] Calculated as IV = {(C16:1) × 0.95 + (C18:1) × 0.86 + (C18:2) × 1.732 + (C18:3) × 2.616 + (C20:1) × 0.785 + (C22:1) × 0.723} where the parentheses indicate concentration, (AOCS, 1998).

Figure 4.1 Mean panelist scores for oxidized[2] flavor intensity for control[3] and treatment[4] foodservice bacon stored frozen for 0-120 days
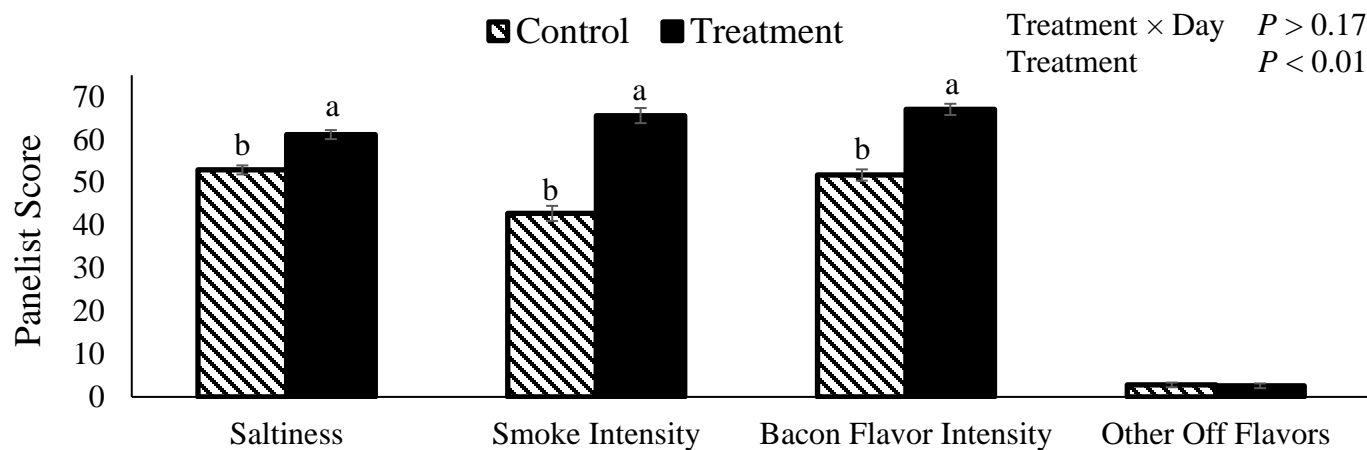

[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment × Day was significant at ($P < 0.05$).

[2] Product of lipid oxidation; Evaluated on a continuous line scale; 0 = no oxidized flavor and 100 = extremely intense oxidized flavor.

[3] Control Bacon = no smoked sugar added

[4] Treatment Bacon = smoked sugar added

Figure 4.2 Mean panelist scores for saltiness[2], smoke intensity[3], bacon flavor intensity[4], and other off-flavors[5] for control[6] and treatment[7] foodservice bacon

[1] Means within the same attribute with different superscripts are significantly different ($P < 0.05$).
[2] Evaluated on a continuous line scale; 0 = extremely not salty and 100 = extremely salty.
[3] Evaluated on a continuous line scale; 0 = extremely not smoky and 100 = extremely smoky.
[4] Evaluated on a continuous line scale; 0 = extremely bland and 100 = extremely intense.
[5] Evaluated on a continuous line scale; 0 = none present and 100 = extremely intense.
[6] Control Bacon = no smoked sugar added
[7] Treatment Bacon = smoked sugar added

Figure 4.3 Mean thiobarbituric acid reactive substances (TBARS)[2] values for control[3] and treatment[4] foodservice bacon stored frozen for 120 days
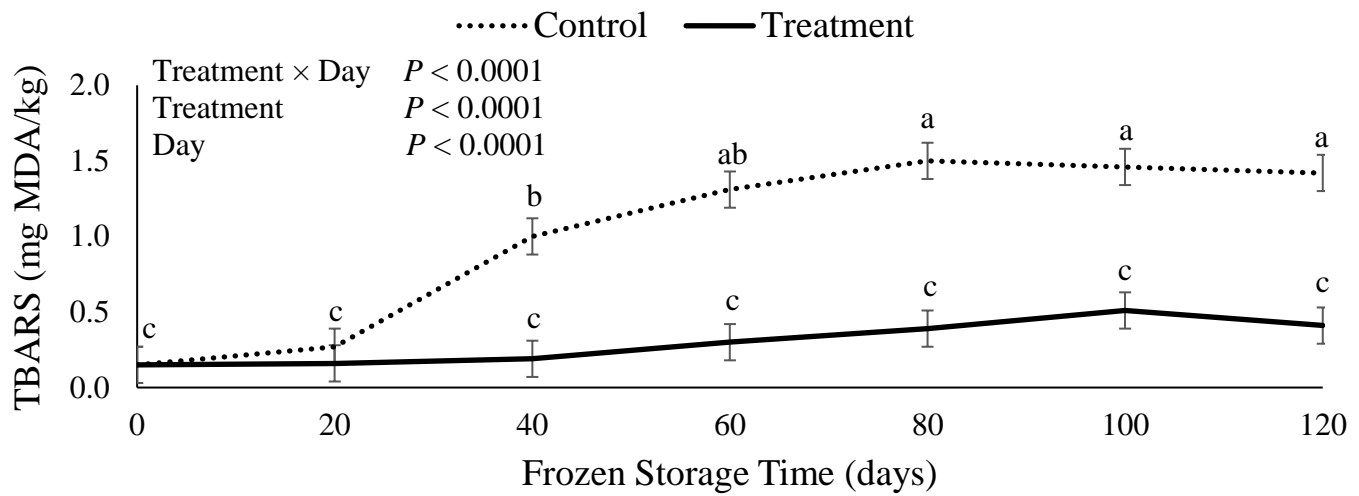
[1] Means with different superscripts are significantly different ($P < 0.05$). The interaction of Treatment × Day was significant at ($P < 0.05$).
[2] Product of lipid oxidation
[3] Control Bacon = no smoked sugar added
[4] Treatment Bacon = smoked sugar added

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Chapter 5 - Appendix

SAS Code for Proximate Analysis

```
PROC IMPORT OUT= WORK.PROX
            DATAFILE= "C:\Users\awhobson\Desktop\SAS LS Nit
PROX.xlsx"
            DBMS=xlsx REPLACE;
          Range="Sheet1$A1:H31";
          GETNAMES=Yes;
Run;

Title "Analysis of Fat Using Unequal Variances for Half
Bellies";
proc mixed data=prox method=REML covtest plots=none;
  class Rep BN Half Trt;
  model F=Half|trt/ddfm=KR;
  random rep;
  repeated /subject=bn(rep) type=CSH;
  lsmeans half trt/pdiff adjust=Tukey;
  lsmeans half*trt;
run;

Title "Analysis of Moisture Using Unequal Variances for Half
Bellies";
proc mixed data=prox method=REML covtest plots=none;
  class Rep BN Half Trt;
  model M=Half|trt/ddfm=KR;
  random rep;
  repeated /subject=bn(rep) type=CSH;
  lsmeans half trt/pdiff adjust=Tukey;
  lsmeans half*trt;
run;

Title "Analysis of Protein Using Unequal Variances for Half
Bellies";
proc mixed data=prox method=REML covtest plots=none;
  class Rep BN Half Trt;
  model P=Half|trt/ddfm=KR;
  random rep;
  repeated /subject=bn(rep) type=CSH;
  lsmeans half trt/pdiff adjust=Tukey;
  lsmeans half*trt;
run;
```

SAS Code for Fatty Acid Analysis

```
PROC IMPORT OUT= WORK.FA
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FA.xlsx"
           DBMS=xlsx REPLACE;
        Range="Sheet1$A1:H31";
        GETNAMES=Yes;
Run;

Title "Analysis of C221n9 Using Unequal Variences";
proc mixed data=FA method=REML covtest maxiter=99 plots=none;
  class Rep BN Half Trt;
  model C221n9=Half|trt/ddfm=KR;
  random rep;
  repeated /subject=bn(rep) type=CSH;
  lsmeans half trt/pdiff adjust=Tukey;
  lsmeans half*trt;
run;
```

SAS Code for TBARS Analysis

```
PROC IMPORT OUT= WORK.TBARS
          DATAFILE= "C:\Users\awhobson\Desktop\SAS LS Nit
TBARS.xlsx"
           DBMS=xlsx REPLACE;
        Range="Sheet1$A1:H31";
        GETNAMES=Yes;
Run;



Title "Analysis of TBARS as Repeated Measures";
proc mixed data=Tbars method=REML covtest plots=none;
  class Rep BN Trt Day;
  model TBAR=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=CSH;
  lsmeans trt day;
  lsmeans trt*day/pdiff slice=day adjust=tukey;
run;
```

SAS Code for GCMS Analysis

```
PROC IMPORT OUT= WORK.GC
```

```
              DATAFILE= "C:\Users\vahl\OneDrive - Kansas State
University\KSU Consulting\Allison Hobson\Bacon\SAS LS Nit
GC.xlsx"
              DBMS=xlsx REPLACE;
                 Range="Sheet1$A1:M73";
                 GETNAMES=Yes;
RUN;

data gc; set gc;
  loghex=log(hex);
  log2chl=log(_2chl);
  lognon=log(non);
  logcre=log(cre);
  logsyr=log(syr);
run;

Title "Analysis of Log HEX as Repeated Measures";
proc mixed data=GC method=REML covtest plots=none;
  class Rep BN Trt Day;
  model loghex=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=UN;
  lsmeans trt*day/pdiff slice=day adjust=tukey;
  ods output lsmeans=hex_lsm;
run;

data hex_lsm; set hex_lsm;
  mean=exp(estimate);
  se_mean=stderr*mean;
run;

proc print data=hex_lsm;
run;

Title "Analysis of Log Cre as Repeated Measures";
proc mixed data=GC method=REML covtest plots=none;
  class Rep BN Trt Day;
  model logcre=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=cs;
  lsmeans trt trt*day/pdiff slice=day adjust=tukey;
  ods output lsmeans=cre_lsm;
run;

data cre_lsm; set cre_lsm;
  mean=exp(estimate);
  se_mean=stderr*mean;
```

```
run;

proc print data=cre_lsm;
run;
```

SAS Code for Sensory Analysis

```
PROC IMPORT OUT= WORK.TBARS
            DATAFILE= "C:\Users\awhobson\Desktop\SAS LS Nit
Sensory.xlsx"
            DBMS=xlsx REPLACE;
          Range="Sheet1$A1:H31";
          GETNAMES=Yes;
Run;



Title "Analysis of Salt as Repeated Measures";
proc mixed data=Sensory method=REML covtest plots=none;
  class Rep BN Trt Day;
  model SA=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=csh;
  lsmeans trt/pdiff adjust=tukey;
  lsmeans day trt*day;
run;

Title "Analysis of Smoke as Repeated Measures";
proc mixed data=Sensory method=REML covtest plots=none;
  class Rep BN Trt Day;
  model SM=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=csh;
  lsmeans trt/pdiff adjust=tukey;
  lsmeans day trt*day;
run;

Title "Analysis of Bacon Flavor as Repeated Measures";
proc mixed data=Sensory method=REML covtest plots=none;
  class Rep BN Trt Day;
  model BF=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=csh;
  lsmeans trt/pdiff adjust=tukey;
  lsmeans day trt*day;
run;

Title "Analysis of Oxidation as Repeated Measures";
```

```
proc mixed data=Sensory method=REML covtest plots=none;
  class Rep BN Trt Day;
  model OX=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=csh;
  lsmeans trt/pdiff adjust=tukey;
  lsmeans day;
  lsmeans trt*day/pdiff slice=day;
run;

Title "Analysis of Off Flavor as Repeated Measures";
proc mixed data=Sensory method=REML covtest plots=none;
  class Rep BN Trt Day;
  model OF=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=csh;
  lsmeans trt/pdiff adjust=tukey;
  lsmeans day;
  lsmeans trt*day/pdiff slice=day;
run;
```

SAS Code for Microbial Analysis

```
PROC IMPORT OUT= WORK.Micro
          DATAFILE= "C:\Users\awhobson\Desktop\SAS LS Nit
MICRO.xlsx"
          DBMS=xlsx REPLACE;
        Range="Sheet1$A1:I151";
        GETNAMES=Yes;
Run;
Title "Analysis of APC as Repeated Measures";
proc mixed data=MICRO method=REML covtest plots=none;
  class Rep BN Trt Day;
  model APC=trt|day/ddfm=KR;
  random rep bn(rep);
  repeated Day/subject=bn*trt(rep) type=CSH;
  lsmeans day trt trt*day/pdiff slice=day adjust=tukey;
run;
```

Chromatogram for naturally smoked sugar with all phenolic compounds

File   : C:\HPCHEM\1\DATA\22618.D
Operator   : Faris
Acquired   : 26 Feb 2018 10:30   using AcqMethod SCOUTING
Instrument :   CSS Instr
Sample Name: Smoke sample 1:10 dilution
Misc Info : Scan, 5 min in the injector
Vial Number: 1