8-2023

Investigation of Consumer Freezing Practices, Condition and Duration on Palatability of Beef

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Investigation of Consumer Freezing Practices, Condition and Duration on Palatability of Beef

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

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Oklahoma State University
Bachelor of Science in Food Science, 2021

August 2023
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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Abstract

The objective of these studies was to determine: the impact of extended periods of frozen storage duration and packaging type on palatability traits of cooked beef steaks from two muscles and the impact of freezing type and storage duration on objective and subjective measures of ground beef palatability traits.

In study 1, no differences were elicited from any of the possible interactions (P= 0.95) for SSF or CL: package × muscle × freezing duration, package × muscle, package × duration or muscle × duration. However, freezing duration did impact both SSF ($P < 0.01$) and CL ($P < 0.001$). Generally, for both SSF and CL steaks that were frozen for 9-months elicited both the highest SSF values as well as the greatest percentage of CL among all treatments. In comparison, EM was impacted by the interaction ($P = 0.059$) of packaging type × muscle × freezing duration. Samples from OW GM stored for 9-months elicited the highest percentage of EM while fresh OW LL samples resulted in the lowest. Additionally, expressible moisture was impacted by the interaction ($P = 0.047$) of muscle × freezing duration. Among all treatments, GM steaks that were frozen for 9-months elicited the highest EM values and fresh LL samples the lowest EM values. Contrastingly, there was no significant difference observed in expressible moisture for the interaction ($P \geq 0.18$) of package type × freezing duration or for the interaction ($P = 0.70$) of package type × muscle. Generally, fresh OW GM steaks resulted in the greatest concentration of lipid derived volatile compounds such as aldehydes, hydrocarbons and alcohols which contrasted sensory ratings of trained panelists that indicated oxidized and refrigerator-stale ratings increased as storage time increased for OW steaks.

In study 2, The interaction of freezer treatment × storage duration impacted gumminess ($P = 0.05$), a TPA attribute. In greater detail, samples stored in RF for 6-months resulted in the

greatest gumminess values ($P < 0.001$), while those stored in the CF for 12-months elicited the lowest ($P < 0.001$). Similarly, flavor development was also impacted by the interaction ($P = 0.05$) of freezer treatment × storage duration. Three lipid derived compounds were of greatest concentration among RF patties stored for 1-month. In contrast, the interaction of freezer treatment × storage duration elicited no impact on consumer ratings, SF or TBARS. Nonetheless, frozen storage duration impacted TPA, flavor development, consumer ratings, SF and TBARS as a main effect ($P < 0.05$), especially in regard to tenderness and juiciness.

Moreover, beef flavor development, tenderness and juiciness are impacted by freezing duration, muscle, freezer type and packaging, however these factors are not necessarily independent of one another. Furthermore, the retail display period is a critical period for fresh beef steaks, especially when packaged in aerobic conditions. For optimal eating experience, beef products should be stored for extended periods in a vacuum packaging, in a designated freezer that is not opened frequently to allow for optimal air flow regulation and minimize freezer burn to improve tenderness and juiciness.

Acknowledgments

I am immensely grateful for the two years I have had the opportunity to spend at the University of Arkansas. My time here has been instrumental in my growth as a scientist, student and overall person. I would like to thank each and every individual who has helped make the years in Fayetteville all that they have been, but I would like to especially thank my major advisor and incredible mentor, Dr. Kelly Vierck. Your mentorship has without question, helped deepen my passion for research while also shaping me into a successful meat scientist. Your devotion to research and teaching has challenged me to think critically while also building my confidence and giving me the power to believe in myself as a young researcher. I look forward to your continuous mentorship, friendship and future collaborations. I will forever be thankful to have had you as my master's advisor.

I would also like to extend my sincere thankfulness to Dr. Yancey and Dr. Setyabrata who not only served as members of my committee but have also been instrumental in my journey at Arkansas. Each of you were there to answer questions, assist in the lab when needed and provide a fresh perspective. Thank you for always encouraging me and believing in me, I could not have made it to this point without the support from each of you.

Additionally, I have a great amount of gratitude for each of the graduate and undergraduate students that I have had the opportunity to work with. From the long days in the lab, at the plant or the abattoir I am thankful for the graduate students who were there alongside of me proving help, support and many laughs. To the undergraduates who took a chance by joining the quiz bowl team, thank you. Thank you for taking a chance, your unwavering dedication and willingness to learn. Thank you for the opportunity to not only serve as your

coach, but to also mentor you in the lab – you each have truly inspired me to pursue my PhD in hopes of establishing a career in academia.

Finally, I would be remiss not to express my gratitude for the greatest pieces of my foundation, that being my parents, George and Cindy, and siblings, Mary Grace, Luci and Rollins. The past year of our lives has not been easy, and it's come with many questions and uncertainties however, the support and encouragement from you all remained resilient. From Stillwater, OK to Fayetteville, AR my parents have always encouraged me to chase my dreams, however far they may take me. Thank you for giving my wings and teaching me to fly – I am who I am because of all that you taught me to be.

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Chapter 1

Review of Literature

Meat Palatability

The summation of tenderness, juiciness and flavor combines to describe the overall eating experience associated with food products and is referred to as palatability (Platter et al., 2003; O'Quinn et al., 2018; Vierck et al., 2020). In determining the individual input of each factor to the overall palatability, O'Quinn et al. (2018) reported that flavor accounted for 49.4% of the overall eating experience, tenderness for 43.4% and juiciness for 7.4%. It was further discovered that the consumer eating experience is driven by all three factors independently as well as the interaction between traits (O'Quinn et al., 2018). As the attributes interact, a beef cut that excels consumer expectations in one or more areas may still not satisfy the consumer due to the dissatisfaction associated with another trait (O'Quinn et al., 2018). Comparatively, a product could be considered satisfactory by consumers as a result of the exceptional quality of a single palatability trait, despite unsatisfactory quality of any of the additional traits (O'Quinn et al., 2018). Thus, purchasing decisions made by consumers at the retail level are partly driven by palatability (Clarborn et al. 2011; Wilfong et al., 2016). Despite consumer demands, the ability for the supply chain to provide consistent and high-quality beef remains difficult (Hocquette et al., 2020).

Among palatability traits, research has historically denoted tenderness as the most important factor of palatability. This came as a result of multiple National Beef Quality Audits in which tenderness was identified as the challenge of greatest importance regarding the beef industry. (Savell et al., 1999; Egan et al. 2001; O'Quinn et al., 2018). Based on reports from the

2016 Beef Tenderness Survey, major tenderness improvements resulted from the greater emphasis placed on the further research and advancement of beef tenderness (Savell et al., 2016; O'Quinn et al., 2018). Additionally, 49 – 55% of consumers more recently identified flavor as the most important palatability trait which is compared to the 36 – 40% that indicated tenderness as the most important factor (Chail et al., 2017; McKillip et al., 2017; O'Quinn et al., 2018; Ponce 2019; Vierck 2020). This is coupled with findings that further indicate a strong correlation between flavor and overall consumer liking (O'Quinn et al., 2012; Legako et al., 2015). As a result, the improvement of beef tenderness has allowed for further investigation into flavor as the largest factor impacting palatability (Lucherk et al., 2016; O'Quinn et al., 2018; Vierck et al., 2020).

Meat palatability is often measured through sensory evaluation with trained or consumer panelists, or through instrumental measurements (Hocquette et al., 2020). The individual palatability factors are further influenced by a variety of production and post-mortem practices that impact muscle biochemical characteristics and eating quality (Hocquette et al., 2020).

Muscle Types

Skeletal muscle tissues are a system comprised mainly of muscle fiber, connective tissues and fat (Listrat et al., 2016). Muscle tissues are classified into three types; striated, smooth and cardiac. Skeletal muscle is a form of striated muscle that both supports the body cavity and generates voluntary movement (Listrat et al., 2016). Primarily, skeletal muscles are divided among two functional groups: support and locomotion (Bailey, 1972). The differences in anatomical location and physiological function yield a specific muscle-fiber configuration and in turn, a specified metabolic pathway (Hunt and Hendrick, 1977; Kirchofer et al., 2002; Suman et

al., 2014). The contractile and metabolic characteristics of the muscle-fiber configuration further correlates to aspects of meat quality (Listrat et al., 2016).

Previous research has found that meat quality is impacted by the muscle fiber – type composition (Cassens and Cooper, 1971; Ashmore, 1974; Seideman and Theer, 1986; Kirchofer et al., 2002). In comparison, each of the contractile fiber types undergo ATPase activity that corresponds to contraction speed; slow (type I) and fast (types IIa, IIx and IIb) (Listrat et al., 2016). For muscle contraction to occur, energy is required in the form of ATP however the amount of energy necessary is variable among muscle fiber types (Listrat et al., 2016). An increased amount of Type-II fibers correlates to skeletal muscle tissue that has more connective tissue and less marbling and is in turn less tender than those muscles that have increased Type-I fibers, largely due to differences in muscle function (Melton et al., 1974, 1975; Calkins et al., 1981; Kirchofer et al., 2002). Myosin heavy-chain isoforms identified within the thick filament drive contractile properties and are frequently described as type I, IIa, IIx and IIb (Listrat et al., 2016). More specifically, type I fibers are considered the marathon fibers, more resistant to fatigue due to low-intensity contractions (Listrat et al., 2016). Additionally, type 1 fiber represent ß- red fibers that are more susceptible to oxidative metabolism as a result of a higher lipid concentration (Cassens and Cooper, 1971; Listrat et al., 2016). Furthermore, ATP regeneration occurs in the muscle through two major pathways: oxidative (aerobic) and glycolytic (anaerobic) (Listrat et al., 2016). Because of difference in the oxidative pathway between fiber types the fibers elicit different fiber colors (Listrat et al., 2016). Oxidative metabolic fibers are red due to the myoglobin concentration, which is the pigment responsible for red color that carries oxygen bound to the sixth ligand (Listrat et al., 2016). In comparison, glycolytic fibers are generally white due to limited oxidative activity and thus less myoglobin (Listrat et al., 2016).

Muscle type can also influence observed differences in color stability as product with a greater number of oxidative fibers inherently possess a higher concentration of mitochondria within the muscle (Hood, 1980; Kirchofer et al., 2002). The intact mitochondria rival myoglobin for the intake of oxygen which alters the oxymyoglobin layer depth resulting in a dark surface color on the muscle (Kirchofer et al., 2002). Oxidative muscles are typically categorized as color labile, while glycolytic muscles are considered color stable, especially during periods of retail display (McKenna et al., 2005). In color labile muscles, oxygen consumption is increased due to a greater depth of oxygen penetration, however there is reduced rate of metmyoglobin reducing activity (O'Keefe and Hood, 1982; Leward, 1992; Suman et al., 2014). In comparison, muscles considered as color stable have an increased reducing capacity (Reddy and Carpenter, 1991; Suman et al., 2014). The work of Mckenna et al. (2005) further categorized muscles based on the rate of discoloration including: high color stability (i.e., Longissimus lumborum), intermediate color stability (i.e., Gluteus medius), low color stability (i.e., Biceps femoris) and very low color stability (i.e., Psoas major).

Packaging System

In order to meet the growing demands of consumers, the technology behind packaging systems is continuously progressive in nature. More importantly packaging is vital to the quality, shelf-life and preservation of meat products (McMillin, 2017; Ponce et al., 2019; Vierck et al., 2020). Packaging, in combination with color, drives perceived quality and wholesomeness of meat and in turn, dictates consumer purchasing decisions (Issanchou, 1996; Carpenter et al., 2001; Ramanathan et al., 2022). Furthermore, the biochemical changes that occur within various packaging systems can elicit altered eating experiences as well as the visual appearance of the meat product (Ponce et al., 2019; Vierck et al., 2020; Reyes et al., 2022). As reported in the 2018

National Meat Case Study, 34% of all meat at the retail level was displayed in an overwrap package. Such trends are contradictory to studies of Ponce et al. (2019) and Vierck et al. (2020) that found the vacuum packaging of beef to have greater advantages regarding beef palatability than overwrap methods.

The anomalies between overwrap packaging systems and vacuum technology are due in large parts to the stark differences in structure and therefore permeability. Since the mid-1950's, traditional methods of overwrap packaging using polyvinyl chloride (PVC) have been used in fresh beef mainly due to consumer preference (Mize and Kelly, 2004; Martin et al., 2013). Following extended storage or retail display, the quality of PVC overwrapped beef diminishes (Jeremiah and Gibson, 2001; Martin et al., 2013). The enticement to use PVC overwrap technology is the inexpensive, easy to use technology that provides an environment in which fresh meat products will bloom to appear bright, cherry-red in color (Ponce et al., 2019). However, the high permeability to both oxygen and moisture counteracts the benefits of PVC overwrap resulting in a more short-term shelf-life not suitable for extended storage in combination with declining quality factors (Ponce et al., 2020).

Vacuum packaging is unique in the fact that an anoxic environment is created through the removal of ambient air and use of double-layer film that is impermeable to both gas and moisture. Seideman and Durland (1983) attributed, among many factors, the moisture loss prevention and increased color stability to the extension of storage-life for meat products packaged in a vacuum system. McMillin (2008) more specifically described that vacuum packaging systems can extend long-term storage to 60 – 90 days and retail display from 30 – 60 days, in storage conditions of 4º C, while also remaining the most cost-efficient. However, during the retail display period, the lack of oxygen within the packaging system results in fresh

meat that appears purple due to the prevention of oxygenation of deoxymyoglobin, thus the product is deemed unsatisfactory to consumers (Mancini and Hunt, 2005). Despite appearance however, vacuum packaging systems have continued to increase at the retail level as a result of extended shelf-life capabilities as removing oxygen from the system has been proven effective in preserving factors that influence meat color during retail, including oxygen consumption and moisture loss (Ponce et al., 2019). Notably, packaging systems also have an impact on the rate of lipid oxidation (Clausen et al., 2009). By removing oxygen from the environment, vacuum packaged systems reduce oxidative rancidity by way of sufficient reducing capacity (Ladikos and Lougovois, 2003).

Freezing Impact

Freezing is a common practice that has existed for hundreds of years with the purpose to extend the shelf-life of meat products (Leygonie et al., 2012). However, throughout storage periods, the internal structure of muscles is altered, resulting in a deterioration of meat quality characteristics (Coombs et al., 2017; Listrat et al., 2016; Setyabrata and Kim, 2019). The meat system is complex and homeostatic conditions are easily distorted as the concentration of all other solutes (proteins, carbohydrates, lipids, vitamins and minerals) increases with the freezing of water within the meat system, manipulating the meat quality (Leygonie et al., 2012).

Meat is comprised largely of water approximately 75%, thus the changes in meat quality throughout extended periods of frozen storage are due largely to changes in the water fraction within the meat (Leygonie et al., 2012; Setyabrata and Kim, 2019; Dang et al., 2021). The altered water composition ultimately results in the occurrence of ice crystallization within both the intracellular and extracellular environment of meat (Leygonie et al., 2012; Setyabrata and Kim, 2019; Nakazawa & Okazaki, 2020). As osmotic pressure aggregates water molecules, the muscle

structure is weakened resulting in ice crystal formation (Setyabrata and Kim, 2019). As ice forms, the muscle tissue expands and in order to return to a balanced state, cells must immediately undergo either dehydration or intracellular ice formation (Bao et al., 2021).

Water freezes within a meat system through a three-step series beginning with the cooling of the meat system until it reaches the freezing point of 0°C (Dang et al., 2021). Once the freezing point has been reached, the latent heat is removed through a transitional phase and finally, through tempering, meat will come to the final storage temperature (Dang et al., 2021). In the transitional phase, the water molecules are aligned in a crystalline formation, resulting in ice crystals forming on the meat surface (Dang et al., 2021). The size of such ice crystals is largely determined by the initial cooling rate of freezing (Dang et al., 2021). More specifically, a slower rate of cooling allows for the formation of larger ice crystals which further damages the myofibrillar structure and leads to the deterioration of meat quality aspects (Dang et al., 2021; Bao et al., 2021). As ice crystals form in the extracellular space, mainly the meat surface, moisture is removed from the intercellular space resulting in dehydration of the muscle fibers (Wang et al., 2020; Zhang et al., 2022). The freezing induced denaturation of proteins in the dehydrated state is a direct result of disrupted hydrogen bonds which increases the surface area of hydrophobic regions on the meat surface (Zhang et al., 2022).

Much literature has determined that the freezing and subsequent thawing of meat products improves tenderness when being measured instrumentally through peak force (Farouke et al., 2003; Leygonie et al., 2012; Setyabrata and Kim, 2019). This increase in tenderness has been largely attributed to the effect of aging prior to storage and the duration of freezing (Setyabrata and Kim, 2019; Leygonie et al, 2012). Ice crystallization that occurs on the meat surface during frozen storage has also been recorded as having a positive interaction with meat

tenderness (Leygonie et al., 2012; Dang et al., 2021) The improved tenderness of frozen meat is largely attributed to enzyme-initiated proteolysis as well as extracellular ice crystallization (Leygonie et al., 2012). Specifically, the formation of ice crystals on the meat surface breaks down the myofibrillar structure which allows for tenderization of the muscle tissue (Leygonie et al., 2012). However, the common practice of freezing and later thawing prior to cooking, can result in biochemical alterations that damage the muscle ultrastructure (Leygonie et al., 2012). The result of such damage are residual amino acids that are involved in protein oxidation (Leygonie et al., 2012). More specifically, these amino acids are embedded in the myofibrillar protein structure, which is the structure that determines a great majority of the meat physiochemical properties (Leygonie et al., 2012). The occurrence of protein oxidation decreases the tenderness of the meat through destabilization of the protein matrix (Leygonie et al., 2012). Furthermore, an environment that enhances the oxidative conditions can also negatively influences shear force values and inherent tenderness of meat products (Bao et al., 2021).

Described as tenderization, enzymatic activity breaks down the muscle fibers during both proteolysis and aging while concurrently, the muscle structure is disrupted through ice crystallization (Leygonie et al., 2012). During extended freezing periods, the formation of large ice crystals on the meat surface breaks down myofibrils resulting in an increase in tenderness (Leygonie et al., 2012). Lagersted et al. (2008) contradicted the instrumental theory that freezing duration improved tenderness when comparing the sensory results of freeze/thaw samples to that of chilled samples. The loss of tenderness was explained by the loss of water throughout the thaw period that resulted in muscle dehydration, increasing the quantity of muscle fibers per surface area, which inherently resulted in more toughness as perceived by trained sensory panelists (Lagersted et al., 2008). Throughout the thaw cycle however, muscles continue to elicit

proteolytic enzyme activity (Crouse and Koohmaraie, 1990). Throughout freezing, the proteolytic activity was stopped as a result of suppressed calpain activity, however the calpains were not destroyed allowing for the calcium-dependent proteases to be re-activated during thawing (Crouse and Koohmaraie, 1990; Dransfield, 1994). Furthermore, thawing results in an increased rate of proteolysis due to a more rapid tenderization (Koohmaraie, 1992). Extensive research into the biochemical muscle mechanisms that alter meat tenderness has found that both the cathepsin and calpain enzymes contribute to the degradation of muscle structure and subsequently impact tenderness (Warner et al., 2021). Goll (1992) found that more than 90% of postmortem storage tenderization of meat resulted from the calpain system. Both μ-calpains and m-calpains require a calcium input, however they differ in activation level requirements (Shackleford et al., 1991). Moreover, μ-calpains requires less calcium and as increased calcium in the muscle system has been determined to positively influence tenderness, meat tenderization is controlled by the μ-calpain activity (Shackleford et al., 1991).

Freezing or the process of freezing/thawing can also induce protein oxidation which results in permanent physiochemical alterations to meat quality (Bao et al., 2021). Fluctuation in the temperature of frozen storage can also hasten ice crystallization within the muscle tissue (Kumar, 2020; Bao et al., 2021). Furthermore, frozen storage temperature that are less than – 18 ºC are attributed to diminished myofibrillar protein solubility (Farouk et al., 2003) However, temperature that exceed – 10 ºC have previously been attributed to accelerated protein oxidation and an overly damaged muscle cell structure (Huff-Lonergan et al., 2010). Biochemical alteration such as protein oxidation inherently result in less tender meat because of cross-link formation that inhibits proteolysis by inactivating the calpain-1 (Rowe et al., 2004).

Furthermore, the process of ice recrystallization throughout frozen storage is rapid due to temperature fluctuation.

Additionally, meat is susceptible to the occurrence of "freezer burn" under specific conditions when the vapor pressure of ice on the meat surface is greater than the vapor pressure of water in the external environment (Schmidt & Lee, 2010; Bao et al., 2021). The difference in vapor pressure among the two environments allows for dehydration to occur, which alters the form of the frozen water state on the surface of the meat (Bao et al., 2021). Freezer burn is perceived to negatively impact quality factors such as color, texture and flavor of meat products (Bao et al., 2021). These changes in sensory attributes are due in large part to the oxidation acceleration elicited from freezer burn due to a localized concentration of prooxidants and soluble proteins on the meat surface (Bao et al., 2021). Extended periods of frozen storage also have various influences on color stability of beef (Coombs et al., 2017). Farouk et al. (2003) observed that 6 – 12 months of frozen storage elicited beef that had increased L* values when compared to beef that had been frozen for only 3 months, or not frozen at all (fresh). It was further interpreted that the increased darkness of the meat resulted from both increased protein oxidation as well as lipid oxidation throughout longer frozen storage durations (Farouk et al., 2003; Coombs et al., 2017). Oxidation is dependent upon the water fraction as chemical reactions continue throughout frozen storage that initiate peroxidation which serves as a catalyst for the occurrence of secondary lipid oxidation as the meat is thawed and results in negative changes in color, odor and flavor (Owen and Lawrie, 1975; Leygonie, 2012). While much is understood about the impact of overall freezing on meat quality, little is known regarding the influence of various freezer types on factors of beef quality and palatability.

Water Holding Capacity and Moisture Retention

Muscle is comprised of nearly 75% water, 10 – 15% of which is intensely bound to the muscle protein (Bailey, 1972). Given the high percentage of free or immobilized water, the loss of moisture within meat systems is an unavoidable postmortem shift (Bailey, 1972; Huff-Lonergan & Lonergan, 2005, Leygonie et al., 2012). The change in water retention after harvest is due in large part to the instantaneous pH decline as well as the loss of ATP and occurrence of the steric effect as the myofibrils shrink due to rigor mortis (Huff-Lonergan & Lonergan, 2005, Leygonie et al., 2012). In the case that the pH declines too rapidly, the net electric charge of the proteins is also reduced resulting in a decrease in the water-holding capacity (Listrat et al., 2016). These factors catalyze the release of both immobilized and bound water within the muscle, which are then redistributed into the sarcoplasmic and extracellular environments (Huff-Lonergan & Lonergan, 2005; Leygonie et al., 2012). The ability of meat to retain intrinsic water is best described as water-holding capacity (Listrat et al., 2016).

While water-holding capacity is largely influenced by the rate and extent of pH decline in the post-mortem muscle (Listrat et al., 2016), water holding capacity and moisture retention are also disrupted by extrinsic factors such as the freezing and subsequent thawing of meat products (Leygonie et al., 2012). Extended frozen storage also greatly deteriorates the water-holding capacity of fresh meat as water is lost throughout the thaw process due largely to disruption of the muscle fibers during frozen storage (Coombs et al., 2017; Zhang et al., 2022). This disruption of the water holding capacity is due in large part to the extrinsic stress placed on the myofibrillar structure of meat throughout freezing due to ice crystallization (Leygonie et al., 2012; Dang et al., 2021). Throughout frozen storage, water within the meat product will freeze, which inherently increases the concentration of all other meat product solutes among unfrozen water

molecules and in turn disrupts the homeostatic conditions of the meat system (Leygonie et al., 2012; Dang et al., 202; Zhang et al., 2022). Ice crystallization results in additional water being removed from within the muscle fibers which in turn correlates to more thaw loss (Dang et al., 2021; Zhang et al., 2022). Comparatively, fast freezing results in the formation of small ice crystals that cause less mechanical aggravation of the muscle structure and therefore generates less thaw loss than that of larger ice crystals formed throughout slow freezing (Kim et al., 2018; Zhang et al., 2022). This more rapid increase in ice crystal size has been attributed greatly to the quick heat conduction of extracellular ice in comparison to factors such as the immobilized water that is extruded from between the muscle fibers (Zhang et al., 2022) Moreover, throughout the thaw process, the ice crystals melt, and the free moisture is absorbed by the muscle tissue in order to restore the pre-freezing state (Nakazawa & Okazaki, 2020; Zhang et al., 2022). However, under conditions of aggressive protein denaturation, the distorted muscle tissue cannot absorb the released water (Nakazawa & Okazaki, 2020). In such a case, empty space remains within the muscle tissue following thawing which is predictive of the failed restoration of the muscle tissue and a decrease in the WHC and inherent sensory factors of flavor and juiciness (Nakazawa & Okazaki, 2020).

As the WHC is altered, juiciness is a major meat quality factor that become distorted (Dang et al., 2021). Typically, the juiciness of meat is improved with an increased WHC, however a decrease in WHC due to extended freezing durations would decrease the overall product juiciness (Dang et al., 2021). The work of Lagerstedt et al., (2008), observed that sensory panelists perceived samples of cooked beef that had been frozen to be significantly less juicy than fresh, never frozen beef.

Lipid Oxidation

The postmortem biochemical alterations in meat that result in color deterioration along with decreased palatability and the production of off-flavors and rancidity can be described through lipid oxidation (Bekhit et al. 2013; Legako et al., 2015). Following exsanguination, the body attempts to rebuild homeostatic conditions through ATP production (Yu, Q et al., 2019). Eventually, ATP is depleted resulting in an increase in the fatty acid metabolism due largely to the lack of energy in the postmortem muscle (Cônsolo et al., 2021). As these fatty acids are metabolized, there is a subsequent increase in the lipid oxidation, which diminishes final product palatability (Cônsolo et al., 2021). However, lipid oxidation is also critical to the development of the meaty aromatics associated with meat products (Khan et al., 2015).

Chemical reactions in which one or more electrons are transferred from the electron donor, or reductant, to an electron acceptor, or oxidant, which inherently transforms both the reductant and oxidant are commonly referred to as oxidative processes (Bekhit et al., 2013). Despite the necessity of oxygen for basic life function, oxygen impairs a variety of cells due to the increased production of reactive oxygen species, or ROS (Min and Ahn, 2005). Lipid oxidation emerges as the most dominant non-microbial causative reaction that diminishes meat quality (Domínguez et al., 2019). In simple terms, lipid oxidation is the reaction between unsaturated fatty acids and oxygen (Domínguez et al., 2019). Occurring over a three-step series, lipid oxidation is a free radical chain reaction in which oxygen is the most vital factor, that corresponds to negative attributes within a meat system (Min and Ahn, 2005; Bekhit et al., 2013). Free radicals are molecules that possess the ability to exist independently, carrying unpaired electrons in the valence orbit (Bekhit et al., 2013). These molecules lack thermostability, thus they work to create a more stable environment through a reaction chain

(Bekhit et al., 2013). The main product of the lipid oxidation reaction are hydroperoxides, which are incredibly unstable compounds that decompose rapidly and result in secondary compounds such as hydrocarbons, aldehydes, ketones, alcohols, esters and acids, which all have linkage to negative meat quality attributes (Domínguez et al., 2019; Ross and Smith, 2006).

Beginning with initiation, a free radical with sufficient reactivity will extract a labile hydrogen atom from the lipid chain resulting in unpaired electrons of the carbon chain (Min and Ahn. 2005). To stabilize the carbon radical, a conjugate diene that can experience various reactions based on the nature of the aerobic environments is formed through molecular rearrangement (Min and Ahn. 2005). Combined with the conjugate diene formation is the configuration alteration of the double bond from cis to trans resulting in more unsaturated fatty acids and a distinct marker of peroxidation in meat (Min and Ahn. 2005). The processes of initiation are continued through propagation, during which lipid peroxides extract a hydrogen from relative fatty acids to form a prominent non-radical product of primary lipid oxidation, lipid hydroperoxide (Min and Ahn, 2005). Propagation continually disrupts the lipid structure forming a number of secondary oxidative products (Min and Ahn, 2005). Termination of lipid oxidation occurs at a point in which all substrate has been depleted through propagation, thus the lipid peroxides react with each other in a manner of destruction resulting in the formation of non-radical products (Min and Ahn, 2005).

The further production of lipid oxidation products results in metmyoglobin formation (Faustman, et al., 2010; Lynch and Faustman, 2000). The result of lipid oxidation is the production of a variety of secondary products, especially through propagation and termination (Min and Ahn, 2005; Bekhit et al., 2013). Of these secondary products, aldehydes are produced in the greatest concentration (Min and Ahn, 2005; Domínguez et al., 2019). Hexanal, the

strongest volatile compound produced through oxidation, stimulates oxymyoglobin oxidation which in turn reduces the metmyoglobin reducing activity, aiding in the production of metmyoglobin within the meat system (Min and Ahn, 2005; Faustman et al., 2010).

Lipid oxidation is susceptible to occur based on several intrinsic and extrinsic factors, meaning that the oxidative stability of a meat system is dependent on the proportion of anti– and prooxidant compounds (Bekhit et al., 2013; Domínguez et al., 2019). Overall, all meat processing processes to some extent disrupt the membrane of skeletal muscles which forces oxidative reactions to occur as the interaction between phospholipids and prooxidant compounds such as oxygen is increased (Domínguez et al., 2019). Regarding intrinsic oxidative conditions, several muscles and communicated meat products are more labile to lipid peroxidation (Bekhit et al., 2013). Processing techniques such as chopping, grinding and cooking associated with the formation of communicated products including ground beef results in the acceleration of lipid oxidation (Cheng, 2016). Further processing deteriorates the muscle structure opening the door for unsaturated fatty acids to react with oxygen within the air (Cheng, 2016). Furthermore, when comparing the fiber type of whole muscles, Type-I contain a higher lipid concentration than Type-II, therefore they are more readily equipped for oxidative metabolism (Cassens and Cooper, 1971).

The characteristic of unfrozen water within a meat system that remains throughout frozen storage is an important factor of oxidation under certain conditions (Leygonie et al., 2012). During extended durations of frozen storage, lipid oxidation is prevalent to occur at an accelerated rate due to high reactivity in conditions that are favorable for lower water activity (Leygonie et al., 2012; Bao et al., 2021). When frozen, certain chemical reactions occur that result in primary lipid oxidation and can result in radical secondary lipid oxidation once thawed

(Owen & Lawrie, 1975; Leygonie et al., 2012). From the consumer perspective, the shelf-life of a meat products ends upon recognition of oxidation which is imparted through changes in aroma and the onset of volatile rancidity as well as visual shifts in fresh meat color (Domínguez et al., 2019).

Flavor Development

The flavor of cooked meat is a derivative of complex reaction pathways that result in volatile compounds, induced through heating in which non-volatile compounds of lean and fatinteract (Mottram, 1998; Khan et al., 2015). Flavor development begins in fresh meat, through reaction pathways of non-volatile compounds that are precursors to both flavor and taste of the cooked product (Khan et al., 2015). Both lipids and water-soluble compounds are distinct precursors of this cooked flavor development (Mottram, 1998). Of the reactions that impart unique flavor, the two that are attributed to developing most of the volatile compound composition of cooked meat are the Maillard reaction and lipid degradation (Mottram, 1998).

A non-enzymatic browning reaction, the Maillard reaction involves the interaction of a reducing sugar and an amino acid under conditions of high temperature (Mottram, 1998). Early segments of the Maillard reaction involves the introduction of heat, causing the carbonyl component of the reducing sugar to condense with the amine group of the amino acid resulting in the production of glycosylamine through rearrangement (Hodge, 1953; Mottram, 1998). The following dehydration of glycosylamine produces several intermediate compounds that subsequently interact with amino acids, aldehydes, hydrogen sulfide and other reactive compounds to produce the distinct aromas of cooked food products (Hodge, 1953; Mottram, 1998). The final products of the Maillard reaction are dependent upon the sugar and amine group present in the reaction (Calkins and Hodgen, 2007). For instance, cysteine and glucose produce

more pyrazines and furans under oxidized conditions compared to the high number of sulfur compounds produced from cysteine and glucose otherwise (Tai and Ho, 1997; Calkins and Hodgen, 2007). Specifically, the Maillard interaction intermediate products, especially reductones and dehydroreductones are interact with reactive compounds to form distinct aromas mainly though the pathway of Strecker degradation (Hodge, 1953; Mottram, 1998, Khan et al., 2015).

During Strecker degradation, the α −amino acid of the Maillard reaction undergoes decarboxylation and deamination resulting in the production of aldehydes as well as α −aminoketones and α −aminoalcohols, all of which are significant markers of thiazole and pyrazine production (Mottram, 1998). However, the α −amino acids that are elicited are reliant upon the amino acid that is degraded, with specific exception to proline and hydroxyproline (Hodge, 1953; Mottram, 1998). Because their structure does not include an additional amino group on the pyrroline ring, proline and hydroxyproline are emitted from Strecker degradation and instead undergo heterocyclization to produce nitrogen-based compounds that have negative correlation to meat flavor when present in high concentration (Hodge, 1953; Mottram, 1993, 1998). Additionally, during Strecker degradation, the sulfur group is removed from cysteine and methionine which then react to form sulfur-containing volatile compounds (Hodge, 1953; Mottram, 1993, 1998). While these compounds are critical to meat flavor development, they have a low odor threshold resulting in negative impacts on flavor at high concentrations (Hodge, 1953; Mottram, 1993, 1998).

Volatile compounds that form from lipid sources correlate to species specific flavor development due to the differences in unsaturated fatty acid concentrations (Khan et al., 2015). Species with more unsaturated fatty acids produce a greater number of volatile carbonyls, which

are integral products of lipid degradation (Perez-Alvarez et al., 2010; Kahn et al., 2015). Moreover, lipids are critical in the aromatic development through the reduction of the vapor pressure of flavor compounds (Khan et al., 2015). A number of secondary products are formed throughout the propagation and termination phases of lipid oxidation such as carbonyls, alcohols, hydrocarbons and furans that all produce negative, rancid off-flavors in meat (Mottram, 1998; Min and Ahn, 2005). More specifically, lipid oxidation produces aldehydes in the greatest concentration (Min and Ahn, 2005). Palamand and Dieckmann (19474) observed that autoxidized hexanal, an aldehyde, produced a variety of compound imparting flavor including esters and carboxylic acid. During extended storage durations, the reactions of lipid degradation can result in rancid off-flavors, however cooking causes lipid degradation to occur more rapidly which results in more positively associated flavor profile (Mottram, 1998).

Flavor Perception

Often, consumers describe flavor as taste, or taste as flavor in the form of interchangeable words (Prescott, 1999; Smith and Margolskee, 2001). However, flavor and taste represent independent attributes of the sensory experience (Prescott, 1999) Flavor is a complex multisensory summation of odor, aroma and basic taste and how each trait interacts with the olfactory, somatosensory and gustatory systems of an individual (Small and Prescott, 2005, Spence, 2015). While flavor is mainly perceived through taste and odor, texture and juiciness can also influence overall flavor perception (Small and Prescott, 2005; Khan et al., 2015).

Via taste receptors spread out across the tongue, flavor is interpreted through the five basic tastes: sweet, salty, sour, bitter and umami (Smith and Margolskee, 2001). Within the body, the chemical components of food are detected and identified through pathways of the gustatory system (Vincis and Fontanini, 2019). Through mastication, small tastant molecules are dissolved

in the saliva then bind to the taste receptors of the taste buds, which creates an electric stimulant to the brain (Vincis and Fontanini, 2019). Simultaneously, olfactory pathways create signals relevant to odor and aroma, which when combined with the signal of the tongue, create the neural sensation of flavor (Smith and Margolskee, 2001). Individual nerve fibers transmit the electrical stimulants to specific regions of the brain (Smith and Margolskee, 2001). As each neural region processes the gustatory stimulate, multimodal neurons function in response to the overlapping sensory attributes (Small and Prescott, 2005). Furthermore, a singular neuron of the gustatory system may elicit response stimuli of multiple taste profiles as well as non-gustatory information relevant to the mouthfeel (Vincis and Fontanini, 2019). Additionally, neurons of the gustatory system can interpret information encapsulated by the psychological and cognitive attributes of food, better describes as the memories of eating which further builds upon the complexity of flavor (Prescott, 1999; Vincis and Fontanini, 2019).

Texture

The texture of meat products is the summation of juiciness and tenderness that creates a certain mouthfeel (Winger and Hagyard, 1994; Purchas, 2014; Warner et al., 2021). When evaluating cooked meat, texture combines the factors of tenderness, hardness, chewiness and graininess (Bruce and Aalhus, 2022). Furthermore, these distinct attributes can be categorized into segments: first-bite, mastication or chewing and after-feeling properties (Purchas, 2014; Warner et al., 2021). More specifically, these textural characteristics of meat are attributed to the muscle structure, being fibrous proteins (Warner et al., 2021).

The adhesiveness, cohesiveness and chewiness each reflect objective measurements of meat texture and are calculated using texture profile analysis measurements (Warner et al., 2021). The work of Sasaki et al., (2010) determined that the terminology associated with

properties of meat texture was best defined in accordance with the International Organization for Standardization. The terminology provided for standardization are internationally recognized for both sensory analysis and methodology (Warner et al., 2021). It was found that "hardness," is defined as the force required achieve a given deformation (Sasaki et al., 2010; Warner et al., 2021). The textural factor, "chewiness," describes the length of time required for mastication to a state for swallowing (Sasaki et al., 2010; Warner et al., 2021).

Meat texture is easily manipulated through cooking and the inherent heat denaturation of proteins that impacts attributes such as WHC, an integral component of meat texture (Hughes et al., 2014; Warner et al., 2021). The denaturation of specific proteins within a meat system is temperature dependent (Davey and Gilbert, 1974; Warner et al., 2021). As internal temperature rises from 40 – 50°C, denaturation of the contractile system occurs, which especially breaks down the myofibrillar structure, myosin (Davey and Gilbert, 1974; Warner et al., 2021). At a temperature threshold of 65 – 75°C, collagen within the muscle tissue essentially melts to form gelatin which results in a softened meat texture (Hamm, 1966; Davey and Gilbert, 1974; Warner et al., 2021). Muscle type also drives texture as intact muscle has a rather unique texture that similarly to comminuted meat, cannot necessarily be copied (Warner et al., 2021). The uniqueness of the products that are ground, chopped or minced stems from the structural integrity provided through the additional processing steps (Warner et al., 2021).

Slice Shear and Warner-Bratzler Shear Force

Shear force is the instrumental measurement in determining the tenderness of meat (Miller et al., 2001). Moreover, tenderness describes the ease of mastication, or chewing by a consumer and is a vital attribute of meat palatability and quality (Miller et al., 2001). Tenderness

is quantified through both subjective and objective, mechanical methods that can be correlated to one another with a high degree of confidence.

Shear force was developed as an instrumental method by which tenderness attributes being evaluated using sensory analysis could also be quantified in an objective measurement (Warner et al., 2021). Furthermore, this included the development of devices that reflect action of the human jaw, by measuring the peak force required to cut through a sample using blades (Warner, 1929; Bratzler, 1932; Warner et al., 2021). Of the devices, Warner – Bratzler Shear Force (WBSF) is the most widely used method in assessment of basic mechanical properties of meat tissue and is considered the ideal device for predicting sensory tenderness (Bourne, 2002; Warner et al., 2021). However, the accepted protocols vary greatly among institutions, limiting the ability to compare WBSF values to determine the tenderness or toughness of meat (Wheeler et al., 1994).

In order to provide an objective tenderness measurement that provided a high degree of acceptability and repeatability, Shackleford (1999b) developed the methodology for Sliced Shear Force (SSF). Initially, SSF was developed as an "in-line" method of sorting carcasses in the plant based on tenderness parameters (Shackleford et al., 1999a). The utilization of "hot" samples, compared to the "cold" samples of WBSF correlated more strongly to the ratings of trained sensory analysis (Shackelford, 1999b).

The work of Vaskoska et al., (2020) found that cathepsins are active throughout the addition of heat and likely resulting in the shrinking of the muscle fibers and additional cook loss that increases toughness. As the last step that occurs before consumption, cooking can have a large impact on the overall eating experience, especially tenderness (Warner et al., 2021).

Throughout cooking, collagen is gelatinized which results in decreased shear force values (Hamm, 1966; Warner et al., 2021).

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Chapter 2

Evaluating the impact of freezing duration and packaging type on the palatability of beef steaks

Abstract

The objectives of this study were to evaluate the impact of extended periods of frozen storage duration and packaging type on palatability traits of cooked beef steaks from two muscles. Paired beef strip loins and top sirloin butts were collected from USDA Choice carcasses (n = 20) and wet aged at 2° C in the dark. Subprimals were then fabricated into 2.54 cm thick steaks of the Longissimus lumborum (LL) and Gluteus medius (GM). Steaks were assigned at random to a packaging treatment of vacuum (VAC) or PVC overwrap (OW), and immediately transported from Canyon, TX to Fayetteville, AR to simulate a case-ready plant to retail scenario. Upon arrival, steaks were placed in a 3 d simulated retail display and allotted at random to one of five frozen storage duration treatments: fresh (not frozen), 1-week, 1-month, 6-months and 9-months (n = 64/duration) with frozen storage being maintained at -20° C. Steaks were all designated to analyses including, slice shear force (SSF), trained sensory panels, expressible moisture (EM), cook loss and volatile compound analysis via grass chromatography-mass spectrometry.

There were no differences elicited from any of the possible interactions (P= 0.95) for SSF or CL: package × muscle × freezing duration, package × muscle, package × duration or muscle × duration. However, freezing duration did impact both SSF (P < 0.01) and CL (P < 0.001). Generally, for both SSF and CL steaks that were frozen for 9-months elicited both the highest SSF values as well as the greatest percentage of CL among all treatments. Furthermore, SSF (P < 0.005) and CL (P < 0.001) were each impacted independently by the main effect of muscle type, with the GM having both higher SSF values and a greater percentage of CL than the

LL steaks. Neither SSF nor CL were impacted by packaging type ($P = 0.93$) as a main effect. In comparison, EM was impacted by the interaction ($P = 0.059$) of packaging type × muscle × freezing duration. Samples from OW GM stored for 9-months elicited the highest percentage of EM while fresh OW LL samples resulted in the lowest. Additionally, expressible moisture was impacted by the interaction ($P = 0.047$) of muscle × freezing duration. Among all treatments, GM steaks that were frozen for 9-months elicited the highest EM values and fresh LL samples the lowest EM values. Contrastingly, there was no significant difference observed in expressible moisture for the interaction ($P \geq 0.18$) of package type × freezing duration or for the interaction ($P = 0.70$) of package type × muscle. Generally, fresh OW GM steaks resulted in the greatest concentration of lipid derived volatile compounds such as aldehydes, hydrocarbons and alcohols which contrasted sensory ratings of trained panelists that indicated oxidized and refrigerator-stale ratings increased as storage time increased for OW steaks. These results indicate that while frozen storage duration, muscle type and packaging system each impart a difference on beef palatability, how each attribute is impacted is independent of another. Furthermore, this data concludes that while lipid oxidation is of greater incidence in steaks that are OW GM and ice crystallization increases with time, the physiochemical changes that occur impact the consumer eating experience greatest after extended periods of frozen storage duration.

Introduction

Freezing as a mechanism of meat preservation has been widely practiced dating back to the 1950s, despite known negative impacts on palatability (Setyabrata and Kim, 2019; Dang et al., 2021). The differences in quality between fresh steaks and those subjected to the freeze/thaw cycle are largely attributed to disruptions of the muscle structure induced from ice crystallization (Leygonie et al., 2012; Dang et al., 2021). In contrast, early ice crystal formation has been shown to break down myofibrillar proteins, resulting in improved meat tenderness after frozen storage (Leygonie et al., 2012; Setyabrata and Kim, 2019; Dang et al., 2021). Furthermore, variations in packaging method are also utilized to extend the shelf-life and quality of fresh meat but could also greatly impact the consumer eating experience, especially after extended periods of frozen storage (Vierck et al., 2020). Several research studies have indicated advantages in meat quality from vacuum packaging, especially when compared to PVC overwrap. Nonetheless, the 2018 National Beef Case Study reported that 34% of all meat was packaged in overwrap (Ponce et al., 2019; Vierck et al., 2020). Currently, little is known about the impact of extended periods of frozen storage in relation to beef palatability and more specifically, flavor development. Therefore, the objectives of this study were to determine the impact of extended frozen storage duration and packaging type on the palatability of beef steaks from two muscles.

Materials and Methods

Product Collection & Fabrication

Paired beef strip loins (NAMI IMPS #180) and top sirloin butts (NAMI IMPS #184) were collected from the side of USDA Low Choice beef carcasses during fabrication (n = 20 subprimals). Collected subprimals were individually vacuum packaged, boxed and transported to West Texas A&M University. Upon arrival, vacuum packaged subprimals were wet aged for 14 d at 0 – 2 °C in the dark. Immediately after aging, subprimals were portioned into 2.54 cm steaks and individually packaged. Representative steaks of the *Longissimus lumborum* (LL) muscle from the strip loin and the *Gluteus medius* (GM) muscle from the top sirloin butt were cut and transported from Canyon, TX to Fayetteville, AR under refrigeration to simulate product shipment from a case-ready plant to retail scenario. Upon arrival, steaks were assigned at random to packaging treatments: vacuum packaging (VAC) or PVC overwrap packaging (OW). Once packaged, steaks were subjected to a 3-d simulated retail display in a Hill Phoenix open-front, multideck case (Colonial Heights, VA) and allotted at random to one of five freezing treatments: fresh (never frozen), 1-week freeze, 1-month freeze, 6-month freeze or 9-month freeze. Frozen storage was maintained at – 20 °C in a commercial blast freezer, until further analysis.

Expressible Moisture

Prior to analysis, frozen steaks were thawed in accordance with their designated freezing duration treatment at 2 – 4 °C for 12 – 24 hours. Following expressible moisture (EM) methods outlined by Pietrasik and Janz (2009), from each steak designated for volatile compound analysis a 5 g ± 0.05 sample was removed prior to cooking. Raw samples were placed in a 50 mL conical tube filled with 25 g ± 0.1 boiling beads. Samples were then centrifuged at 900 g for 10 minutes. After centrifugation was complete, samples were removed and reweighed.

The proportion of weight lost following centrifugation to the initial weight of the raw sample was used to determine the percent of expressible moisture.

Proximate Analysis

Following each designated freezing treatment, steaks assigned for proximate analysis were thawed at 2 – 4 °C for 24 h in refrigeration. Once thawed, steaks were trimmed free of external fat and connective tissue. Trimmed steaks were then cubed into pieces measure 2.5 cm × 2.5 cm and ground through a 4-mm plate. Proximate analysis was conducted on raw steaks in accordance with AOAC official methods (Anderson, 2007) using a near infrared spectrophotometer (FoodScan, FOSS NIRsystems, Inc., Laurel, MD). Percentages of fat, moisture and protein were determined on an individual steak basis.

Cooking Method

Steaks designated for cooked analysis were thawed and trimmed free of any excess fat. After the removal of the EM samples, steaks were cooked on closed clamshell grills (Model GR-150 Griddler, Cuisinart, Stamford, CT) set at 176.6°C. Steaks were removed from the grill and set to rise to a peak internal temperature of 71°C . Temperature was monitored by placing a thermometer at the geometric center of each steak with individual peak internal temperatures being routinely recorded. Additionally, steaks were set to rest for 3 min prior to sliced shear force evaluation. Furthermore, all raw and cooked weights were recorded for determining overall cook loss for each steak.

Slice Shear Force

In accordance with the AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Meat (2015) and following the previously described

cooking methods, steaks were subjected to slice shear force analysis (SSF). Using a SSF sizing box (ZB – 150; Tallgrass Solution, Manhattan, KS), a 1 cm
thick, 5 cm long portion was removed from the lateral end, parallel to the muscle fibers of each steak. A second cut was made that measured between 1 – 2 cm across the width of the muscle from the lateral end. A final sample was cut in the SSF sizing box measuring 5 cm across the width of the steak, parallel to the initial cut. The final 5 cm section was positioned in the SSF slice box (CB – 150; Tallgrass Solution, Manhattan, KS), to allow for the 45 ° slots to line up with the direction of the muscle fibers. A slice was removed from the center of the section using a double-bladed knife (DK – 150; Tallgrass Solution, Manhattan, KS). The slice sample was then positioned on the SSF testing machine (GR – 152; Tallgrass Solutions, Manhattan, KS), to allow the blade to cut perpendicular to the muscle fibers. The final slice shear force was recorded in peak kg/f.

Homogenization

Immediately after sliced shear force evaluation, steaks designated for volatile compound analysis were further portioned into cube size pieces and flash frozen by liquid nitrogen. Once frozen, sampled were homogenized (Nutribullet, Ninja, Mesa, AZ) and packaged in labeled individual Whirl-Pak bags (Model S-19794 6 × 9” White Block Whirl-Pak® Bags – 24 oz, Whirl-Pak®, Atkinson, WI). All homogenized samples were stored in -80 °C until time of designated analysis.

Volatile Compound Analysis

The volatile compound composition of each steak was identified using the methods of Gardner and Legako (2018). Immediately following cooking and the subsequent sliced shear force evaluation, steaks were flash frozen, homogenized and stored in -80 °C until volatile

compound analysis. Immediately prior to analysis, homogenized samples were removed from the freezer and a 5.0 g ± 0.1 sample was portioned into a labeled 20 mL glass vial (Gerstel Inc Linthicum, MD) with a polytetrafluorethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc, Linthicum, MD). Upon analysis, 10 µL of internal standard (1, 2-dichlorobenzene, 2.5 mg/µL) was pipetted into the glass vial and then sealed with the screw cap. Each sample was then loaded using a Gerstel automatic sampler (MPS; Gerstel, Inc.) and set to a 5-minute incubation time in the Gerstel agitator set at 65 °C. Following a 20-minute extraction setting, an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA) collected the volatile compounds from the sample headspace by solid-phase microextraction (SPME). The extracted compounds were positioned on a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Compound identities were identified through retention time correlation and confirmation of authentic standards (Sigma-Aldrich, St. Louis, MO).

Trained Sensory Panels

Panelists were trained at West Texas A&M (Canyon, TX) in accordance with the AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness (2015). Prior to panel sessions, eight panelists were identified and trained for 5-d period following the methods and anchors outlined by Adhikari (2011).

Trained panelists evaluated samples on a continuous 100 mm line scale for specific characteristics of: beef flavor identity, brown/roasted, bloody/serum, fat-like, umami, overall tenderness, overall juiciness and off-flavors. The notable off-flavors included: liver-like, fishy, oxidized, cardboard, rancid, refrigerator/stale, bitter and sour. Each panelist was provided with

an electronic tablet with a digital ballot (Version 2417833; Qualtrics Software, Provo, UT) that included a line scale for each trait. Verbal anchors were provided on the line scale at the midpoint and each endpoint, where 0 = extremely dry/extremely tough/none/un-beef-like/bland; 50 = neither dry or juicy/neither tough nor tender/neither un-beef like nor beef-like; 100 = extremely juicy/ extremely tender/extremely abundant/ extremely beef-like/extremely intense.

A total of five panel sessions were completed in line with the corresponding freezing treatment. Each session consisted of the eight trained panelists who evaluated four samples indicative of steaks from a VAC LL, VAC GM, OW LL and OW GM. Before receiving the treatment samples each session, panelists were provided anchors under white fluorescent light. Following anchor sampling, panelists were served in individual sensory booths under red incandescent lighting conditions. During each session, panelists were provided water, unsalted crackers and apple slices to use for palate cleansing between each sample. An expectorant cup and napkin were also provided.

All steaks were cooked using the previously described cooking methods, on a clamshell grill to a medium degree of doneness (71 °C) with internal temperature measured by a thermometer inserted at the geometrical center of each steak). Samples were then portioned into a 1 × 1 – cm^3 cube. Immediately following cooking, steaks were served to the panelists in a randomized order in a closed 2-oz container that was labeled with a blind identification number that correlated to the sample.

Statistical Analysis

Statistical analysis was performed using the PROC GLIMMIX procedure of SAS (SAS version 9.4; Cary, NC). Data were analyzed as a completely randomized design with a 2 × 2 × 5 factorial arrangement. Packaging type, freezing duration and muscle type were included in the

design as fixed effects. Peak temperature was included in the model for cooked analyses as a covariate. For sensory evaluation, panel session and number served as random effects. Acceptability data was measured in a binomial error distribution model. Individual steaks were treated as the experimental unit and blocked by animal number. The Kenward-Roger adjustment was used for all statistical analysis. Means were separated at $\alpha < 0.05$.

Results and Discussion

Expressible moisture

Expressible moisture was impacted by the interaction of packaging type × muscle × freezing duration ($P = 0.059$; Table 2.1). In comparison, GM samples packaged in OW for 9 months elicited the highest percentage of expressible moisture while fresh LL samples in OW resulted in the lowest percentage of expressible moisture ($P < 0.01$). Additionally, expressible moisture was impacted by the interaction ($P = 0.047$) of muscle × freezing duration (Table 2.2). Among all treatments, GM samples placed in frozen storage for 9-months resulted in the highest expressible moisture values ($P < 0.01$) while fresh LL samples resulted in the lowest ($P < 0.01$). Generally, frozen storage resulted in an increase in the percent of expressible moisture when compared to fresh, never frozen samples. However, there was no significant difference observed in expressible moisture for the interaction ($P \geq 0.18$) of package type × freezing duration or for the interaction ($P = 0.70$) of package type × muscle (Table 2.3). Steaks stored in OW resulted in a higher percentage of expressible moisture when compared to VAC ($P < 0.01$).

Extended storage durations of steaks, specifically those of the GM, resulted in an increase in the percentage of expressible moisture. Previous studies found that extrinsic factors, such as freezing and/or thawing act as deterrents to the water holding capacity and moisture retention of meat products, largely due to the disruption of the muscle fibers ensued through each process (Leygonie et al., 2012). Previous research has also indicated that frozen storage conditions provide a basis for ice crystallization, through which water is removed from within muscle fibers, resulting in an increase in thaw loss (Dang et al., 2021; Zhang et al., 2022). After

extended periods of frozen storage, the size of the ice crystals present increases, which further agitates the muscle structure (Dang et al., 2021). The observed increase in expressible moisture (Table 2.3) as storage time increased is likely reflective of an increase in ice crystallization over time. Furthermore, the difference in expressible moisture between fresh (never frozen) steaks and steaks frozen for 9-months corresponds to the work of Nakazawa and Okazaki (2020) which determined that free moisture elicited from ice crystals formed throughout freezing would inherently melt away during thawing and only readily be absorbed by muscle tissue that has not undergone aggressive protein denaturation. Additionally, Nakazawa and Okazaki (2020) concluded that when the released water is not absorbed back into the muscle tissue, the failed restoration of the pre-freezing state decreases the water-holding capacity which is an objective precursor of juiciness.

The observed differences in expressible moisture among packaging systems is most easily explained through physical differences of the packaging types. The OW steaks were placed on a Styrofoam tray with an individual soaker pad, whereas VAC steaks were stored only in the film system, devoid of oxygen. It is likely that some moisture lost throughout storage for OW packaged steaks was absorbed in the soaker pad compared to the VAC steaks in which moisture was absorbed back into the muscle tissue. Additionally, the differences in parameters of thaw loss among muscle types are supported by previous findings in which the beef GM elicited higher thaw loss than samples derived from the LL (Hergenreder et al., 2013).

Proximate Composition

Table 2.4 contains the least-squares means for proximate analysis of LL and GM steaks. As expected, the LL was higher for fat percentage and lower for moisture than the GM ($P <$

0.01). These results indicate that steaks from the LL possessed a greater amount of intramuscular fat compared to steaks of the GM. This is similar to previous observations which found the LL to produce a higher fat percentage among USDA Low Choice samples compared to the GM (Legako et al., 2015).

Cook loss

No difference in cook loss was observed for the interaction of packaging type × muscle × freezing duration ($P \geq 0.17$), nor for the interaction between packaging type × muscle ($P \geq 0.50$). Additionally, no difference was elicited in cook loss between the interaction of muscle × freezing duration ($P \geq 0.71$) nor for the interaction of packaging type × freezing duration ($P \geq 0.14$;). However, cook loss was impacted by the main effect of freezing duration ($P < 0.001$) and muscle ($P < 0.001$). The main effect of packaging type had no effect on the percentage of cook loss ($P = 0.059$; Table 2.5). In comparison, samples that were subjected to a freezing duration of 9-months resulted in the greatest percentage of cook loss ($P < 0.001$), while samples frozen for 6-months elicited the lowest percentage of cook loss ($P < 0.001$). Furthermore, the GM resulted in a higher percentage of cook loss ($P < 0.001$) than samples from the LL ($P < 0.001$).

The results of the instrumental measures of expressible moisture and cook loss support the concept that the extended freezing, and freeze/thaw cycle each impact beef palatability, especially as it relates to juiciness. Furthermore, after extended periods of frozen storage, the size of the ice crystals present increases, which further agitates the muscle structure (Dang et al., 2021). The observed increase in cook loss (Table 2.4) as storage time increased is reflective of an increase in ice crystallization over time.

Slice shear force

No difference in slice shear force was observed for the interaction of packaging type × muscle × freezing duration ($P \geq 0.83$; Table 2.5) , packaging type × muscle ($P \geq 0.79$), muscle × freezing duration. ($P \geq 0.596$) nor for the interaction of packaging type × freezing duration ($P \geq 0.955$). Contrastingly, objective tenderness was impacted by the main effect of freezing duration ($P < 0.01$) and muscle type ($P = 0.005$). In comparison, samples that experience a freezing duration of only 1-week resulted in the highest slice shear force values ($P < 0.001$) and were similar to samples frozen for 9-months, while samples frozen for 1-month had the lowest slice shear force values ($P < 0.001$). Also, the GM resulted in higher shear force values ($P < 0.001$) than samples from the LL. Packaging type imparted no difference on slice shear force values as a main effect ($P = 0.93$).

Much research has supported the understanding that the frozen storage of meat products improves the instrumental peak force measurements of beef tenderness (Farouke et al., 2003; Leygonie et al., 2012; Setyabrata and Kim., 2019). The work of Leygonie et al. (2012) and Dang et al. (2021) attributed the positive tenderization to extracellular ice crystallization as well as enzyme-initiated proteolysis. Such findings support the observation that the lowest shear force values were recorded from steaks that were allotted to 1-month of frozen storage. As previously shown by Leygonie et al. (2012), the formation of small ice crystals on the meat surface breaks down the muscle ultrastructure, creating more tender muscle tissue. However, the significant change in objective tenderness measurements after 1-month can likely be explained through protein oxidation. Bao et al. (2021) observed that freezing, as well as the freeze/thaw cycle can lead to protein oxidation during which meat palatability is permanently altered. An unstable

protein matrix is illustrated by a decrease in slice shear force values (Leygonie et al., 2012), similar to those elicited from treatments of 6- and 9-month frozen storage.

Additionally, muscle type also imparts a significant impact on objective measures of beef tenderness, due largely to known differences in structure and function. In comparison, the LL represents a support muscle, which is inherently more tender due to a shorter sarcomere length, while the GM is considered a muscle of locomotion and is associated with greater toughness due to a greater amount of connective tissue. Previously, results have concluded that the LL is overall more tender than the GM and that such differences increase with storage duration (Colle et al., 2015; Vierck et al., 2020).

Volatile compound analysis

Of the 72 compounds evaluated for beef flavor development, 32 volatile compounds elicited a packaging type × muscle × freezing duration interaction ($P < 0.05$), 3 compounds were impacted by a packaging type × freezing duration interaction ($P < 0.05$) and 2 compounds were impacted by an interaction of muscle × freezing duration ($P < 0.05$). Additionally, the main effect of duration solely imparted a difference on 9 compounds ($P < 0.05$), while 3 compounds were impacted by muscle type alone ($P < 0.05$) and 5 compounds were impacted by packaging type as a main effect ($P < 0.05$). In total, 19 compounds were not impacted by packaging system, muscle or frozen storage duration ($P > 0.05$).

Interaction of packaging type, muscle and freezing duration

In evaluating the interactive effects of packaging type, muscle and frozen storage duration, OW GM steaks that were fresh, never frozen elicited the greatest concentration of lipid derived compounds including; 4 aldehydes (hexanal; nonanal; dodecanal; decanal), 4 alkenes (1-octene;

toluene; p-xylene; 2,4-dimethyl 1-heptene) 3 ketones (butyrolactone; 2-butanone; 2-propanone), 2 alcohols (2,3-butanediol; 1-octanol) 2 alkanes (tetradecane; 4-methyl-heptane), 2 esters (nonanoic acid, methyl ester; hexanoic acid, methyl ester), 2 carboxylic acids (acetic acid; butanoic acid) and 1 furan (2-pentyl furan). Additionally, fresh steaks of the OW GM also resulted in the greatest concentration of some Maillard derived products, those being; 3 Strecker aldehydes (acetaldehyde; isobutyraldehyde; phenylacetaldehyde), 2 ketones (2,3-butanedione; 3-hydroxy-2-butanone), 1 pyrazine (trimethylpyrazine) and methional. In comparison, fresh steaks from the VAC LL produced the highest concentration of pentane ($P = 0.03$) and pentanal ($P = 0.02$) both of which are products of lipid degradation. Fresh, VAC LL steaks also elicited the greatest concentration of the Maillard reaction product, methanethiol ($P= 0.008$). Furthermore, the sulfur-containing compound, dimethyl sulfide ($P =0.005$) derived from the Maillard reaction was of greatest concentration among VAC GM steaks subjected to 9-months of frozen storage.

The lipid-derived compounds of the packaging type × muscle × freezing duration interaction are known secondary products of lipid oxidation (Ross and Smith, 2006; Ponce, 2019; Vierck, 2020). Thus, it can be concluded that the combination of the aerobic environment of OW with the oxidation labile muscle of the GM as well as the intensified photo-oxidation from the retail lighting further enhanced the occurrence of lipid oxidation resulting in inflated concentration of lipid derived compounds. These results support the conclusion that the increased concentration of products elicited from lipid oxidation could be attributed to the oxygen permeability of OW which increased the likelihood of lipid oxidation occurrence (Ponce et al., 2019; Vierck et al., 2020). Furthermore, the permeability of the OW combined with the difference in known muscle and lipid stability of the GM and the LL would impact the development of lipid degradation compounds (Ponce et al, 2020). The GM contains less intramuscular fat, and a greater area of lean

tissue and therefore is more susceptible to lipid oxidation and oxidative rancidity due to decreased chemical stability from an increase in unsaturated fatty acid content (Ponce et al., 2020). Additionally, the GM is known to produce increased concentrations of Maillard intermediate products, specifically ketones that are closely associated with buttery flavors (Vierck et al., 2020).

Interaction of packaging type and freezing duration

The interaction of packaging type × freezing duration resulted in the highest concentration of the lipid-derived ketone, 2-pentanone ($P = 0.04$) among fresh OW steaks. Comparatively, VAC steaks in frozen storage for 1-month produced the highest concentration of the alcohol, 1-penten-3-ol ($P = 0.0002$) as well as the hydrocarbon, nonane ($P = 0.01$), both of which are secondary derivatives of lipid degradation. For both 1-penten-3-ol and nonane, concentration between VAC 1-month samples and OW fresh samples were similar ($P < 0.0001$)

The increased concentration of lipid-derived products among OW steaks from no or limited frozen storage durations further supports the conclusion that the aerobic environment of the packaging system, in combination with the impacts of the lighting conditions during retail display continues to have an overall negative impact on the rate of lipid oxidation. As reported by Ercolini et al. (2011) and Ponce et al., (2019), there is a difference in the development of lipid oxidation among products that are stored in anaerobic systems, in the absence of light compared to products that are packaged under aerobic conditions such as OW resulting in differences in overall flavor development. Additionally, the production of intermediate hydrocarbons and alcohols has previously been correlated to an increase in the incidence of lipid oxidation, especially in aerobic packaging systems (Hur et al., 2004; Vierck et al., 2020).

Interaction of muscle type and freezing duration

Two lipid-derived compounds were impacted by the interaction of muscle type × freezing duration (Table 2.). Among all treatments, fresh GM steaks elicited the highest concentration of the lipid-derived hydrocarbon, nonane ($P = 0.03$) while LL steaks from 1-month of frozen storage produced the greatest concentration of the lipid-derived alcohol, 1-penten-3-ol ($P = 0.004$).

The increased concentrations of lipid degradation products among fresh GM steaks provides further indication of the impact of muscle function and packaging integrity on beef flavor development. In alignment with the findings of Vierck and others (2020), the combination of aerobic conditions from OW packaging with the decreased oxidative stability of the GM results in increased lipid oxidation and therefore a higher concentration of compounds associated with less desirable flavors. More specifically, alcohols such as 1-penten-3-ol have previously been identified as compounds derived from the degradation of lipids via oxidation that negatively impact flavor (Garcia et al., 1991; Ponce et al., 2020). In cooked beef products, the undesirable off-flavors and aromas are closely linked to differences in the proportions of phospholipids and prooxidants that determine lipid oxidation product formation among differing muscle types (Legako et al., 2016; Ponce et al., 2020).

Impact of Duration, Muscle and Packaging as Main Effects

Certain volatile compounds were impacted only by the main effect of freezing duration (Table 2.). More specifically, specific products of the Maillard reaction were elicited at the highest concentration after 6-months of frozen storage, those being, 1 sulfur-containing compound (carbon disulfide, $P= 0.01$;) and 1 pyrazine (methyl pyrazine, $P= 0.04$). Contrastingly, fresh steaks also resulted in the highest concentration of a number of Maillard products, 3-methylbutanal ($P = 0.02$),

a Strecker aldehyde as well as 2,5 dimethylpyrazine (P = 0.001) and the sulfur-containing compound dimethyl sulfone (P = 0.003). Furthermore, lipid-derived products including an aldehyde (octanal, $P < 0.0001$), hydrocarbon (decane, $P < 0.0001$) and 2 alcohols (1-octen-3-ol; 1-pentanol) were also greatest among fresh steaks that were subjected to no period of frozen storage

Packaging type also elicited an impact on flavor development as a main effect. Steaks that were packaged in OW resulted in the highest concentration of Butanoic acid, methyl ester (P =0.03;), as well as decane (P =0.01) and octanal (P =0.02), all of which are products of lipid degradation

Additionally, muscle type impacted volatile compound concentrations as a main effect. In greater detail, LL steaks elicited the highest concentration of two lipid-derived products, methyl-propionate (P = 0.007), a carboxylic ester as well as the aldehyde 2-methylbutanal (P = 0.001).

These results further indicate that duration, muscle and packaging all impart an impact on the development of beef flavor, however these effects are not necessarily independent.

Trained Panel Sensory Evaluation

An interaction was observed between muscle × frozen storage duration for beef flavor identity, oxidized and sour attributes ($P \leq 0.04$; Table 2.6). The GM frozen 6-months rated lower than all other treatments for beef flavor identity ($P < 0.01$) but similar to GM frozen 9-months ($P > 0.05$). No difference was observed for beef flavor identity in LL across all freezing durations ($P > 0.05$). Generally, oxidized ratings increased alongside an increase in freezing duration: Fresh < 1 Week < 1 Month < 6-month < 9-month. The GM steaks were higher than LL steaks for oxidized ratings after 9-months of frozen storage (P = 0.04). The GM also rated higher for sour

after both 6- and 9-months frozen storage in comparison to the LL ($P < 0.01$). In contrast, the GM elicited the lowest oxidized ratings in fresh steaks ($P < 0.05$) and was similar to LL steaks after fresh (not frozen) and 1-week frozen storage ($P > 0.05$).

A package type × frozen storage duration interaction was observed for beef flavor identity, oxidized, refrigerator-stale and sour (Table 2.7; $P < 0.01$). Beef flavor identity ratings for OW steaks generally decreased with each freezing duration: Fresh > 1 Week > 1 Month > 6 Month > 9 Month. Comparatively, as predicted, oxidized and refrigerator-stale ratings increased with each cold storage duration for OW steaks: Fresh < 1 Week < 1 Month < 6 Month < 9 Month. For both OW and VAC steaks, sour ratings were higher after 6-months of frozen storage than all other treatments and especially lower in fresh (not frozen) steaks ($P < 0.01$). Also, VAC steaks following 6- and 9-months freezing duration, were rated to be more sour than GM steaks ($P < 0.01$). There was no muscle type × frozen storage duration interaction for ratings of brown roasted, bloody-serumy, fat like, liver like, fishy, cardboard, rancid, refrigerator stale, umami, bitter, overall tenderness, or overall juiciness ($P > 0.05$). In addition, no interaction was observed for packaging type × muscle in regard to beef flavor identity, brown roasted, bloody-serumy, fat-like, liver like, fishy, oxidized, cardboard, rancid, refrigerator stale, umami, bitter, sour, or overall tenderness ($P > 0.05$).

Overall juiciness was impacted by the interaction of packaging type × muscle (Table 2.8; $P < 0.01$). Ratings for overall juiciness were lowest for OW GM compared to all other treatments ($P < 0.01$). Juiciness ratings for OW LL, VAC GM and VAC LL were all similar in value ($P > 0.05$).

The main effect of packaging type is presented in Table 2.9. Steaks packaged in OW were rated as more brown roasted, with more fishy and cardboard off-flavors ($P < 0.05$). Additionally, VAC steaks rated higher for attributes of bloody-serumy, umami, and tenderness compared to OW steaks ($P < 0.01$). Packaging type did not affect ratings of beef flavor identity, fat like, liver like, rancid, or bitter ($P > 0.05$).

The main effect of muscle type is represented in Table 2.9. Steaks of the LL rated higher than GM for traits of bloody-serumy, fat-like, umami, and tenderness. Additionally, GM steaks were higher for liver like, rancid, and refrigerator stale ratings ($P < 0.05$). There was no significant difference in muscle for brown roasted, fishy, cardboard, or bitter ($P > 0.05$).

The main effect of frozen storage duration is represented in Table 2.10. As storage time increased, ratings for bloody-serumy, fat-like and umami generally decreased. Bloody-serumy displayed the highest ratings in fresh (not frozen) steaks and the lowest ratings in steaks previously frozen for 6 and 9 months ($P < 0.01$). Fat-like and Umami traits decreased as freezing duration increased: Fresh = 1 Week > 1 Month > 6 Month > 9 Month ($P < 0.01$). Liver-like displayed the highest rating after 6 months of frozen storage ($P < 0.01$). Cardboard off-flavors were highest after 9 months of frozen storage, but similar to 1-week and 1-month freezing durations ($P < 0.02$). Compared to all other duration treatments, steaks that were frozen for 6 months elicited the highest ratings for rancidity ($P < 0.01$). Furthermore, as frozen storage duration increased, bitter ratings also increased: Fresh < 1 Week < 1 Month < 6 Month < 9 Month ($P < 0.01$). Steaks frozen for 9-months, 6-months and stored fresh (not frozen) were rated to be more tender than 1-month frozen steaks. Finally, fresh and 9-Month frozen steaks were rated higher for overall juiciness than all other treatments ($P < 0.01$).

These results suggest that aerobic packaging conditions in combination with extended periods of frozen storage and muscle type result in an overall negative impact on subjective beef flavor. The differences observed during trained panel analysis can largely be attributed to packaging type and muscle function. Similar to Ponce et al. (2019) and Vierck et al. (2020), packaging systems such as OW, are likely to produce increased ratings in oxidized, cardboardy and sour flavors as a result of an increase in lipid oxidation due to packaging permeability. More specifically, while concentrations of lipid derived volatile compounds were greatest among fresh OW samples, the increase in oxidative off-flavors after extended periods of frozen storage can likely be attributed to ice crystallization and the increased incidence of freezer-burn (Dang et al., 2021). Furthermore, the increase in tenderness ratings after 6 and 9 months of frozen storage supports the findings of Setyabrata and Kim. (2019), which observed improved tenderness from frozen storage. As expected, samples from the LL rated more tender than those from the GM, regardless of packaging type or freezing duration. This observation is supported by Vierck et al. (2020) in which trained panelists rated steak from the LL to also be more tender than those from the GM.

Conclusion

The results of this study indicate that while frozen storage duration, muscle type and packaging system each impacts tenderness and juiciness the individual treatments were not independent of one another in terms of beef flavor development. Furthermore, the impact of specific factors on attributes of beef palatability was dependent upon measurement type, whether that be instrumental or subjective techniques. Therefore, it can be concluded that individual attributes of beef palatability are impacted differently based upon storage duration, muscle type and packaging system as well the inherent interaction of each of these factors.

Based on the results, it is evident that throughout frozen storage, certain physiochemical changes occur that could impact final beef palatability and therefore the consumer eating experience. These disruptions to the muscle structure are due in large part to the behavior of ice crystallization that occurs throughout extended periods of frozen storage, which would especially impart a difference in factors such as juiciness throughout the freeze/thaw cycle. Despite the impact of frozen storage duration on factors of tenderness and juiciness however, it is evident that beef flavor development is most influenced by the interaction of duration, muscle and packaging, especially in the extreme scenario of an OW GM steak fresh that was only subjected to retail display and never frozen. It is clear that palatability traits are impacted differently and that in terms of beef flavor development, no consumer handling practices are independent of one another.

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Tables and Figures

Table 2.1 Interaction of package type[1] × muscle[2] × freezing duration[3] on percent expressible moisture of frozen – thawed beef steaks *(*n = 320*)* from the *Longissimus lumborum* and *Gluteus medius*

	% EM	
	Gluteus medius	*Longissimus lumborum*
VAC		
Fresh	15.05^{bcde}	9.99^{gh}
1-week	18.00^{abc}	16.32^{abcde}
1-month	14.40^{cdef}	15.59^{bcde}
6-month	18.61^{ab}	16.96^{abcd}
9-month	15.10^{bcde}	15.34^{bcde}
SEM[4]	1.37	1.35
OW		
Fresh	10.78^{fgh}	8.68^{h}
1-week	15.25^{bcde}	16.12^{bcde}
1-month	12.75^{efg}	14.41^{cdef}
6-month	15.29^{bcde}	16.48^{abcd}
9-month	20.01^{a}	13.70^{defg}
SEM	1.37	1.44
P-value	< .0001	< .0001

[1]Package types include both vacuum packaging (VAC) and PVC overwrap (OW).
[2]Muscles include beef steaks from both the *Gluteus medius* and *Longissimus lumborum.*
[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[4]SE (largest) of the least square means in the same main effect (packaging type or muscle).
^{abcdefgh}Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.2 Interaction of muscle[1] × freezing duration[2] on percent expressible moisture of frozen – thawed beef steaks (n = 320*)* from the *Longissimus lumborum* and *Gluteus medius*

	% Expressible Moisture
Gluteus medius	
Fresh	12.91[a]
1-week	16.63[ab]
1-month	13.58[cd]
6-month	16.95[ab]
9-month	17.55[a]
SEM[3]	0.97
P-value	<.0001
Longissimus lumborum	
Fresh	9.34[e]
1-week	16.22[ab]
1-month	15.00[abcd]
6-month	16.72[ab]
9-month	14.52[bcd]
SEM	0.99
P-value	< .0001

[1]Muscles include steaks from the *Gluteus medius* and *Longissimus lumborum.*
[2]Freezing durations include Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[3]SE (largest) of the least square means in the same main effect (muscle).
[abcde]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.3 Least square means for expressible moisture of beef steaks (n = 320*)* from two muscles[1] stored in two packaging types[2] for five different freezing durations[3]

Treatment	% Expressible Moisture
Duration	
Fresh	11.13[c]
1-week	16.43[a]
1-month	14.29[b]
6-month	16.83[a]
9-month	16.04[ab]
SEM	0.71
P-value	< .0001
Muscle	
GM	15.52
LL	14.36
SEM	0.42
P-value	0.05
Packaging	
OW	14.35[b]
VAC	15.54[a]
SEM	0.42
P-value	0.04
Packaging Type × Freezing Duration	
P-value	0.18
Packaging Type × Muscle	
P-value	0.70

[1]Muscles include beef steaks from both the *Gluteus medius* and *Longissimus lumborum.*
[2]Packaging includes OW and VAC.
[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[4]SE (largest) of the least square means in the same main effect (packaging type or freezing duration).
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.4 Least-square means for proximate analysis of raw USDA[1] Low Choice beef *Longissimus lumborum* and *Gluteus medius* steaks

Muscle Type	Fat %	Moisture %	Protein %
Longissimus lumborum	5.81[a]	70.58[b]	23.78
Gluteus medius	3.81[b]	72.21[a]	23.57
SEM[2]	0.44	0.34	0.16
P-value	<0.01	<0.01	0.33

[1]United States Department of Agriculture.
[2]SEM (largest) of the least-squares means

Table 2.5 least square means for cook loss of beef steaks (n = 320) from two muscles[1] stored in two packaging types[2] for five different freezing durations[3]

Treatment	% Cook Loss
Duration	
Fresh	18.5[bc]
1-week	19.9[b]
1-month	19.3[bc]
6-month	17.8[c]
9-month	25.4[a]
SEM[4]	0.7
P-value	< .0001
Muscle	
GM	21.2[a]
LL	19.1[b]
SEM	0.4
P-value	0.0008
Package	
OW	19.6
VAC	20.7
SEM	0.4
P-value	0.05
Packaging Type × Freezing Duration	
P-value	0.14
Packaging Type × Muscle	
P-value	0.50
Muscle × Freezing Duration	
P-value	0.71
Package Type × Muscle × Freezing Duration	
P-value	0.17

[1]Muscles include beef steaks from both the *Gluteus medius* and *Longissimus lumborum.*
[2]Packaging includes OW and VAC.
[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[4]SE (largest) of the least square means in the same main effect (freezing duration or muscle).
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.6 Least Square Means for Sliced Shear Force of Cooked Beef Steaks (n = 320) from Two Muscles[1] Stored in Two Packaging Types[2] for Five Different Freezing Durations[3]

Treatment	SSF (kg/f)
Duration	
Fresh	11.7[b]
1-week	14.5[a]
1-month	4.6[c]
6-month	11.8[b]
9-month	13.1[ab]
SEM[4]	0.68
P-value	< .0001
Muscle	
GM	12.0[a]
LL	16.4[b]
SEM	0.40
P-value	0.0054
Package	
OW	11.2
VAC	11.1
SEM	0.40
P-value	0.93
Packaging Type × Freezing Duration	
P-value	0.95
Packaging Type × Muscle	
P-value	0.79
Muscle × Freezing Duration	
P-value	0.59
Package Type × Muscle × Freezing Duration	
P-value	0.83

[1]Muscles include beef steaks from both the *Gluteus medius* and *Longissimus lumborum.*
[2]Packaging includes OW and VAC.
[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[4]SE (largest) of the least square means in the same main effect (freezing duration or muscle).
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$

Table 2.7 Interaction of Packaging Type [1] × Muscle[2] × Frozen Storage Duration [3] on Volatile Compound Analysis of Lipid Derived Aldehydes and Ketones from Cooked Beef Steaks from the *Longissimus lumborum* and *Gluteus medius*

	Aldehydes (ng/g sample)					Ketones (ng/g sample)		
Treatment	*Hexanal*	*Nonanal*	*Dodecanal*	*Decanal*	*Pentanal*	*Butyrolactone*	*2-Butanone*	*2-Propanone*
VAC, GM								
Fresh	58.97^{de}	27.33^{bc}	43.03^{b}	243.35^{bcd}	27.44^{bcd}	447.26^{cd}	7.27^{bcd}	31.29^{b}
1-week	44.09^{de}	18.93^{bcde}	28.53^{b}	227.80^{bcde}	22.08^{cdefg}	398.71^{d}	5.69^{cde}	17.61^{bcd}
1-month	54.03^{de}	19.65^{bcde}	35.55^{b}	249.85^{bc}	22.51^{cdef}	448.67^{cd}	5.80^{cde}	23.97^{bcd}
6-month	70.61^{de}	11.27^{def}	24.95^{b}	125.25^{def}	6.64^{gh}	372.93^{d}	3.12^{e}	15.67^{bcd}
9-month	58.12^{de}	13.06^{def}	22.36^{b}	142.23^{cdef}	6.87^{fgh}	326.20^{d}	3.53^{de}	20.79^{bcd}
SEM[4]	40.32	4.81	15.72	46.4	27.44	329.10	1.50	7.11
OW, GM								
Fresh	303.14^{a}	54.19^{a}	116.37^{a}	466.48^{a}	39.73^{ab}	1888.55^{a}	11.77^{a}	83.05^{a}
1-week	40.12^{de}	11.28^{def}	23.44^{b}	128.24^{def}	9.03^{efgh}	312.18^{d}	3.43^{de}	20.37^{bcd}
1-month	64.82^{de}	10.62^{ef}	16.05^{b}	174.37^{cdef}	7.53^{efgh}	385.13^{d}	3.38^{de}	18.47^{bcd}
6-month	35.82^{de}	11.39^{def}	17.52^{b}	168.33^{cdef}	6.31^{gh}	362.28^{d}	3.66^{de}	22.07^{bcd}
9-month	19.87^{e}	7.13^{ef}	11.66^{b}	143.21^{cdef}	3.58^{h}	310.49^{d}	3.09^{e}	14.80^{bcd}
SEM	32.33	4.98	15.72	46.41	39.73	208.14	1.50	7.11
VAC, LL								
Fresh	169.91^{bc}	31.03^{b}	52.01^{b}	315.40^{b}	45.82^{a}	1232.44^{b}	11.18^{ab}	30.35^{bc}
1-week	54.66^{de}	19.43^{bcde}	31.33^{b}	206.36^{bcdef}	23.14^{cde}	868.88^{bcd}	5.58^{cde}	12.51^{bcd}
1-month	180.52^{b}	23.94^{bcd}	94.55^{a}	261.62^{bc}	32.78^{abc}	1186.38^{bc}	8.55^{abc}	16.98^{bcd}
6-month	73.40^{de}	8.95^{ef}	21.29^{b}	116.78^{ef}	6.80^{gh}	524.72^{cd}	3.14^{e}	6.36^{d}
9-month	64.57^{de}	8.47^{ef}	17.81^{b}	102.84^{f}	7.31^{efgh}	490.09^{cd}	3.72^{de}	8.55^{d}
SEM	34.92	4.66	15.72	44.84	45.82	248.78	1.45	6.88
OW, LL								
Fresh	80.98^{cde}	14.68^{cdef}	18.97^{b}	164.60^{cdef}	13.84^{defgh}	586.52^{cd}	4.47^{de}	12.35^{bcd}
1-week	69.66^{de}	11.77^{def}	21.82^{b}	152.31^{cdef}	12.19^{defgh}	432.75^{d}	4.12^{de}	11.54^{cd}
1-month	115.90^{bcd}	12.68^{def}	19.52^{b}	175.12^{cdef}	13.72^{defgh}	633.99^{cd}	5.09^{cde}	12.54^{bcd}
6-month	25.86^{de}	4.11^{f}	12.56^{b}	94.81^{f}	2.48^{h}	409.26^{d}	2.79^{e}	7.94^{d}
9-month	40.81^{de}	9.17^{ef}	14.37^{b}	153.80^{cdef}	5.26^{h}	615.30^{cd}	3.80^{de}	13.87^{bcd}
SEM	36.47	4.98	16.27	46.41	13.84	198.46	1.55	7.36
P-Value	0.0008	0.002	0.04	0.004	0.02	0.005	0.02	0.0003

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3] Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.
[4]SE (largest) of the least square means in the same column.
[a-h]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.8 Interaction of packaging type [1] × muscle[2] × frozen storage duration [3] on volatile compound analysis of lipid derived hydrocarbons and alcohols from cooked beef steaks of the *Longissimus lumborum* and *Gluteus medius*

	Hydrocarbons (ng/g sample)						Alcohols (ng/g sample		
Treatment	*1-Octene*	*Pentane*	*Toluene*	*p-Xylene*	*2.4 Dimethyl 1-Heptene*	*Tetradecane*	*4-methyl-heptane*	*2,3-Butanediol*	*1-Octanol*
VAC, GM									
Fresh	14.38^{bc}	19.67^{bc}	37.77^{bcde}	3614.70^{c}	651.24^{bc}	21.57^{bc}	2.57^{b}	13.46^{b}	7.76^{bc}
1-week	10.35^{cde}	15.94^{c}	30.74^{bcde}	3113.48^{c}	506.30^{bcd}	11.95^{cde}	1.97^{bc}	10.24^{b}	2.95^{de}
1-month	12.07^{cde}	16.37^{c}	37.42^{bcde}	3166.71^{c}	600.66^{bc}	16.17^{bcde}	2.27^{bc}	13.45^{b}	4.32^{cde}
6-month	6.01^{cde}	4.84^{c}	10.173^{e}	2956.94^{c}	327.99^{cd}	8.95^{cde}	1.05^{bc}	11.67^{b}	3.42^{cde}
9-month	5.84^{cde}	12.31^{c}	2.92^{e}	2924.00^{c}	532.58^{bcd}	5.91^{de}	0.76^{bc}	11.90^{b}	3.09^{cde}
SEM[4]	3.46	7.97	13.24	1057.12	153.13	5.03	0.74	4.47	1.70
OW, GM									
Fresh	26.59^{a}	34.88^{ab}	134.^{a}	10967.00^{a}	1246.31^{a}	41.83^{a}	5.17^{a}	48.73^{a}	13.62^{a}
1-week	6.70^{cde}	6.34^{c}	50.75^{b}	6656.52^{b}	448.48^{cd}	8.62^{cde}	0.95^{bc}	16.06^{b}	2.88^{de}
1-month	4.60^{de}	4.94^{c}	46.59^{bc}	4808.45^{bc}	341.39^{cd}	9.38^{cde}	0.69^{bc}	15.53^{b}	3.06^{de}
6-month	6.34^{cde}	5.54^{c}	6.15^{e}	3399.84^{c}	353.01^{cd}	6.35^{de}	0.80^{bc}	16.37^{b}	2.74^{e}
9-month	3.90^{e}	4.68^{c}	7.97^{e}	3298.52^{c}	218.77^{cd}	5.26^{e}	0.67^{bc}	8.88^{b}	2.74^{e}
SEM	3.46	8.40	13.24	1084.87	200.49	4.86	0.77	4.47	1.76
VAC, LL									
Fresh	23.78^{a}	41.45^{a}	57.60^{b}	5327.98^{bc}	927.95^{ab}	26.06^{b}	4.67^{a}	16.55^{b}	10.11^{ab}
1-week	13.62^{bcd}	17.33^{bc}	31.40^{bcde}	2963.16^{c}	562.08^{bcd}	15.45^{bcde}	2.49^{b}	9.68^{b}	5.06^{cde}
1-month	22.63^{ab}	21.35^{bc}	44.50^{bcd}	4192.08^{bc}	923.17^{ab}	19.18^{bcd}	4.86^{a}	19.71^{b}	7.43^{bcd}
6-month	7.43^{cde}	5.49^{c}	3.50^{e}	3326.32^{c}	410.75^{cd}	6.87^{de}	1.07^{bc}	9.11^{b}	2.85^{de}
9-month	4.95^{de}	4.79^{c}	7.33^{e}	2941.77^{c}	346.22^{cd}	5.69^{de}	0.51^{bc}	8.34^{b}	2.90^{de}
SEM	3.34	7.27	12.81	1057.12	159.94	5.03	0.71	4.32	1.70
OW, LL									
Fresh	11.08^{cde}	9.61^{c}	47.54^{bc}	5279.40^{bc}	503.95^{bcd}	11.70^{cde}	1.90^{bc}	13.33^{b}	2.98^{de}
1-week	8.67^{cde}	8.07^{c}	45.08^{bcd}	5161.15^{bc}	484.10^{cd}	8.05^{de}	1.31^{bc}	13.01^{b}	3.35^{cde}
1-month	10.06^{cde}	8.99^{c}	54.72^{b}	5404.60^{bc}	500.46^{bcd}	13.23^{bcde}	1.47^{bc}	14.91^{b}	3.97^{cde}
6-month	4.34^{de}	1.99^{c}	5.02^{e}	2974.66^{c}	173.41^{d}	5.41^{e}	0.72^{bc}	8.23^{b}	2.70^{e}
9-month	6.30^{cde}	4.55^{c}	13.45^{cde}	4197.10^{bc}	356.90^{cd}	6.61^{de}	1.13^{bc}	11.51^{b}	2.68^{e}
SEM	3.59	7.97	13.70	1122.95	167.74	5.03	0.77	4.62	1.76
P-Value	0.02	0.03	0.008	0.003	0.02	0.03	0.02	0.004	0.02

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).

[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.

[3]Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.

[4]SE (largest) of the least square means in the same column.

[a-e]Least square means in the same column without a common superscript differ ($P < 0.05$)

Table 2.9 Interaction of Packaging Type [1] × Muscle[2] × Frozen Storage Duration [3] on Volatile Compound Analysis of Lipid Derived Products from Cooked Beef Steaks of the *Longissimus lumborum* and *Gluteus medius*

	Esters (ng/g sample)		Carboxylic Acids (ng/g sample)		Furan (ng/g sample)
Treatment	*Nonanoic acid, methyl ester*	*Hexanoic acid, methyl ester*	*Acetic acid*	*Butanoic acid*	*2-pentyl furan*
VAC, GM					
Fresh	0.40^{bc}	1.40^{bcd}	7.83^{bcd}	14.62^{b}	4.42^{bc}
1-week	0.34^{bcd}	1.36^{bcd}	6.56^{bcd}	6.94^{b}	3.76^{bcde}
1-month	0.3372^{bcd}	1.29^{bcd}	8.10^{bcd}	12.35^{b}	3.07^{bcdef}
6-month	0.26^{d}	0.77^{cd}	6.47^{bcd}	13.69^{b}	1.52^{ef}
9-month	0.24^{d}	0.80^{cd}	4.99^{d}	18.27^{b}	2.04^{cdef}
SEM[4]	0.34	0.45	2.51	5.06	0.90
OW, GM					
Fresh	0.643^{a}	4.02^{a}	24.94^{a}	54.16^{a}	9.74^{a}
1-week	0.26^{d}	1.86^{bc}	5.69^{d}	17.45^{b}	2.21^{cdef}
1-month	0.31^{bcd}	1.48^{bcd}	5.68^{d}	17.26^{b}	1.83^{def}
6-month	0.31^{bcd}	0.87^{bcd}	6.38^{bcd}	21.39^{b}	1.68^{def}
9-month	0.24^{d}	0.91^{bcd}	4.24^{d}	10.44^{b}	1.46^{ef}
SEM	0.64	0.45	2.51	5.98	0.90
VAC, LL					
Fresh	0.43^{b}	2.03^{b}	12.77^{bc}	15.08^{b}	4.89^{b}
1-week	0.35^{bcd}	1.21^{bcd}	6.09^{cd}	7.67^{b}	2.83^{bcdef}
1-month	0.37^{bcd}	1.20^{bcd}	12.97^{b}	17.59^{b}	4.09^{bcd}
6-month	0.25^{d}	0.92^{bcd}	5.31^{d}	12.47^{b}	1.31^{ef}
9-month	0.27^{cd}	0.71^{d}	4.89^{d}	12.21^{b}	1.37^{ef}
SEM	0.060	0.45	2.43	5.06	0.90
OW, LL					
Fresh	0.33^{bcd}	1.58^{bcd}	6.36^{bcd}	12.56^{b}	2.77^{bcdef}
1-week	0.34^{bcd}	1.34^{bcd}	6.07^{cd}	13.01^{b}	1.92^{cdef}
1-month	0.28^{cd}	1.61^{bcd}	6.97^{bcd}	12.43^{b}	1.91^{cdef}
6-month	0.26^{d}	0.62^{d}	4.55^{d}	12.03^{b}	0.80^{f}
9-month	0.25^{d}	0.89^{bcd}	4.81^{d}	13.13^{b}	2.02^{cdef}
SEM	0.34	0.43	2.60	5.25	0.93
P-Value	0.01	0.01	0.001	0.007	0.004

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3] Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.
[4]SE (largest) of the least square means in the same column.
[a-f]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.10 Interaction of packaging type [1] × muscle[2] × frozen storage duration [3] on volatile compound analysis of Maillard reaction Strecker aldehydes and ketones from cooked beef steaks of the *Longissimus lumborum* and *Gluteus medius*

	Strecker Aldehydes (ng/g of sample)			Ketones (ng/g of sample)	
Treatment	*Acetaldehyde*	*Phenylacetaldehyde*	*Isobutyracetaldehyde*	*2,3-butanedione*	*3-hydroxy-2-butanone*
VAC, GM					
Fresh	17.92^{cd}	0.50^{bc}	1.12^{bc}	27.75^{b}	17.57^{cd}
1-week	11.71^{cd}	0.38^{c}	1.79^{bc}	23.17^{bcde}	14.58^{d}
1-month	16.63^{cd}	0.44^{c}	1.74^{bc}	26.34^{bc}	19.13^{cd}
6-month	8.36^{d}	0.36^{c}	1.04^{bc}	10.65^{ef}	20.26^{cd}
9-month	7.22^{d}	0.39^{c}	1.08^{bc}	11.68^{ef}	24.81^{bcd}
SEM[4]	6.03	0.50	0.46	4.78	6.60
OW, GM					
Fresh	50.27^{a}	1.02^{a}	5.34^{a}	56.41^{a}	69.08^{a}
1-week	5.88^{d}	0.32^{c}	1.02^{c}	18.25^{bcdef}	26.29^{bcd}
1-month	8.38^{d}	0.33^{c}	1.21^{bc}	17.28^{bcdef}	22.94^{bcd}
6-month	6.54^{d}	0.41^{c}	1.34^{bc}	16.12^{bcdef}	40.46^{b}
9-month	4.37^{d}	0.25^{c}	1.16^{bc}	13.66^{def}	32.72^{bc}
SEM	6.03	0.12	0.65	4.78	6.60
VAC, LL					
Fresh	36.24^{ab}	0.81^{a}	1.37^{bc}	24.85^{bcd}	18.47^{cd}
1-week	15.60^{cd}	0.39^{c}	2.12^{b}	13.96^{cdef}	10.71^{d}
1-month	24.98^{bc}	0.78^{ab}	1.66^{bc}	23.20^{bcde}	24.10^{bcd}
6-month	6.18^{d}	0.40^{c}	1.32^{bc}	8.61^{f}	16.66^{cd}
9-month	7.56^{d}	0.35^{c}	1.61^{bc}	8.15^{f}	14.39^{d}
SEM	5.84	0.81	0.46	4.94	6.39
OW, LL					
Fresh	10.63^{cd}	0.30^{c}	1.40^{bc}	11.27^{ef}	14.59^{d}
1-week	9.87^{cd}	0.33^{c}	1.43^{bc}	10.92^{ef}	15.11^{cd}
1-month	13.66^{cd}	0.32^{c}	1.56^{bc}	12.12^{def}	17.26^{cd}
6-month	2.52^{d}	0.27^{c}	1.43^{bc}	8.61^{f}	20.88^{cd}
9-month	8.70^{d}	0.30^{c}	1.61^{bc}	11.37^{ef}	26.00^{bcd}
SEM	6.24	0.32	0.41	4.94	6.83
P-Value	0.0008	0.003	0.001	0.003	0.01

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3] Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.
[4]SE (largest) of the least square means in the same column.
[a-f]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.11 Interaction of packaging type [1] × muscle[2] × frozen storage duration [3] on volatile compound analysis of Maillard reaction products from cooked beef steaks of the *Longissimus lumborum* and *Gluteus medius*

	Compound ng/g sample		
Treatment	*Trimethylpyrazine*	*Methional*	*Methanethiol*
VAC, GM			
Fresh	0.22^{bc}	72.01^{bcd}	0.84^{b}
1-week	0.20^{bc}	63.17^{bcdef}	0.39^{b}
1-month	0.21^{bc}	60.17^{bcdef}	0.83^{b}
6-month	0.32^{bc}	28.00^{ef}	0.24^{b}
9-month	0.15^{c}	27.34^{ef}	0.38^{b}
SEM[4]	0.32	16.29	1.95
OW, GM			
Fresh	1.07^{a}	197.83^{a}	7.78^{a}
1-week	0.09^{c}	86.44^{b}	0.20^{b}
1-month	0.16^{c}	69.53^{bcde}	0.32^{b}
6-month	0.18^{c}	27.89^{ef}	0.45^{b}
9-month	0.12^{c}	27.67^{ef}	0.12^{b}
SEM	0.13	16.29	1.88
VAC, LL			
Fresh	0.59^{b}	97.79^{b}	8.57^{a}
1-week	0.32^{bc}	58.61^{bcdef}	0.34^{b}
1-month	0.46^{bc}	64.37^{bcde}	1.38^{b}
6-month	0.22^{bc}	28.25^{def}	0.21^{b}
9-month	0.37^{bc}	28.54^{def}	0.31^{b}
SEM	0.15	15.77	1.82
OW, LL			
Fresh	0.22^{bc}	69.95^{bcde}	0.41^{b}
1-week	0.16^{c}	72.49^{bc}	0.69^{b}
1-month	0.27^{bc}	66.56^{bcde}	0.68^{b}
6-month	0.25^{bc}	19.16^{f}	0.14^{b}
9-month	0.19^{c}	33.14^{cdef}	0.79^{b}
SEM	0.15	16.86	0.69
P-Value	0.001	0.001	0.008

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3] Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.
[4]SE (largest) of the least square means in the same column.
[a-f]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.12 Interaction of packaging type [1] × frozen storage duration [2] on volatile compound analysis of lipid degradation products from cooked beef steaks of the *Longissimus lumborum* and *Gluteus medius*

	Compound ng/g of sample		
Treatment	*2-pentanone*	*1-penten-3-ol*	*Nonane*
OW			
Fresh	0.05^{a}	0.08^{ab}	0.54^{ab}
1-week	0.01^{bc}	0.03^{c}	0.38^{bcd}
1-month	0.02^{abc}	0.03^{c}	0.32^{cd}
6-month	0.02^{abc}	0.05^{bc}	0.25^{d}
9-month	0.02^{abc}	0.03^{c}	0.24^{d}
SEM[3]	0.01	0.01	0.06
VAC			
Fresh	0.01^{abc}	0.03^{c}	0.46^{bc}
1-week	0.04^{ab}	0.07^{ab}	0.48^{abc}
1-month	0.04^{ab}	0.09^{a}	0.64^{a}
6-month	0.01^{bc}	0.03^{c}	0.27^{d}
9-month	0.01^{c}	0.03^{c}	0.26^{d}
SEM	0.01	0.01	0.06
P-value	0.04	0.0002	0.01

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2] Frozen storage duration includes fresh (not frozen), 1-week, 1-month, 6-months and 9-months.
[3]SE (largest) of the least square means in the same column.
[a-d]Least square means in the same column without a common superscript differ ($P < 0.05$)

Table 2.13 Interaction of muscle type [1] × frozen storage duration [2] on volatile compound analysis of lipid degradation products from cooked beef steaks of the *Longissimus lumborum* and *Gluteus medius*

	Compound ng/g of sample	
Treatment	*1-penten-3-ol*	*Nonane*
LL		
Fresh	0.03^{c}	0.40^{bcd}
1-week	0.06^{ab}	0.48^{abc}
1-month	0.08^{a}	0.57^{ab}
6-month	0.04^{abc}	0.24^{e}
9-month	0.04^{abc}	0.26^{ed}
SEM[3]	0.01	0.06
GM		
Fresh	0.08^{a}	0.60^{a}
1-week	0.03^{bc}	0.39^{cde}
1-month	0.04^{abc}	0.40^{bcd}
6-month	0.04^{bc}	0.29^{ed}
9-month	0.02^{c}	0.23^{e}
SEM	0.01	0.06
P-value	0.004	0.03

[1]Muscles include steaks from the *Gluteus medius* and *Longissimus lumborum.*
[2]Freezing durations include Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[3]SE (largest) of the least square means in the same main effect (muscle).
[a-e]east square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.14 Least square means for volatile compound analysis of Maillard derived products from beef steaks from two muscles[1] stored in two packaging types[2] for five different freezing durations[3]

	Compounds ng/g of sample					
Treatment	*Carbon Disulfide*	*Methyl Pyrazine*	*3-Methylbutanal*	*2,5 dimethylpyrazine*	*2-Methylbutanal*	*Dimethyl Sulfone*
Duration						
Fresh	2.73^{ab}	0.04^{b}	0.12^{a}	0.30^{a}	0.02	0.60^{a}
1-week	0.69^{c}	0.02^{b}	0.05^{b}	0.08^{c}	0.01	0.28^{b}
1-month	1.25^{bc}	0.04^{b}	0.08^{ab}	0.14^{bc}	0.02	0.39^{b}
6-month	2.90^{a}	0.20^{a}	0.08^{ab}	0.21^{ab}	0.01	0.18^{b}
9-month	1.97^{abc}	0.16^{ab}	0.07^{b}	0.23^{ab}	0.01	0.23^{b}
SEM	0.53	0.05	0.02	0.04	0.004	0.07
P-value	0.01	0.04	0.02	0.0013	0.63	0.003
Muscle						
GM	2.22	0.07	0.08	0.18	0.01^{b}	0.31
LL	1.60	0.12	0.88	0.21	0.02^{a}	0.36
SEM	0.33	0.03	0.01	0.02	0.002	0.05
P-value	0.19	0.20	0.71	0.31	0.001	0.57
Packaging						
OW	2.20	0.09	0.09	0.19	0.01	0.30
VAC	1.62	0.10	0.07	0.19	0.01	0.37
SEM	0.33	0.03	0.01	0.02	0.002	0.06
P-value	0.22	0.96	0.39	0.99	0.80	0.38
Packaging × Muscle						
P-value	0.90	0.32	0.29	0.56	0.75	0.26

[1]Muscles include beef steaks from both the *Glueteus medius* and *Longissimus lumborum.*

[2]Packaging includes OW and VAC.

[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.

[4]SE (largest) of the least square means in the same main effect (packaging type or freezing duration).

[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.15 Least square means for volatile compound analysis of lipid derived products from beef steaks from two muscles[1] stored in two packaging types[2] for five different freezing durations[3]

	Compounds ng/g of sample					
Treatment	*Methyl Propinate*	*Butanoic acid, methyl ester*	*Octanal*	*Decane*	*1-Octen-3-ol*	*1-pentanol*
Duration						
Fresh	0.73	0.05	9.98[a]	77.35[a]	64.91[a]	2.39[a]
1-week	0.72	0.06	5.86[b]	43.71[b]	35.68[b]	1.59[b]
1-month	0.77	0.07	5.88[b]	53.37[b]	43.70[b]	1.64[b]
6-month	0.77	0.11	2.57[c]	18.53[c]	17.30[c]	1.24[b]
9-month	0.72	0.05	2.79[c]	18.50[c]	17.10[c]	1.00[b]
SEM[4]	0.03	0.02	0.76	6.35	5.23	0.32
P-value	0.61	0.49	<.0001	<.0001	<.0001	0.001
Muscle						
GM	0.70[b]	0.07	5.64	42.54	37.24	1.53
LL	0.78[a]	0.07	5.20	42.04	34.23	1.62
SEM	0.02	0.01	0.48	4.01	3.30	0.16
P-value	0.007	0.88	0.52	0.93	0.51	0.71
Packaging						
OW	0.75	0.09[a]	6.17[a]	35.17[b]	29.39[b]	1.40
VAC	0.73	0.04[b]	4.66[b]	49.41[a]	42.09[a]	1.75
SEM	0.02	0.01	0.48	4.02	3.26	0.17
P-value	0.39	0.03	0.02	0.01	0.006	0.12
Packaging × Muscle						
P-value	0.90	0.46	0.07	0.08	0.05	0.23

[1]Muscles include beef steaks from both the *Glueteus medius* and *Longissimus lumborum.*

[2]Packaging includes OW and VAC.

[3]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.

[4]SE (largest) of the least square means in the same main effect (packaging type or freezing duration).

[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.16 Interaction of muscle[1] × freezing duration[2] for trained panel ratings[3] of palatability traits of cooked beef steaks

Muscle Type/Duration	Beef Flavor Identity	Oxidized	Sour
Longissimus lumborum			
Fresh	58.88^{b}	0.06^{d}	0.00^{f}
1 Week Frozen	59.87^{a}	0.32^{cd}	0.69^{ef}
1 Month Frozen	58.23^{ab}	0.63^{bc}	1.30^{de}
6 Month Frozen	58.69^{ab}	0.83^{bc}	3.04^{b}
9 Month Frozen	60.64^{a}	1.06^{c}	2.66^{bc}
Gluteus medius			
Fresh	60.65^{a}	0.10^{d}	0.05^{f}
1 Week Frozen	59.48^{a}	0.80^{bc}	1.35^{de}
1 Month Frozen	58.36^{ab}	0.73^{bc}	1.97^{cd}
6 Month Frozen	54.89^{c}	1.10^{b}	4.75^{a}
9 Month Frozen	56.64^{bc}	2.11^{a}	4.43^{a}
SEM[4]	0.95	0.19	0.34
P-value	<0.01	0.04	<0.01

[1]Muscles include beef steaks from both the *Glueteus medius* and *Longissimus lumborum.*
[2]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[3]Sensory scores: 0 = extremely dry/tough/unflavored; 100 = extremely juicy/tender/flavored.
[4]SE (largest) of the least square means in the same main effect (freezing duration or muscle).
[abcdef]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.17 Interaction of packaging type[1] × freezing duration[2] for trained panel ratings[3] of palatability traits of cooked beef steaks

Package Type/Duration	Beef Flavor Identity	Oxidized	Refrigerator-Stale	Sour
Overwrap				
Fresh	59.63^{abc}	0.04^{d}	0.01^{e}	0.01^{d}
1 Week Frozen	59.43^{abc}	0.66^{bc}	0.22^{de}	1.08^{c}
1 Month Frozen	58.30^{bcd}	0.91^{bc}	0.43^{cd}	1.57^{c}
6 Month Frozen	57.20^{cd}	1.12^{b}	0.77^{ab}	3.20^{b}
9 Month Frozen	56.14^{d}	2.48^{a}	0.99^{a}	2.95^{b}
Vacuum				
Fresh	59.90^{ab}	0.12^{d}	0.00^{e}	0.04^{d}
1 Week Frozen	59.91^{ab}	0.46^{cd}	0.11^{e}	0.97^{c}
1 Month Frozen	58.29^{bcd}	0.45^{cd}	0.24^{de}	1.71^{c}
6 Month Frozen	56.38^{d}	0.77^{bc}	0.57^{bc}	4.60^{a}
9 Month Frozen	61.14^{a}	0.69^{bc}	0.09^{e}	4.14^{a}
SEM[4]	0.95	0.19	0.11	0.34
P-value	<0.01	<0.01	<0.01	<0.01

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.
[3] Sensory scores: 0 = extremely dry; 100 = extremely juicy.
[4]SE (largest) of the least square means in the same main effect (freezing duration or muscle).
[abcde]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.18 Interaction of packaging type[1] × muscle[2] for trained panel ratings[3] of palatability traits of cooked beef steaks

Packaging Type/Muscle Type	Juiciness
PVC Overwrap	
Longissimus lumborum	51.26[a]
Gluteus medius	45.73[b]
Vacuum	
Longissimus lumborum	53.83
Gluteus medius	52.93
SEM[4]	0.94
P-value	<0.01

[1]Packaging types include PVC Overwrap (OW) and Vacuum (VAC).
[2]Muscles include beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3] Sensory scores: 0 = extremely dry; 100 = extremely juicy.
[4]SE (largest) of the least square means in the same main effect (freezing duration or muscle).
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.19 Least-square means of trained panel ratings[1] of cooked beef steaks previously frozen in two packaging types[2]

Packaging Type	Brown Roasted	Bloody Serumy	Fat like	Liver like	Fishy	Cardboard	Rancid	Umami	Bitter	Tenderness
Overwrap	52.12[a]	18.99[b]	10.62	1.45	0.51[a]	0.25[a]	0.11	25.83[b]	0.20	54.60[b]
Vacuum	47.68[b]	23.43[a]	11.14	1.37	0.16[b]	0.14[b]	0.28	27.27[a]	0.20	60.2[a]
SEM[3]	0.89	0.85	0.34	0.22	0.11	0.06	0.15	0.51	0.06	0.95
P value	<0.01	<0.01	0.13	0.72	<0.01	0.04	0.27	<0.01	0.95	<0.01

[1]Sensory scores: 0 = extremely dry/tough/unflavored; 100 = extremely juicy/tender/flavored.

[2]Packaging Types include PVC Overwrap (OW) and Vacuum (VAC).

[3]SEM (largest) of the least-squares means.

[ab]Least-square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.20 Least-square means of trained panel ratings[1] of cooked beef steaks of two muscle types[2]

Muscle Type	Brown Roasted	Bloody Serumy	Fat like	Liver like	Fishy	Cardboard	Rancid	Refrigerator Stale	Umami	Bitter	Tenderness
Longissimus lumborum	50.71	22.07[a]	11.52[a]	0.99[b]	0.31	0.20	0.01[b]	0.01[b]	27.45[a]	0.17	59.19[a]
Gluteus medius	49.09	20.35[b]	10.23[b]	1.82[a]	0.36	0.20	0.38[a]	0.48[a]	25.66[b]	0.24	55.59[b]
SEM[3]	0.67	0.63	0.28	0.16	0.08	0.04	0.19	0.05	0.51	0.09	0.67
P value	0.07	0.04	<0.01	<0.01	0.68	0.98	0.02	<0.01	<0.01	0.21	<0.01

[1]Sensory scores: 0 = extremely dry/tough/unflavored extremely; 100 = extremely juicy/tender/flavored extremely.
[2]Muscle types included beef steaks from the *Longissimus lumborum* and *Gluteus medius*.
[3]SEM (largest) of the least-squares means.
[ab]Least-square means in the same column without a common superscript differ ($P < 0.05$).

Table 2.21 Least-squares means of Trained Panel Palatability Ratings[1] of Cooked Beef Steaks from Five Frozen Storage Durations[2]

Duration	Brown Roasted	Bloody Serumy	Fat like	Liver like	Fishy	Cardboard	Rancid	Umami	Bitter	Tenderness	Juiciness
Fresh	48.14	31.55^{a}	15.00^{a}	1.11^{b}	0.01^{c}	0.05^{c}	0.00^{c}	39.25^{a}	0.00^{c}	58.57^{ab}	54.55^{a}
1-Week Frozen	51.77	26.19^{b}	14.11^{ab}	1.09^{b}	0.44^{ab}	0.25^{ab}	0.00^{bc}	36.74^{a}	0.13^{bc}	55.65^{bc}	49.25^{b}
1-Month Frozen	49.07	24.37^{b}	13.41^{b}	1.31^{b}	0.51^{ab}	0.28^{ab}	0.00^{b}	33.14^{b}	0.30^{ab}	54.84^{c}	46.18^{c}
6-Month Frozen	50.07	11.96^{c}	7.07^{c}	2.63^{a}	0.56^{a}	0.10^{bc}	1.62^{a}	13.40^{c}	0.26^{ba}	57.56^{abc}	50.11^{b}
9-Month Frozen	50.46	11.98^{c}	4.79^{d}	0.89^{b}	0.16^{bc}	0.30^{a}	0.20^{b}	10.24^{d}	0.40^{a}	60.33^{a}	54.61^{a}
SEM[3]	1.12	1.05	0.48	2.63	0.13	0.06	0.25	0.99	0.12	1.07	1.05
P-value	0.20	<0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Sensory scores: 0 = extremely dry/tough/unflavored extremely; 100 = extremely juicy/tender/flavored extremely.

[2] Freezing durations includes frozen storage times of Fresh (not frozen), 1-week, 1-month, 6-month and 9-month.

[3]SEM (largest) of the least-squares means.

[abcd]Least-squares means in the same column without a common superscript differ ($P < 0.05$

Chapter 3

Impact of extended frozen storage duration and freezer type on palatability of ground beef patties

Abstract

The objectives of this study were to evaluate the impact of freezing type and storage duration on objective and subjective measures of ground beef palatability traits. 80:20 ground beef was procured from a commercial purveyor. Ground beef was finely ground through a 0.95 cm plate to create 10 batches, and then immediately formed into 130 g patties using an automatic patty maker. Patties were assigned at random to one of three freezer types (n = 30): commercial blast freezer (BF), chest freezer (CF) and refrigerator top-freezer (RF). Prior to frozen storage, patties (n = 360) were subjected to a 5-d simulated retail display under continuous fluorescent lighting. Following display, patties were randomly allotted to a freezing duration: 1-month, 6-months, 9-months or 12-months. Upon completion of the assigned storage duration, patties designated for consumer sensory evaluation (n = 120) were shipped to Manhattan, KS. Consumers (n = 120) evaluated each sample on an unstructured 100-point continuous line scales for flavor-liking, tenderness, texture, juiciness, undesirable and overall liking. At the end of each session, panelists were instructed to answer questions pertaining to household freezing habits for fresh meat products. Furthermore, raw patties were assigned to thiobarbituric acid reactive substances assay (TBARS) following each frozen storage duration as well as cooked volatile compound analysis via gas chromatography-mass spectrometry, shear force (SF) and texture profile analyzation (TPA).

The interaction of freezer treatment × storage duration impacted gumminess (P = 0.05), a TPA attribute. In greater detail, samples stored in RF for 6-months resulted in the greatest gumminess

values ($P < 0.001$), while those stored in the CF for 12-months elicited the lowest ($P < 0.001$). Similarly, flavor development was also impacted by the interaction ($P = 0.05$) of freezer treatment × storage duration. Three lipid derived compounds were of greatest concentration among RF patties stored for 1-month. In contrast, the interaction of freezer treatment × storage duration elicited no impact on consumer ratings, SF or TBARS. Nonetheless, frozen storage duration impacted TPA, flavor development, consumer ratings, SF and TBARS as a main effect ($P < 0.05$), especially in regard to tenderness and juiciness. Furthermore, despite notable impact on tenderness and juiciness, a majority of consumers (50.0%) indicated a primary use of a refrigerator freezer as well as 56.6% indicating that they store the product in the retail packaging. The negative effect RF storage has on tenderness and juiciness is likely a direct result of reduced water-holding capacity that occurs as a result of increased ice crystallization and incidence of freezer burn as a result of air fluctuations due to the dense nature and time spent open of an RF compared to both a BF and CF.

Introduction

As a result of the COVID-19 pandemic, consumer demand for meat, more specifically beef, shocked the global production system, creating industry bottlenecks that resulted in bare grocery store shelves. In order to preserve food for later consumption, freezing has long been utilized to maintain freshness and wholesomeness for extended periods (Huyck and Messing, 2021). While much freezing research has been found to improve tenderness, extended periods of frozen storage can also increase the negative behavior of ice crystallization resulting in freezer burn, a surface dehydration that impacts both tenderness and juiciness (Setyabrata and Kim, 2019; Dang et al., 2021). Nonetheless, despite variability in the impact of extended freezing durations on beef palatability, the 2019 global pandemic also elicited an increase in the purchase of household chest freezers, likely for the long-term storage of meat (Ortiz, 2020; Selyukh, 2020; Tyko, 2020). Commercially, a majority of freezing occurs through blast freezing. However, knowledge is limited in regards to the impact of consumer freezing methods. Limited research has been conducted in regard to consumer freezing methods and the impact on palatability especially when compared to commercial methods of freezing. Nonetheless, the 2016 Beef Quality Audit reported that eating satisfaction ranked second only to food safety when evaluating quality priorities of the industry. Beef palatability however, is readily impacted by product type. Ground beef, a comminuted meat product, is derived from the trimmings, or parts of a beef carcass that are trimmed away and not utilized in retail cuts such as steaks or roasts (Schulz et al., 2021). At the retail level, ground beef accounts for more than 46% of total retail beef consumption in the United States (Schulz et al., 2021). In comparison, the comminuted state of ground beef creates a product that is less shelf-stable in comparison to whole-muscle product.

Therefore, the objective of this study is to determine the impact of consumer freezing methods and frozen storage duration on the palatability of ground beef.

Materials and Methods

Product Collection, Fabrication and Retail Display

Coarse ground 80:20 beef was purchased from a commercial beef processor and shipped to the University of Arkansas Red Meat Laboratory (Fayetteville, AR). Upon arrival, the ground beef was split among 10 batches, fine ground through a 0.95 cm plate and then immediately formed into 130 g patties using the Hollymatic Corporation (Countryside, IL) automatic patty machine. Patties were randomly assigned to one of three freezer types: commercial blast freezer, chest freezer or refrigerator freezer (n = 30/treatment) then placed on individual Styrofoam trays with a soaker pad and overwrapped in polyvinyl-chloride (PVC) film. Once packaged, patties were subjected to a 5 d simulated retail display in an open-front, multideck case (Hill Phoenix, Colonial Heights, VA) under continuous fluorescent lighting. Following display, patties were randomly assigned to a freezing duration: 1-month, 6-month, 9-month and 12-months (n = 10). Additionally, patties were designated for thiobarbituric reactive substances assay (TBARS) analysis, modified ground beef shear force, texture analysis, consumer sensory analysis and volatile compound analysis. Frozen storage was maintained at – 20 °C in each freezer system by internal temperature monitoring.

Thiobarbituric Reactive Substances Assay (TBARS)

Upon completion of the designated freezing duration, samples were flash frozen, homogenized and stored at -80°C until further analysis. Following thiobarbituric reactive substances assay (TBARS) modified methodology of Buege and Aust (1978) as described by Luque et al. (2011), 10 g ± 0.1 was weighed out in a 50 mL conical tube. Each tube was then filled with an additional 30 mL of cold, deionized water, vortexed and immediately homogenized in the conical tube for 30 sec before being re-vortexed. Homogenized samples were then

centrifuged for 10 minutes at 3000 rpm. After centrifugation, 2 mL of supernatant was removed from each tube as pipetted in 50 mL conical tubes with 4 mL of trichloroacetic acid/thiobarbituric acid reagent as well as 100 µL of butylated hydroxyanisole. Samples were then heated in a water bath set at 100°C for 15 minutes prior to being submerged in an ice bath for 10 minutes. Samples were allowed time to return to ambient temperature before being recentrifuged for another 10 minutes at 3000 rpm. Upon the final centrifugation step, 1 mL of supernatant was removed from each sample and placed in a 96-well plate. All samples were subjected to spectrophotometric analysis at 531 nm. Sample absorbance was recorded, and concertation determined based on a standard curve that was prepared each day of analysis.

Cooking Method

Following each designated freezing treatment, patties assigned for cooked analysis were thawed at 2 – 4 °C for 24 hours. Once thawed, patties were cooked on closed clamshell grills (Model GR- 150 Griddler, Cuisinart, Stamford, CT) set at 176.6°C. Patties were removed from the grill and set to rest until they reached a peak internal temperature of 74 °C. Temperature was routinely monitored by inserting a thermometer to the geometric center of each patty. Peak temperature was recorded for each sample. Additionally, all raw and cooked weights were recorded for determining overall cook loss for each patty.

Modified Ground Beef Shear Force

In accordance with the AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Meat (2015), following a 3 min rest period, cooked patties were subjected to modified ground beef shear force analysis. One strip (2.54 cm in length) was removed from the center of each patty, which was determined by measure 2.54 cm from the patty edge. The slice sample was then positioned on the testing machine (GR – 152;

Tallgrass Solutions, Manhattan, KS), equipped with a straight edge slice shear force blade. Each slice was sheared three times, with each shear being recorded in peak kg/f. A final average was taken of all three slices for data analyzation.

Texture Profile Analysis

Upon completion of the designated freezing analysis, samples assigned for texture profile analysis (TPA) were cooked to the previously described standard and allowed to cool to ambient temperature prior to texture evaluation. In line with the AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Meat (2015) in combination with methods determined by Bourne (1978), three cores, each measuring 2.54 cm in diameter, were removed from the relative center of each ground beef patty. Each cored sampled was placed on the TPA instrument (MODEL) programmed for a 5 kg load cell and cross-head speed of 100 mm/min and compressed twice to reflect 70% of the original height of the core. The peak force area of each core was averaged between the three cores of each sample. Additionally, the time that elapsed between the first and second compression was measured for each core and averaged among individual samples. Using the calculated averages, hardness was measured as the peak force during the first compression cycle and cohesiveness as the ratio of the peak force area during the second compression to the peak force area of the first compression (Area1/Area2). Springiness, previously referred to in literature as elasticity was determined as the height that the sample recovered during the time elapsed between the end of the first compression and start of the second. Furthermore, gumminess was evaluated as the product of hardness and cohesiveness and chewiness as the product of gumminess and springiness.

Homogenization

Immediately after modified ground beef shear force evaluation, patties assigned to volatile compound analysis were cubed and flash frozen using liquid nitrogen. Once all cubed pieces were frozen, patties were homogenized (Nutribullet, Ninja, Mesa, AZ) and packaged in labeled individual Whirl-Pak bags (Model S-19794 6 × 9" White Block Whirl-Pak® Bags – 24 oz, Whirl-Pak®, Atkinson, WI). All homogenized samples were stored in -80 °C until time of designated analysis.

Volatile compound analysis

For each patty, the volatile compound composition was determined in accordance with the methods of Gardner and Legako (2018). Immediately after cooking and shear force evaluation, patties were homogenized using the previously described method and stored in -80 °C until time of analysis. Upon analyzation, stored sampled were removed from storage. From each patty, a labeled 20 mL glass vial (Gerstel Inc, Linthicum, MD) was filled with a 5.0 g ± 0.1 sample and closed with a polytetrafluorethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc, Linthicum, MD). Prior to instrumental analysis, the glass vial containing a portion of each sampled was pipetted with 10 µL of internal standard (1, 2-dichlorobenzene, 2.5 mg/µL) and sealed again with the screw cap. Samples were initially subjected to an incubation time of 5 minutes in the Gerstel agitator programmed to 65 °C. The vials were loaded by the Gerstel automatic sampler (MPS; Gerstel, Inc.) prior to agitation. Volatile compounds were extracted from the sample headspace through solid phase microextraction (SPME) with an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA) at an extraction setting of 20 minutes. isolated compounds were immediately aligned on a VF-5 MS capillary column (30 m × 0.25 mm

× 1.0 μm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Retention times in combination with confirmation of authentic standards (Sigma-Aldrich, St. Louis, MO) were used to identify and classify volatile compounds.

Consumer Sensory Panels

Consumer sensory panels were conducted at Kansas State University (Manhattan, KS) in accordance with the methodology of Vierck et al. (2018) and Drey et al. (2019) and similar to the AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness (2015).

Upon completion of the designated freezer treatment, samples (n = 30) were packaged in dry ice and shipped from Fayetteville, AR to Manhattan, KS. Upon arrival, patties were thawed for 12 -14 hours at 2 – 4 °C in refrigeration. Samples were prepared for each session in line with cooking methods previously described, on a closed clamshell grill until reaching a peak internal temperature of 74 °C which was determined by placing a thermometer at the geometric center of each patty. Once cooked, samples were portioned in 1 cm^3 pieces and two portions from each patty were placed in 2 oz plastic cups that were labeled with a blind code that correlated to the sample.

Panels were conducted as an incomplete block design, with each panelist receiving a randomly assigned order of samples during each session. Electronic tablets (iPad, Apple, Inc., Cupertino, CA) were programmed with 100-point unstructured line scales using electronic survey software (Qualtrics, Provo, UT). Initial ballots were comprised of a series of questions regarding consumer demographics, purchasing motivators and sample ballots. Panelists evaluated each sample for flavor-liking, tenderness, juiciness and overall liking using. Verbal anchors were provided at each endpoint as: extremely dislike/tough/dry or extremely

like/tender/juicy with a neutral term midpoint set at 50. Additionally, panelists were instructed to rate each sample as acceptable or unacceptable for each previously described trait and assign each sample a quality level: unsatisfactory, every day, better than every day or premium quality. At the conclusion of each session, panelists were asked questions pertaining to their habits, specifically in terms of freezing meat products after purchase, the types of meat products purchased, the time elapsed before freezing and estimated frozen storage time.

A total of five panel sessions were completed in line with the corresponding freezing treatment. Each session consisted of the 20 consumer panelists who evaluated two samples indicative of patties from a commercial blast freezer, chest freezer and refrigerator freezer. Panels were conducted in a lecture-style classroom and during each session, panelists were provided water, apple juice and unsalted crackers to use for palate cleansing between each sample. An expectorant cup, toothpick, plastic fork and napkin were also provided.

Statistical Analysis

Statistical Analysis was performed using the PROC GLIMMIX procedure of SAS (SAS version 9.4; Cary, NC). Data were analyzed as a completely randomized design with a 3×5 factorial arrangement. Freezing duration and freezer type were included in the design as fixed effects. Peak temperature was included in the model for cooked analysis as a covariate. For sensory evaluation, panel session and number served as random effects. Acceptability data was measured in a binomial error distribution model. Individual patties were treated as the experimental unit and blocked by animal number. Kenward-Roger adjustment was used for all statistical analysis. Means were separated at a level of $P \leq 0.05$.

Results and Discussion

Thiobarbituric Acid Reactive Substances (TBARS)

No difference was observed from the interaction of freezer treatment × storage duration for malondialdehyde (MDA) concentration via TBARS ($P \geq 0.05$; Table 3.1). Furthermore, MDA concentration was not influenced by duration or freezer type independently as main effects ($P \geq 0.05$; Table 3.1). Generally, the MDA concentration increased as frozen storage duration increased (Table 3.1). The increase in oxidation activity over time could be attributed to loss of vacuum throughout frozen storage. Due to an increased incidence of packages deemed to be "leakers" after 12-months of frozen storage, it is plausible that the abrupt transition from anaerobic to aerobic conditions at some point during storge could result in an increase in lipid oxidation and therefore a general increase in MDA concentration and surface discoloration.

Shear Force

The interaction ($P \geq 0.05$; Table 3.2) of freezer treatment × storage duration elicited no impact on shear force values. Instrumental tenderness was however influenced by frozen storage duration as a main effect ($P < 0.001$; Table 3.2). In greater detail, patties that were frozen for 12-months resulted in the greatest shear force values ($P < 0.001$), while those frozen for 6-months resulted in the lowest ($P < 0.001$). Generally, shear force values increased with time, with storage durations of 1-month and 6-months being similar.

The shear force values at 6-months aligns with the work of Setyabrata and Kim (2019) that concluded that frozen storage improved instrumental tenderness of beef steaks. Furthermore, the decrease in shear force values, which would correspond to an increase in toughness can be attributed to the negative impacts of prolonged ice crystallization and freezer burn formation (Dang

et al., 2021). The difference in peak force measurements from 1 to 12-months indicates that as icecrystal size increases with time, greater exogenous stress is placed on the muscle fibers resulted in increased incidence of protein oxidation and a perceived decrease in product tenderness (Zhang et al., 2022). Additionally, due to the comminuted state of ground beef patties, muscle structure is already disrupted which could explain the further deterioration of palatability with extended periods of frozen storage.

Texture Profile Analysis

The texture attribute of gumminess was impacted by the interaction of freezer treatment × storage duration ($P = 0.05$; Table 3.3). Moreover, patties that were stored in RF for 6-months resulted in the greatest gumminess values ($P < 0.001$), compared to patties from the CF that were frozen for 12-months, which resulted in the lowest gumminess values ($P < 0.001$). Furthermore, all other texture attributes were impacted by frozen storage duration as a main effect ($P \leq 0.05$; Table 3.4). For springiness, samples that were frozen for 9-months resulted in the greatest measurement values ($P < 0.001$), while 1-month frozen samples resulted in the lowest values for springiness ($P < 0.001$). Similarly, 9-month frozen patties also resulted in the greatest values for cohesiveness ($P < 0.001$) and 1-month frozen samples, the lowest ($P < 0.001$). Contrastingly, for chewiness, patties that were frozen for 6- values ($P < 0.001$), while those frozen for 12-months resulted in the lowest chewiness values ($P < 0.001$). Finally, resilience was greatest among samples frozen for 9-months ($P < 0.001$) and lowest among 12-month frozen samples ($P < 0.001$).

The results of TPA align with known alterations of water-holding capacity throughout frozen storage of meat products. Throughout extended periods of frozen storage, the prolonged ice crystallization results in a greater amount of moisture being removed from the muscle tissue (Dang et al., 2021; Zhang et al., 2022). During the thaw process, the ice crystals formed on the meat

surface melt, however a disputed myofibrillar structure, such as that of ground beef is unable toabsorb the release moisture, therefore resulting in a cooked product that is less juicy, with greater texture variation. Therefore, these results further indicated the impact of prolonged ice crystallization on surface dehydration and attributes of palatability.

Volatile compound analysis

Of the 72 compounds evaluated for beef flavor development, 3 volatile compounds elicited a freezer type × freezing duration interaction ($P < 0.05$). Additionally, the main effect of frozen storage duration solely imparted a difference on 10 compounds ($P < 0.05$) In total, 59 compounds were not impacted by freezer type or frozen storage duration ($P \geq 0.05$). The freezer type × freezing duration interaction impacted a number of lipid-derived compounds (Table 3.5). More specifically, patties that were stored in RF for 1-month resulted in the greatest concentration of ethanol ($P < 0.001$), 2-propanone ($P = 0.02$) and p-xylene ($P = 0.02$)

Furthermore, volatile compound analysis was impacted by frozen storage duration as a main effect ($P < 0.05$). More specifically, patties that were frozen for 1-month resulted in the greatest concentration of a number of Maillard reaction products (Table 3.6), including, acetaldehyde ($P= 0.02$), a Strecker aldehyde, 3-methylbutanal ($P= 0.02$), and the sulfur-containing compounds, carbon disulfide ($P < 0.0001$) and methional ($P < 0.001$). Additionally, 1-month frozen patties also elicited the greatest concentration of pentanal ($P < 0.001$), an alcohol as well as the hydrocarbons toluene ($P < 0.001$), decane ($P = 0.0001$) and nonane ($P = 0.02$), all of which are secondary lipid degradation products. Contrastingly, samples that were designated for 6-months of frozen storage resulted in the greatest concentration of a Maillard derived ketone, butyrolactone ($P = 0.02$) and pyrazine, 2-ethyl-3,5/6-dimethylpyrazine ($P = 0.02$), while samples that were frozen for 9-months resulted in the greatest concentration of the lipid derived ester, nonanoic acid, methyl

ester ($P = 0.04$). No compounds were impacted by freezer type as a main effect ($P \geq 0.05$). These results indicate that extended periods of frozen storage, and the type of freezer has little impact on beef flavor development. It is likely that the increased concentrations of lipid-derived compounds after 1-month of frozen storage are the result of increased lipid oxidation throughout retail display. During retail display, patties were packaging in PVC overwrap, which is an oxygen permeable film. The packaging system in combination with the oxidative labile characteristics of ground beef would result in greater initial concentrations of lipid-derived products upon allotment to frozen storage. Those lipid degradation compounds that increased in concentration as storage time increased are likely a result of "leaker" packages and the subsequent circulation of oxygen in the storage environment. The results of volatile compound concentration is similar to the findings of Al-Dalal et al. (2022) in which concentrations increased within raw marinated beef during storage periods of up to 4 months, and then gradually decreased as time continued to increase

Consumer Sensory Evaluation

The demographics of 120 consumers who participated in sensory panels are presented in Table 3.11. The majority of participants were Caucasian/White (91.2%) from two-person households (51.2%). Additionally, 54.0% of participants were female and 62.1% were married. Moreover, most consumers were 30 – 39 years of age (33.3%) with an income of less than $25,000 (21.8%) or $100,000 – 149,000 (21.8%) and were college graduates (33.3%). A majority of consumers specified flavor as the most important palatability trait when consuming beef (75.9%) followed by juiciness (13.8%) with a preference for steaks to be cooked to a medium degree of doneness (28.6%). Finally, a majority of consumers indicated that they consume beef 3 times per week (28.9%). Finally, based on results of the consumer purchasing motivators survey, price is the attribute that drives consumer purchasing decisions the most while preformed patties are the lowest

motivator of consumer purchasing decisions (Table 3.12). When asked about storage practices of meat products, it was found that 50.0% of consumers store meat in a RF, compared to 38.5% that use a CF and 9.0% that only store product in refrigeration and do not freeze (Table 3.10). Additionally, the survey results indicated that 56.6% of consumers choose to store meat in the same retail packaging system with the next most frequent packaging type being Ziploc (21.3%; Table 3.10). Survey results also indicated that the length of frozen storage duration among consumers is variable with 33.6% stating they stored product from 2 – 4 weeks, 21.3% from 3 – 4 months and 13.9% for 1-–2 months. These results indicate that freezer type, regardless of storage duration, impacts consumer ratings of ground beef tenderness and juiciness, despite RF being the most common method of frozen storage among consumers. The negative impact of RF on tenderness and juiciness is likely as result of an increased incidence of ice crystallization and freezer burn as a result of fluctuations in air flow due to the more densely packed area and time spent open of a RF compared to both a BF and CF.

No impacts were observed from the interaction of freezer treatment × storage duration for any of the consumer ratings evaluated, including all acceptability ratings ($P \geq 0.05$). However, juiciness was influenced by the main effect of freezing treatment ($P = 0.01$; Table 3.8). In comparison, patties from RF were rated as the least juicy ($P < 0.001$), while patties from the BF were rated as the juiciest ($P < 0.001$), but were similar to the CF ($P > 0.05$). Furthermore, freezing type also imparted a difference on consumer tenderness ratings ($P = 0.001$; Table 3.8). Similarly, patties from the BF were rated as the most tender ($P < 0.001$), while RF patties were rated the toughest ($P < 0.001$). Consumer ratings for flavor, texture and overall liking were contrastingly not impacted by the main effects of freezing treatment ($P \geq 0.05$). Additionally, the main effect of

storage duration imparted no impact on any of the consumer ratings evaluated ($P \geq 0.05$; Table 3.8). Consumer acceptability ratings were also not impacted by freezer type or storage duration as main effects ($P \geq 0.05$; Table 3.9). Generally, however, acceptability ratings decreased as frozen storage duration increased (Table 3.9).

despite RF being the most common method of frozen storage among consumers. The negative impact of RF on tenderness and juiciness is likely as result of an increased incidence of ice crystallization and freezer burn as a result of fluctuations in air flow due to the more densely packed area and time spent open of a RF compared to both a BF and CF.

Conclusion

Overall, these results indicate that the impact of freezer type and frozen storage duration on palatability factors of ground beef patties is variable. The likely increase in ice crystal size on the meat surface overtime negatively interferes with the water-holding capacity, mainly impacting juiciness. Nonetheless, despite some instrumental differences in palatability measures, consumers largely do not detect such physiochemical changes as deemed through high acceptability ratings. Therefore, when considering recommendations for frozen storage duration and techniques of beef products, it is of the utmost importance to consider product type (i.e. comminuted vs. whole muscle) as well as importance of specific palatability attributes in order to best improve the consumer eating experience.

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Tables

Table 3.1 Least square means for thiobarbituric acid reactive substances measurements of ground beef patties stored in three freezer types[1] for four freezing durations[2]

Treatment	*MDA ng/g sample*
Duration	
1-month	0.35
6-month	0.35
9-month	0.39
12-month	0.53
SEM[3]	0.08
P-value	0.13
Freezer	
Blast	0.38
Chest	0.42
Refrigerator	0.42
SEM	0.05
P-value	0.85
Freezer Type × Storage Duration	
P-value	0.97

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same main effect.
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.2 least square means for shear force measurement of ground beef patties stored in three Freezer types[1] for four freezing durations[2]

Treatment	Peak Shear Force (kg/f)
Duration	
1-month	1.83[c]
6-month	1.75[c]
9-month	2.10[b]
12-month	2.35[a]
SEM[3]	0.06
P-value	<.0001
Freezer	
Blast	1.80[a]
Chest	1.88[a]
Refrigerator	1.82[a]
SEM	0.014
P-value	0.17
Freezer Type × Storage Duration	
P-value	0.73

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same main effect.
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.3 Interaction of freezer type[1] × storage duration[2] for texture profile analysis of cooked ground beef patties

Freezer Treatment/Storage Duration	*Gumminess*
Blast Freezer	
1-Month	2159.68^{cd}
6-Months	3351.12^{ab}
9-Months	1262.55^{f}
12-Months	1227.56^{f}
Chest Freezer	
1-Month	2420.84^{c}
6-Months	3060.82^{b}
9-Months	1802.46^{de}
12-Months	1227.55^{f}
Refrigerator Freezer	
1-Month	1877.01^{de}
6-Months	3584.45^{a}
9-Months	1727.80^{def}
12-Months	1478.27^{ef}
SEM[3]	197.75
P-value	0.05

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same column.
[a-f]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.4 Least square means for texture profile of ground beef patties stored in three freezer types[1] for four freezing durations[2]

	Texture Attribute			
Treatment	Springiness	Cohesiveness	Chewiness	Resilience
Duration				
1-month	1.13[b]	0.77[c]	2506.26[bc]	0.56[c]
6-months	1.58[b]	0.82[b]	5267.40[a]	0.62[b]
9-months	2.36[a]	0.85[a]	3188.21[b]	0.84[a]
12-months	1.13[b]	0.82[b]	1643.15[c]	0.54[c]
SEM[3]	0.22	0.008	423.41	0.007
P-value	0.0003	<.0001	<.0001	<.0001
Freezer Type				
Blast	1.46[a]	0.81[a]	2681.03[b]	0.64[a]
Chest	1.42[a]	0.82[a]	3066.79[ab]	0.64[a]
Refrigerator	1.78[a]	0.82[a]	3705.94[a]	0.64[a]
SEM	0.19	0.006	362.53	0.006
P-value	0.33	0.76	0.12	0.85
Freezer Type × Storage Duration				
P-value	0.94	0.46	0.87	0.58

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same main effect.
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.5 Interaction of freezer type[1] × storage duration[2] on volatile compound analysis of lipid derived products from cooked ground beef patties

	Compound ng/g		
Treatment	*Ethanol*	*2-propanone*	*p-Xylene*
Blast Freezer			
1-Month	45.63^{bc}	21.15^{bc}	539.91^{bc}
6-Months	19.59^{c}	23.67^{bc}	.
9-Months	17.15^{c}	20.17^{bc}	451.00^{c}
12-Months	35.42^{c}	28.65^{b}	747.97^{ab}
	22.22^{c}		
Chest Freezer			
1-Month	76.27^{b}	26.72^{b}	483.66^{bc}
6-Months	19.00^{c}	21.61^{bc}	.
9-Months	.	17.68^{bc}	585.49^{abc}
12-Months	18.05^{c}	16.83^{bc}	422.28^{c}
Refrigerator Freezer			
1-Month	115.73^{a}	45.02^{a}	989.26^{a}
6-Months	22.67^{c}	18.40^{bc}	.
9-Months	22.53^{c}	18.56^{bc}	553.28^{bc}
12-Months	16.34^{c}	12.05^{c}	506.08^{bc}
SEM	13.50	6.07	163.69
P-value	0.01	0.02	0.02

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same column.
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.6 Least square means of Maillard reaction derived volatile compounds from cooked ground beef patties stored in three freezer types[1] for four storage durations[2]

	Compound ng/g sample					
Treatment	*Acetaldehyde*	*3-methylbutanal*	*Carbon Disulfide*	*Methional*	*Butyrolactone*	*2-ethyl-3,5/6-dimethylpyrazine*
Freezer Type						
Blast	26.79[a]	0.21[a]	7.81[a]	57.81[a]	2138.64[a]	0.15[a]
Chest	23.34[a]	0.22[a]	8.77[a]	56.67[a]	1656.61[a]	0.16[a]
Refrigerator	26.04[a]	0.24[a]	6.58[a]	61.73[a]	2067.82[a]	0.16[a]
SEM[3]	4.56	0.03	0.95	3.24	437.93	0.21
P-value	0.72	0.77	0.26	0.50	0.50	0.93
Duration						
1-Month	33.30[a]	0.33[a]	1.76[c]	100.11[a]	2368.36[ab]	0.16[ab]
6-Months	27.97[ab]	0.23[ab]	10.69[a]	46.54[b]	1284.06[c]	0.22[a]
9-Months	22.81[bc]	0.21[ab]	13.48[a]	47.44[b]	2691.35[a]	0.14[b]
12-Months	17.49[c]	0.14[b]	4.96[b]	40.85[b]	1573.66[bc]	0.11[b]
SEM[3]	3.68	0.046	1.11	3.78	349.66	0.02
P-value	0.02	0.02	<.0001	<.0001	0.02	0.02
Freezer Type × Storage Duration						
P-value	0.57	0.21	0.28	0.13	0.49	0.58

[1]Freezer Type includes a commercial blast freezer, chest freezer and refrigerator top freezer
[2]Freezing durations include 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same column.
[abc]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.7 Least square means lipid derived volatile compounds from cooked ground beef patties stored in three freezer types[1] for four storage durations[2]

	Compound ng/g sample				
Treatment	*Pentanal*	*Nonane*	*Toluene*	*Decane*	*Nonanoic Acid, methyl ester*
Freezer Type					
Blast	6.12[a]	0.76[a]	9.19[a]	12.00[a]	1170.24[a]
Chest	5.05[a]	0.63[a]	7.26[a]	9.99[a]	942.86[a]
Refrigerator	7.05[a]	0.85[a]	8.54[a]	10.88[a]	1100.60[a]
SEM[3]	0.74	0.21	3.07	1.60	278.49
P-value	0.16	0.75	0.86	0.67	0.71
Duration					
1-Month	13.52[a]	1.26[a]	25.75[a]	17.89[a]	1377.36[a]
6-Months	4.13[b]	0.30[b]	1.45[b]	7.97[b]	904.87[ab]
9-Months	3.90[b]	1.00[ab]	3.47[b]	11.30[b]	1383.89[a]
12-Months	2.74[b]	0.44[b]	2.65[b]	6.67[b]	618.83[b]
SEM[3]	0.86	0.27	5.03	2.60	228.02
P-value	<.0001	0.02	<.0001	0.0001	0.04
Freezer Type × Storage Duration					
P-value	0.28	0.77	0.97	0.14	0.56

[1]Freezer Types include commercial blast freezer, chest freezer and refrigerator-top freezer.
[2]Freezing durations includes frozen storage times of 1-month, 6-months, 9-months and 12-months.
[3]SE (largest) of the least square means in the same main effect (freezer type or storage duration).
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.8 Least square means for consumer palatability ratings[1] of cooked ground beef patties from three freezer types[2] and four storage durations[3]

	Attribute					
Treatment	Flavor	Juiciness	Tenderness	Overall Liking	Undesirable	Texture
Freezer Type						
Blast	61.57	70.89[a]	69.51[a]	65.43	23.19	65.21
Chest	62.41	70.36[a]	66.80[a]	63.85	23.67	62.83
Refrigerator	62.45	66.06[b]	63.59[b]	62.38	21.83	62.87
SEM[4]	1.83	1.37	1.83	1.88	2.18	1.89
P-value	0.83	0.01	0.001	0.24	0.65	0.14
Duration						
1-Month	63.61	66.06[b]	63.23[a]	65.06	24.78	65.98
6-Months	59.84	67.02[ab]	65.09[a]	61.50	28.89	61.19
9-Months	63.24	71.37[ab]	66.94[a]	64.13	14.71	61.73
12-Months	61.88	71.96[a]	1.27[a]	64.86	23.21	65.64
SEM	3.12	1.93	3.23	3.22	3.73	3.47
P-value	0.82	0.11	0.37	0.83	0.09	0.66
Freezer Type × Storage Duration						
P-value	0.34	0.30	0.49	0.40	0.62	0.21

[1]Palatability Ratings on 0 – 100 scale with 0 = tough/undesirable and 100 = tender/desirable.
[2]Freezing types include commercial blast freezer, chest freezer and refrigerator-top freezer.
[3]Storage durations include 1-month, 6-months, 9-months and 12-months.
[4]SE (largest) of the least square means in the same main effect (freezer type or duration).
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$).

Table 3.9 Least square means for consumer palatability acceptability ratings[1] of cooked ground beef patties from three freezer types[2] and four storage durations[3]

	Attribute				
Treatment	Flavor	Juiciness	Tenderness	Texture	Overall Acceptability
Freezer Type					
Blast	81.55	88.68	93.02	87.41	86.42
Chest	84.70	90.2	91.62	88.29	88.14
Refrigerator	87.18	87.51	91.29	90.02	88.04
SEM[4]	2.82	2.39	2.29	2.59	2.58
P-value	0.18	0.40	0.74	0.62	0.83
Duration					
1-Month	85.51	89.76	93.89	92.69	8.67
6-Months	84.38	82.24	92.31	83.51	86.69
9-Months	87.04	92.46	91.99	89.24	88.52
12-Months	80.98	91.54	89.73	88.81	86.25
SEM	4.54	3.95	3.79	4.27	3.85
P-value	0.79	0.29	0.88	0.50	0.94
Freezer Type × Storage Duration					
P-value	0.92	0.71	0.16	0.56	0.81

[1]Acceptability Ratings on 0-100 scale, 0 = unacceptable and 100 = acceptable
[2]Freezing types include commercial blast freezer, chest freezer and refrigerator-top freezer
[3]Storage durations include 1-month, 6-months, 9-months and 12-months
[4]SE (largest) of the least square means in the same main effect (freezer type or duration).
[ab]Least square means in the same column without a common superscript differ ($P < 0.05$)

Table 3.10 Exit survey results – consumer handling practices

Handling Practices	*% of Consumers*
Storage Preference	
Refrigerator	9.0
Refrigerator Freezer	50.0
Chest Freezer	38.5
Other	2.5
Storage Packaging Type	
Store Packaging	56.6
Vacuum Seal	12.3
Ziploc	21.3
Butcher Paper	7.4
Other	2.5
Typical Storage Duration	
0-1 Weeks	9.0
2-4 Weeks	33.6
1-2 Months	13.9
3-4 Months	21.3
5-6 Months	10.7
7-8 Months	2.5
9-10 Months	5.7
11-12 Months	3.3

Table 3.11 Consumer demographics exit survey results

Demographic	*% of Consumers*
Gender	
Male	46.0
Female	54.0
Age	
Under 20	11.8
20 – 29 Years	11.8
30 – 39 Years	33.3
40 – 49 Years	11.8
50 – 59 Years	15.1
Over 60 Years	16.1
Ethnicity	
African American	0.0
Asian	0.8
Caucasian/White	91.2
Hispanic	3.2
Mixed Race	1.6
Native American	0.0
Other	3.2
Household Size	
1 Person	16.8
2 People	51.2
3 People	5.6
4 People	12.8
5 People	7.2
6 People	4.0
> 6 People	2.4
Income	
< $25,000	21.8
$25,000 - $34,999	4.8
$35,000 - $49,999	8.9
$50,000 - $74,999	18.5
$75,000 - $99,999	14.5
$100,000 - $149,999	21.8
$150,000 - $199,999	4.8
> $199,999	4.8
Marital Status	
Married	62.1
Single	37.9
Education	
High School Graduate	16.7
Some College/Technical School	27.8
College Graduate	33.3
Post-College Graduate	22.2
Preferred Palatability Trait	
Flavor	75.9
Tenderness	10.3
Juiciness	13.8
Preferred Degree of Doneness	
Rare	3.2
Medium-Rare	19.0
Medium	28.6
Medium-Well	27.0
Well-Done	20.6

Table 3.11 Cont.

Very Well-Done	1.6
Average Weekly Meat Consumption	
1 Time	15.7
2 Times	18.2
3 Times	28.9
4 Times	14.9
5 Times	5.8
6 Times	9.1
7 Times	1.7
8 Times	0.8
9 Times	2.5
10 Times	2.5

Table 3.12 Simple means of consumer purchasing motivators[1]

Purchasing Motivator	*Purchasing Motivator (0-100 Scale)*
Animal Diet	
Grass-Based Diet	37.6
Grain-Based Diet	44.8
Labeling Claims	
No Antibiotics	40.8
Animal Welfare Claims	58.3
Use of Growth Promotant	45.8
Natural or Organic	36.5
Locally Raised	45.1
Product Appearance	
Lean to Fat Ratio	69.7
Color	70.1
Brand of Product	36.9
Fat Content	68.8
Fresh, Never Frozen	40.2
Nutrient Content	61.5
Packaging Type	40.0
Preformed Patties	29.6
Primal Source	49.0
Size, Weight and Thickness	58.0
Price	75.0

[1]Motivators were rated on a 0-100 scale 0 = not important 100 = Important Purchasing Factor