THE EFFECTS OF FRESH AND FROZEN STORAGE ON PALATABILITY, OXIDATIVE RANCIDITY AND COLOR OF PACKAGED BEEF STEAKS

By

MARCIA ANNE DALTON

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THE EFFECTS OF FRESH AND FROZEN

STORAGE ON PALATABILITY,

OXIDATIVE RANCIDITY AND

COLOR OF PACKAGED

BEEF STEAKS

Thesis Approved:

James B. Morgan Thesis Advisor

Christina DeWitt

J. Roy Escoubas

A. Gordon Emslie Dean of the Graduate College

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FORMAT OF THESIS

This Thesis is presented in Journal of Animal Science style format, as outlined by the Oklahoma State University Graduate College Style Manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

Introduction

From *Rodale's Complete Book of Home* Freezing, it is stated that "owning a freezer allows you to use the erratic pattern of meat prices to your advantage, stockpiling meat when it is inexpensive to consume when it is dear" (Hodges, 1984). Many consumers utilize this strategy, especially consumers with families to consider. However, with the introduction of case ready packaging, in particular packaging with modified gaseous atmospheres (MAP), freezing has become a potential issue. Packaging for frozen conditions requires that the package must minimize or prevent the deterioration, both physical and chemical, that frozen meat goes through (Bell, 2001). Unfortunately, little research has been conducted to see how MAP products stand up to frozen conditions when compared to more traditional forms of packaging.

Case-ready packaging systems have fast become the primary means by which many retailers prefer to market meat products to their consumers. Case ready packaging has been called the most significant advance in beef processing since the advent of boxed beef in the late 1960s, and has already reshaped the way beef is processed, packaged and marketed to consumers (NCBA, 2000).

Case-ready packaging systems generally come in one of two major forms: vacuum packaged product, which is in a vacuum environment with tightly fitted

high oxygen barrier plastic packaging, or in modified atmosphere packaging, which entails plastic trays, high oxygen barrier film, and a combination of nitrogen, carbon dioxide (or monoxide), and oxygen gases to "flush" the package. These two types of packaging systems offer many advantages to retailers and consumers alike, when compared to typical retail packaged systems such as air permeable polyvinyl chloride over-wrap with an air permeable polystyrene tray

Some benefits of case ready systems include reduced labor costs in-store; fewer out-of-stock items due to the ability of the retailer to reorder specific cuts when necessary; ability of retailers to guarantee a consistent product to consumers from purchase to purchase; reduce liability risks when and if a food safety issue arises concerning the packaged product because the retailer performs no direct product handling; and, the fact that the packages are "tamperproof" in that tampering results in opening of the package; and extend retail shelf life of the product due to barrier films, lack of oxygen, and/or antimicrobial abilities of some gases.

Vacuum packaging, considered the best packaging system by Romans et al. (2001), provides a system where little to no air is present thereby limiting oxidative processes, is durable, and has no head space in the package, which allows for more cost effective transportation. Unfortunately, vacuum packaging also provides myoglobin in its deoxymyoglobin state, causing it to appear purplish-red in appearance. Since meat color is the number one quality aspect that drives beef sales and deviation from the bright, cherry red is heavily discriminated against (Faustman, 1994), then vacuum packaging is not a

desirable option from a retail standpoint. Until the industry is able to educate the consumer as to why vacuum packaged beef is purplish-red, Faustman (1994) believes that maintaining oxymyoglobin state, or bright, cherry red color in beef cuts, works best at the retail level.

Luño et al. (2000) stated that modified atmosphere packaging is an accepted method for extending the shelf life of a variety of foods, including fresh meat. If tenderness of a beef cut is the number one quality aspect in relation to pleasurable eating experience (Miller et al., 1995), then limiting lipid oxidation and extending shelf life is the key to retail success in beef. According to Faustman (1994), the keys to delaying oxidative processes, and extending shelf life, are refrigerated storage and display, hygienic preparation of the product, selective light use, the use of natural or synthetic antioxidants, and effective packaging.

One additional benefit that MAP consumer-ready packages offer that vacuum packages are not able to offer is the ability to display meat cuts in a appealing manner in an attractive package and with a bright, cherry red color that consumers have come to connect with meat freshness. Modified atmosphere packaging was designed to present fresh meat attractively in a retail display case to consumers, while extending the retail shelf life and supplying all other advantages that case-ready packages provide. Product in these packages were meant to be taken home and prepared soon thereafter.

However, many consumers are taking these packaged products home and freezing them shortly thereafter, rather than preparing them within a few days of

purchase. This may be creating meat quality issues that were unexpected in the MAP packaged product especially. Again, there have not been many studies involving case-ready packaging systems stored in home freezer conditions. The objective of this research, therefore, was to determine the effects of fresh and frozen storage on palatability, oxidative rancidity and color of packaged beef steaks.

CHAPTER II

Literature Review

Basic Slow Freezing

Freezing is used for the purpose of preserving perishables and "results in fewer undesirable changes in qualitative and organoleptic properties than other methods of preservation" (Aberle et al., 2001) and has been used to a great extent commercially since the 1960s (Xiong and Mikel, 2001). Home freezing is a good way to reduce microbiological activity, slow enzyme-indued oxidative rancidity (Faustman, 1994; Romans et al., 2001)) and to increase the storage life of meat products. The quality of frozen meat depends on freezing rate, frozen storage conditions and length of freezing period (van Laack, 1994; Aberle et al., 2001) as well as the lipid composition, especially the degree of unsaturation (Faustman, 1994; Igene et al., 1980). Freezing and frozen conditions affect the size and distribution of ice crystals, which ultimately affect the texture, surface color and water holding capacity of the thawed meat (Bhattacharya & Hanna, 1989). In addition, while freezing can slow oxidative rancidity, extended frozen periods can actually lead to oxidation of not only fats but proteins as well. Sikorski (1978) also noted a hardening of meat frozen for extended periods and attributed this to cross-linking of fibrillary proteins.

Slow freezing of meat, which is done in a typical home freezer, begins with the cooling of the meat surface to below its freezing point, while the center is still

well above freezing. Ice crystals first begin to form at the surface of the meat From there, freezing boundaries continue to form and gradually progress from the exterior surface to the interior of the meat, until the product is thoroughly frozen (Aberle et al., 2001). This freezing in layers also occurs at the cellular level. Extracellular water is more likely to freeze faster than intracellular water because of its lower solute concentration. This creates "pure ice crystals (extracellularly) and increased concentration of solutes in remaining unfrozen solutions" (Aberle et al. 2001). The ice crystals can cause stretching and rupturing of the surrounding muscle tissues (Romans et al., 2001). This is also the point at which recrystallization occurs. Recrystallization is a process by which ice crystals increase in size and decrease in number by coming together and joining (Bell, 2001), which is primarily the result of "energy differences between large and small crystals and differences in free energy due to internal strain" (Ngapo et al., 1999). In other words, intracellular water migrates outside of the muscle fibers and joins with the extracellular ice crystals, increasing their size. This increases intracellular solute concentration and ultimately lowers the intracellular freezing point (Aberle et al., 2001). This can cause further destruction to the cellular structure of the meat, degrading meat texture and negatively affecting water holding capacity, A decline in water holding capacity can eventually influence dryness of the final cooked product due to drip and cook loss, which will be discussed further on. Ultimately, migration ceases and a fully frozen product is achieved. This point is called the eutectic point and comes about when solutes crystallize alongside the ice crystal formation (Aberle et al.,

2001). Unfortunately, no home freezer system is perfect. Fluctuations in freezer temperature are thought to promote recrystallization and be a key reason for quality deterioration due to further disruption of muscle fibers (Bevilacqua and Zaritzky, 1982).

Protein degradation, considered a major issue in frozen meat products, can be directly affected by ice crystallization and solute concentration increases. As mentioned, ice crystal formation can cause ruptures to muscle fibers and can also degrade proteins on a cellular level, but the increased concentration of intracellular solutes can also cause proteins to solubilize (Sikorski, 1978).

During frozen storage, the most visible quality defect is freezer burn, or dehydration of the meat due to sublimation of ice. Freezer burn is denoted by gray or pale patches on the meat surface. According to Jul (1969), meat loses moisture "as a consequence of vapor pressure gradients within the product and between the product and the external environment." This loss of moisture from the meat surface can cause concentration of color pigments and due to loss of water, reduce reflectivity (Aberle et al., 2001). Combined, these issues can make thawed meat appear darker than chilled fresh meat (Jeremiah, 1981; Aberle et al., 2001). According to Bell (2001), the best way to reduce freezer burn is to apply a "tightly-fitting film that is impermeable to water and vapor."

Though lipid oxidation is significantly retarded in a frozen atmosphere when compared to a chilled atmosphere, lipid oxidation still may occur to some degree. It has been found that a gradual decrease in sensory acceptability during frozen storage is primarily due to the oxidation of lipids (Aberle et al.,

2001). Lipid oxidation in frozen storage conditions can also be increased by several different factors. Extreme freezer burn can cause an increase in surface area of the meat, thus enhancing oxygen penetration (Bell, 2001). Also, the salting out of proteins by the increased solute concentrations in pockets can encourage lipid-protein complex formations which in turn can increase lipid oxidation (Sikorski, 1978). Also, according to Khan and Lentz (1977), the accumulation of protein-breakdown products in beef, a result of oxidative deterioration and enzyme activity, can also increase off-flavors and odors.

Moisture loss other than surface sublimation can also be an issue in frozen meat. This moisture, in the form of purge or cooking loss, becomes evident when meat is thawed and cooked. According to Romans et al. (2001), Aberle et al. (2001) and Bell (2001), drip or purge loss is a result of large crystals of extracellular ice melting, and rather than migrating back into the cells, it collects to form unattractive pools at the bottom of packages. This loss of moisture can result in decreased cooking yields and perceived dryness of the product from a sensory aspect (Romans et al., 2001). Loss of nutrients such as salts, proteins, peptides, amino acids and water soluble vitamins (Aberle et al, 2001) coincides with moisture loss.

To manage some of the issues associated with freezing meat, aspects such as storage period, packaging type, and the addition of phosphates for water-binding ability or antioxidants for reduction of oxidative rancidity must be addressed. Storage period has, in the past, been a primary means to control problems with frozen meat quality. It is suggested by Aberle and others (2001)

that beef muscle cuts, held at -12°C, may be stored for up to 4 months without adverse effects on quality. Another source cites that beef muscle cuts and chopped beef, held at -12°C, may be stored from 4 to 12 months and 3 to 4 months, respectively (Anon., 1994).

Enhancement of Meat

Many processed meat products have used spices and additives to improve consumer eating experience and to provide consistent product to consumers. However, the industry use of non-meat ingredients such as sodium tripolyphosphate, sodium chloride and natural antioxidants is relatively new to beef whole muscles. The reason behind this is the consumer's desire for a consistent and pleasurable eating experience with beef cuts that can already be enjoyed with items such as poultry and fresh breakfast sausage. According to the NCBA (2000), inadequate tenderness, flavor, juiciness and overall palatability are all within the top 10 greatest quality challenges that purveyors, retailers and restaurateurs believe must be overcome are. These challenges may be met with the enhancement of beef cuts. Many studies have shown that enhancement with a solution containing phosphate, salt, antioxidant, or a combination thereof has been found to improve tenderness, juiciness and overall eating experience (Vote et al., 2000; Robbins et al., 2003; Lawrence et al., 2004).

Sodium Chloride

For ages, salt has been used as a flavoring agent as well as a method for preserving meat products. Salted, dried meats were a primary source of protein for individuals before refrigeration was introduced.

The three main functions that salt serves currently are flavor enhancement, protein extraction to increase water-holding capacity of processed meats and extension of shelf life by lowering water activities of food products (Claus, et al., 1994).

Flavor enhancement is relatively self explanatory, however, it is because of salt's flavor that it is also self limiting in how much can be added to a product (Claus, et al., 1994). Protein extraction, or salting-in, occurs as a result of salt binding to proteins, causing electrostatic repulsion among molecules within the meat system (Foegeding et al., 1996). This electrostatic repulsion among molecules causes a loosening of the protein structure, which allows more water to enter the matrix, thus increasing water holding capacity of the meat system (Foegeding et al., 1996). Several studies have shown that, with the addition of a phosphate/salt solution to meat, water holding capacity increased (McGee et al., 2003; Lawrence et al., 2004). The ability of salt to lower water activity in a meat product is another of its beneficial characteristics. By lowering water activity, salt is able to slow down microbial growth and ensuing spoilage (Huang and Nip, 2001).

Though salt has many attributes, it may also present a challenge. Salt is a promoter of oxidation in meat products (Toldrá et al., 2001), and may be

especially disadvantageous for fresh or uncooked frozen meat cuts (Claus et al., 1994). Trout (1990) and Chu and others (1987) found that sodium chloride has a significantly negative effect on the oxidative stability of myoglobin. Also, it was recommended by Lee et al. (1997) that since salt has such an effect on oxidative stability of meat, meat processors should attempt to minimize the use of salt in products in order to improve product quality from an oxidative rancidity standpoint.

Sodium Tripolyphosphate

The primary function of phosphates in meat systems is to increase water holding capacity, thus preventing product from becoming too dry when submitted to a heating process. This function may be achieved by increasing ionic strength, increasing pH, and by phosphate anions complexing with myofibrillar proteins and divalent cations (Lindsay, 1996). Increased ionic strength is believed to cause a decreased interaction among proteins up to a point where a colloidal solution is formed, of great significance in comminuted product (Lindsay, 1996).

Meat products generally have an isoelectric point around pH 5 to 5.5 (Martin, 2001). At this point no net charge exists and water retention by the protein complex is at a minimum (Martin 2001). However, when pH shifts away from the isoelectric point, electrostatic repulsion between protein molecules occurs, causing a swelling of the protein complexes, allowing available water to gain access to the complex.

Phosphates often work in conjunction with salts to increase water holding capacity (Martin, 2001). However, unlike salts, phosphates are able to increase yields without providing a strong salty flavor to the product, even though phosphates are the salt form of phosphoric acid. Phosphates are also functional in that they have the ability, as negatively charged compounds at common food pH levels, to seize onto positively charged metal ions in a process called chelation (Miller, 1996). The metal ions often act as catalysts to oxidative reactions (Claus, 1994; Martin, 2001), and chelating them slows oxidative processes. Phosphates have also been noted with improving color retention, and increasing tenderness and juiciness of cooked product. Retention of color may also be directly related to the buffering ability of phosphates.

As functional as phosphates may be, their uses in enhancement solutions are not without problems. Phosphates are often very difficult to get into solution, especially if salt has been added previously to the solution. Product with added phosphate has also been distinguished by having a soapy flavor (Smith et al., 1984; Claus et al., 1994). In this respect, the addition of phosphates to meat products is self limiting.

Natural Antioxidants

Oxidation within a meat system is a general term that describes two processes. It refers to myoglobin oxidation or the loss of an electron from the sixth ligand site of the iron molecule, going from ferrous to ferric state, in myoglobin which causes a browning effect in meat. It also refers to lipid oxidation which is the reaction of oxygen with the double bonds of

polyunsaturated fatty acids (PUFAs) to form peroxides which lead to off-flavors (Faustman, 1994). Off-flavors characteristic to oxidation are created by the presence of aldehydes, acids and ketones produced during the oxidation process (Aberle et al., 2001).

Meat that has not been exposed to oxygen is said to possess deoxymyoglobin pigments and shows a purplish color. The ferrous iron has a water molecule bound to it at the sixth ligand position. Myoglobin that has been exposed to oxygen possesses oxygen at the sixth site of the ferrous iron and is called oxymyoglobin and is a bright, cherry red color. When oxidation of myoglobin occurs, a hydrogen molecule steps into the sixth site and joins the oxygen to form a water molecule. The oxidized form of myoglobin is metmyoglobin and is brown in color.

There are several forms of lipids in meat that can be affected by oxidation processes. Oxidation of triacylglycerols, located within the lipid droplet and fat depots, and more importantly phospholipids, located in the cellular and subcellular membranes, causes the majority of the off-flavors (Faustman, 1994). Oxidation of cholesterol molecules has very little effect on off-flavor development (Faustman, 1994).

Major factors that aid oxidation within meat systems are the presence of metal ions (i.e. iron and copper), salt, surface dehydration, heat, ultraviolet light and low pH (Aberle et al., 2001). Conversely, factors that inhibit, or retard, oxidation of meat components are darker storage space, reduction of air

(oxygen) in contact with the meat and the addition of synthetic or natural antioxidants.

Though there are many antioxidants used in the food industry today, all have the same basic purpose, to delay the onset of lipid oxidation by binding free radicals that cause oxidation reactions. Synthetic antioxidants such as butylated hydroxyanisole (BHA), propyl gallate and butylated hydroxytoluene (BHT) have been the most common antioxidants adopted by the food industry in recent years (Romans et al., 2001), but historically natural antioxidants have been used without knowledge of their antioxidant effects. Currently, the industry is utilizing these natural antioxidants primarily in the form of extracts or oleoresins.

Rosemary is a popular antioxidant and is widely used in the beef industry, while sage is used considerably for pork, especially sausage. Rosemary and sage have been found to be the most effective natural antioxidants, followed closely by thyme and oregano (Coggins, 2001). When compared to other natural antioxidants in a study performed by Sánchez-Escalante and others (2001), it was found that rosemary powder, alone or in conjunction with ascorbic acid, was "highly effective in inhibiting both metmyoglobin formation and lipid oxidation", that ascorbic acid alone or combined with taurine or carnosine had limited antioxidative abilities, and that taurine did not show any antioxidative effect on beef patties in modified atmosphere packaging. In a study conducted by Sebranek and others (2005), it was found that rosemary extract was as effective as BHA/BHT in curbing oxidative rancidity in refrigerated sausage and pre-

cooked frozen sausage and was a more effective antioxidant than BHA/BHT in raw frozen sausage.

Packaging Systems

An old meat packing adage states that a package must protect what it sells and sell what it protects. In the search for the perfect frozen storage package, one must also keep in mind that it would beneficial if this package was also attractive at the retail store. Bell (2001) asserted that there are seven functional requirements of meat packaging: containment, protection, preservation, apportionment, unitization, convenience and communication. There are several packaging systems that are able to achieve the first three requirements in this modern era, but the perfect combination of apportionment, unitization, convenience and communication is still being sought, without lowering the ability to maintain protection and preservation.

Romans et al. (2001) and Aberle (2001) both stated that the ideal packaging system for frozen meat was one that prevented moisture loss, was air impermeable to exclude oxygen and other volatiles, was pressed tightly to minimize air contact with the meat surface and possessed some resistance to scuffing and tearing. Thus, Romans and others (2001) found that vacuum packaging was the ideal packaging system for shelf-life longevity. Jayasingh et al. (2001) also made note that vacuum packaged steaks are perfect for transport because they have very little head space and are very durable.

The problem that arises with vacuum packaging is the purplish-red color of the meat due to deoxymyoglobin meat pigments. Though Faustman (1994) and many others have found that the use of vacuum packaging greatly extends the shelf life of meat when compared to their aerobically packaged counterparts, consumers still believe that bright, cherry red is the only right color for meat. Faustman (1994) found that consumers associated the purplish-red color of vacuum packaged beef with old cow meat, tougher meat, spoiled or temperature abused meat. In a study conducted by Carpenter and others (2001), it was found that conventional polyvinylchloride over-wrap packaging was preferred over vacuum skin packaging which was preferred over a high oxygen modified atmosphere packaging in relation to appearance scores and likelihood of purchase of beef steaks, but that knowing which package the meat came from when performing sensory analysis had no effect on eating experience.

According to Romans et al. (2001), consumers "prefer to buy fresh retail cuts (not trusting frozen raw cuts) and to freeze them at home in inadequate freezers and in inadequate packaging." Currently consumers are purchasing these beef steaks primarily in over-wrapped polystyrene packages, high-oxygen modified atmosphere packages or, in a small percentage of cases, in freezer paper from local butchers. According to Gill (1990), controlled atmosphere packaging greatly extends the shelf life of beef when compared to air permeable packaging. However, in a study performed by Insausti, et al. (1999) it was found that vacuum packaged beef had a considerably longer shelf life than their modified atmosphere packaged equivalents. Jackson and others (1992), found

that beef steaks packaged in high oxygen modified atmosphere packaging, as compared to vacuum packaged beef steaks, produced much stronger off-odors over retail display time. There have been few studies in relation to frozen storage in anything other than vacuum package, but a study comparing vacuum packaged steaks to controlled atmosphere packaged steaks stored frozen for 6 months found that both packaging systems showed similar moisture loss but that the controlled air packaged steaks had higher lipid oxidation levels than the vacuum packaged counterparts (Kenawi, 1993).

The use of carbon monoxide gas in beef to maintain bright, cherry red color without having the oxidative effects of high oxygen packaging is also becoming a prevalent method for maintaining meat for retail display. Carbon monoxide (CO) has been found to have a higher affinity for the iron molecule in myoglobin than oxygen molecules do, thus competing with oxygen for the sixth binding site and delaying lipid oxidation by forming carboxymyoglobin. Lunõ and others (2000) found that the addition of CO to several different combination atmospheres including O_2 , CO_2 and N_2 provided significant aerobic plate count reductions when compared to packaging atmospheres without CO. Also packaged steaks with CO experienced 5 – 10 days of exta shelf life as evidenced by delayed metmyoglobin formation, longer lasting red color, lower TBARS for an extended period and maintained acceptable meat odor for longer periods.

Warner-Bratzler Shear

Beef tenderness was identified by the NCBA (2000), in the National Beef Quality Audit, as the second most important quality challenge according to

purveyors, retailers and restaurateurs, second only to insufficient marbling and tied with lack of uniformity in cuts. Others have found that tenderness is the most important characteristic that determines whether a consumer will have a good eating experience or not (Miller et al., 1995) Packers and retailers, alike, are striving to come up with a beef product that is "guaranteed tender" for every eating experience, and many have turned to methods such as chilled aging, electrical stimulation of carcasses, injections of solutions containing calcium chloride, sodium tripolyphosphate and the like, and mechanical tenderization. It has even been found that frozen storage can produce a slightly tenderizing effect (Jeremiah, et al., 1990; Shanks et al; 2002). From previous discussion, it was learned that enhancing beef with solutions containing phosphate produced more tender and juicy product. The question becomes, how does the industry measure tenderness from an economical standpoint? Consumer panels are expensive and often expansive. Trained sensory panels are good representatives of consumer panels, but are not completely objective.

Much work has been done to develop a method to determine objective meat tenderness. The most popular method is the Warner-Bratzler shear force (WBSF) method which measures the amount of force it takes to shear through a core sample of cooked meat. This method was first developed by K.F. Warner in the late 1920s and was later refined by L.J. Bratzler in the 1930s (Wheeler et al., 1997b). The process of determining WBSF begins with cooked steaks. The AMSA (1995) suggests that steaks be cooked to an internal temperature of 71°C, and Wheeler et al. (1997b) suggest the use of a belt grill, or even possibly an

impingement oven or clam shell griddle for turning out evenly cooked steaks. After steaks have cooled to room temperature, it is suggested by the AMSA (1995) to take at least six "good" cores parallel to the muscle fibers. The core samples are then sheared perpendicular to the muscle fibers to determine the force required to do so. Wheeler and others (1997a) found that though this operation is highly repeatable within institutions, due to the difference in procedures from institution to institution, there is very little correlation between institutions and thus comparisons of actual shear values should not be made.

Still, efforts have been made to come up with a general threshold to distinguish tender cuts from all else, and many institutions use the value of 4.1 kg of shear force (Huffman et al., 1996) as a general threshold of tenderness. Steaks at or below this threshold are found to be acceptable in tenderness 98 % of the time, as recorded by Huffman et al. (1996). Researchers involved with the National Beef Tenderness Survey (Brooks et al., 1998) used a threshold of 3.9 kg to denote likely to be tender product and 4.6 kg to separate intermediate and tough tenderness categories of beef.

Regardless of what values represent which tenderness category, the WBSF must be validated when compared to consumer acceptability and to trained sensory panel scores. Several studies have found that WBSF values correlate highly with consumer panel tenderness scores on equivalent samples (Miller, et al., 2001; Platter et al., 2003). According to Miller (1994), although WBSF values highly correlate to trained sensory panel tenderness scores, there is no explanation within the values for "fracturability, cohesiveness of mass,

springiness, number of chews required to segment a meat sample, initial juiciness, sustained juiciness, connective tissue amount, or muscle fiber tenderness." One can shear a sample and find that it is abnormally tough, but the explanation as to why it is tough (i.e. connective tissue or genetically though muscle fibers) is not located in the shear force value.

A method may some day be developed than can take into account the many factors involved with consumer perceptions of beef including cooking method, degree of doneness, added seasonings and personal preferences in relation to tenderness, juiciness and flavor (Lorenzen et al., 2003). However, WBSF is currently a good method for objectively measuring cooked beef tenderness, one of the most important aspects in relation to beef palatability.

CHAPTER III

THE EFFECTS OF FRESH AND FROZEN STORAGE ON PALATABILITY, OXIDATIVE RANCIDITY AND COLOR OF PACKAGED BEEF STEAKS

Abstract

The purpose of this study was to determine the effects of home storage enhanced tripolyphosphate/sodium period and temperature on (E: non-enhanced chloride/rosemary oleoresin) or (N) Longissimus or Semimembranosus steaks, in modified atmosphere packaging (MAP: 80% O₂) and 20% CO₂), vacuum packaging (VP), or polystyrene trays over-wrapped with polyvinyl chloride (PVC). Storage periods were as follows: 3 d refrigeration (2.2 °C), or 15, 30, 60, or 90 d frozen (-14.4°C). Steaks were evaluated for Warner-Bratzler shear force (WBSF), oxidative rancidity, sensory attributes by a trained panel, odor, packaged oxygen percentages, purge loss, and objective lean color values. It was found that enhanced steaks were more tender than their nonenhanced counterparts throughout the storage periods from a WBSF standpoint and were more acceptable for longer frozen storage periods than non-enhanced from a sensory evaluation perspective. Enhanced MAP steaks were found to be unacceptable after 60 d frozen storage from a sensory aspect. It is recommended that non-enhanced MAP and PVC steaks be used quickly by the consumer and not frozen for any period of time within the original packaging.

Purge loss was significant for frozen steaks stored in VP systems, and MAP *Longissimus* steaks revealed excessive purge loss after 60 d frozen storage.

Introduction

Case-ready packaging systems have fast become the primary means by which many retailers prefer to market meat products to their consumers. It has been estimated that approximately one-half of all beef is retailed in a modified atmosphere packaging (MAP) system (FMI, 2004). The introduction of caseready product, the most significant advance in beef processing since the advent of boxed beef in the late 1960s, has already reshaped the way beef is processed, packaged and marketed to consumers (NCBA, 2000). Retail stores are moving more case-ready products, selling 1.2 billion packages in 2000, more than double the number sold in 1997 (Brody, 2004).

Case-ready packaging systems generally come in one of two major forms: vacuum packaged product, which provides a vacuum environment with tightly fitted high oxygen barrier plastic packaging, or in modified atmosphere packaging, which entails plastic trays, high oxygen barrier film, and a combination of nitrogen, carbon (dioxide or monoxide), and oxygen gases to "flush" the package. These two types of packaging systems offer many advantages to retailers and consumers alike, when compared to a typical retail packaged system such as polyvinyl chloride over-wrap, an air permeable film.

Some benefits that case-ready packaging systems offer retailers are reduced in-store labor costs; fewer out-of-stock items due to the ability of the

retailer to reorder specific cuts when necessary; Further benefits include the ability of retailers to guarantee a consistent product to consumers from purchase to purchase, extension of product retail shelf life due to barrier films, lack of oxygen, and/or antimicrobial abilities of some gases, and the reduction of liability risks for the retailer when and if a food safety issue arises concerning the packaged product This last is valid because the retailer performs no direct product handling, and the packages are "tamper-proof" because tampering results in opening of the package. Luño et al. (2000) stated that modified atmosphere packaging is well known as a method for extending the shelf life of a variety of foods, including fresh meat. One additional benefit that MAP consumer-ready packages offer that vacuum packages are not able to offer is the ability to display meat cuts in an appealing manner in an attractive package and with a bright, cherry red color that consumers have come to associate with meat freshness.

Modified atmosphere packaging was designed to present fresh meat attractively in a retail display case to consumers, while extending the retail shelf life and supplying all other advantages that case-ready packages provide. Product in these packages were meant to be taken home and prepared soon thereafter. However, many consumers are taking these packaged products home and freezing them, rather than preparing them within a few days of purchase. This may be creating meat quality issues especially in MAP packaged product. Unfortunately, there have not been many studies involving case-ready packaging systems stored in homefreezer conditions.

The objective of this study is to determine the quality attributes of beef steaks placed in vacuum, over-wrap or modified atmosphere packaging systems and stored for various periods of time at home refrigeration or frozen storage temperatures. Recommendations for acceptable storage periods for different packaging systems will hopefully be developed to aid retailers in consumer education and to improve consumer home use of product.

Materials and Methods

Experimental Samples.

USDA Select ribeye rolls (IMPS # 112A; n = 20 pairs) and inside rounds (IMPS # 169A; n = 20 pairs) were randomly selected at 48 h post-mortem from the Excel/Cargill plant in Plainview, TX. Subprimals were vacuum packaged and shipped to the Food and Agricultural Product Center (FAPC) at Oklahoma State University for further analysis.

Postmortem Handling.

Upon arrival at the FAPC, one-half of the subprimals were randomly assigned to enhancement protocol, while their remaining paired subprimal were designated as non-enhanced controls. Subprimals designated for enhancement were pumped to 110% of their green weight with an enhancement solution designed to distribute .25% salt, .35% phosphate and .10% rosemary oleoresin in the final product. The subprimals were enhanced using a Metalquimia[®] (Model 120/3000CR) multi-needle spray injector. Following enhancement, subprimals

were allowed to equilibrate (approximately 30 min), and then were fabricated into 2.54 cm steaks.

Steaks were then randomly assigned to one of three packaging systems: modified atmosphere packaging (MAP), polyvinylchloride over-wrap (PVC) or vacuum packaging (VP). Packaged steaks were randomly assigned to one of five storage periods: 3 d refrigeration (2.2 °C), 15, 30, 60 or 90 d frozen (-14.4 °C). All packaging systems were equally represented within each storage period. Refrigeration and freezing were performed in walk-in coolers/freezers, which had been adjusted to household refrigeration conditions (2.2 °C, ± 1°C) or household freezer conditions (-14.4 °C ± 1°C), respectively. A percentage of steaks were set aside for initial objective color analysis (L*, a*, b*) and were also utilized for initial thiobarbituric acid (TBA) analysis samples. MAP packaged steaks were placed in Cryovac[®] solid barrier polypropylene trays [Max. oxygen transmission rate (OTR) of 0.1 cc/tray/24h], flushed with an 80% O₂, 20% CO₂ gas and sealed with a Cryovac[®] oxygen barrier film (Model LID1050; Max. OTR of 25.0 cc/m²/24h) with a G. Mondini[®] MAP machine (Model CVS 0.1-S). PVC packaged steaks were placed in Cryovac[®] barrier polystyrene trays (Max. OTR of 0.1 cc/tray/24h) and over-wrapped with low oxygen barrier PVC film (23,000 cc $O_2/m^2/24h$). VP steaks were placed in Cryovac[®] high abuse barrier vacuum bags (Model BH620; OTR of 15-30 cc/m²/24h) and sealed using an Ultravac[®] vacuum packaging machine (Model Busch RA025004261011).

Warner-Bratzler Shear Force.

Warner-Bratzler shear force (WBSF) measurements were obtained for a percentage of the steaks to determine tenderness. Steaks were randomly selected for WBSF and there was an even distribution of storage periods, packaging systems and enhancement treatments. Frozen steaks were tempered for approximately 36 h at 4 °C before being cooked. Steaks were cooked on a Lincoln[®] impingement oven (Model 1132-00-A) and brought to an internal temperature of 70 °C. Steaks were allowed to cool to room temperature, and then six cores, parallel in orientation to the muscle fibers, were removed from each steak. Cores were then sheared, perpendicular to the muscle fibers, using a WBSF head attachment on an Instron[®] Universal Testing Machine (Model 4502) at a crosshead speed of 200 mm per min. The max load (kg) for each core was recorded utilizing Instron software, and the mean max load for each steak was calculated and analyzed.

Thiobarbituric Acid Assay.

Lipid oxidation estimates were obtained by means of thiobarbituric acid reactive substance (TBARS) assays in the method suggested by Buege and Aust (1978) with modifications. Steak samples used for TBARS analysis were randomly selected from those steaks to be used for sensory analysis. A 10-g sample was obtained from the steak surface, weighed and homogenized with 30 ml of cold, deionized water for 15 s using a Waring[®] commercial blender (Model 33BL79). The homogenate was then centrifuged at 2000 xg for 10 min at 4 °C

(Beckman[®] Induction Drive Centrifuge, Model J-6M). The following test tubes were prepared in duplicate. The supernatant (2 ml) from the centrifuged sample was combined with 4 ml trichloroacetic acid (TCA)/thiobarbituric acid (TBA) reagent, which consisted of 15% TCA and 20 mM TBARS reagent in deionized water. The supernatant then received 100 µl of butylated hydroxyanisole (BHA). The mixture was vortexed and heated for 15 min in a boiling water bath. The mixture was cooled for 10 min in cold water following the hot bath and centrifuged at 2000 xg for 10 min. Absorbance for each sample was then read at 531 nm using a spectrophotometer (Beckman[®], Model DU 7500). Results were recorded as thiobarbituric acid reactive substance (TBARS), which represents the mg malonaldehyde (MDA) equivalents per kg of fresh meat.

Sensory Analyses

Trained taste panels (consisting of 5 to 7 panelists) evaluated steaks from each storage period, with an even representation from packaging system and treatment groups. Panelists were recruited from a group of individuals that had been trained according to the methods outlined by the American Meat Science Association (1995) guidelines.

Before training, panelists went through a screening process to determine sensory acuity; interest in sensory evaluation; sensory discrimination and reproducibility; and panelist cooperation and motivation ability (AMSA, 1995). Selected panelists were then familiarized with the sensory ballot layout. Samples were then analyzed by the panelists and a discussion of the samples' attributes

ensued to improve the panelist's ability to recognize and identify sensory attributes (AMSA, 1995). Before the beginning of each morning session, panelists were given a sample to be analyzed and then discussed. This was performed in an effort to retrain panelists and keep sensory attribute analysis as precise as possible.

Previously frozen steaks were tempered for 36 h at 4 °C prior to cooking. Steaks were cooked to an internal temperature of 70 °C using a Lincoln® impingement oven (Model 1132-00-A). Samples were then placed in plastic bags to keep warm and transported to the sensory evaluation room. Samples were cut into equal serving portions (1 cm³) and served to panelists in individual booths to remove any bias by fellow panelists and under red light to remove any effect of cooked color on acceptability. Panelists were provided an expectorant cup, a cup of water and unsalted crackers to cleanse their palates. Descriptive sensory analysis was used to evaluate samples (AMSA, 1995). Panelists were asked to evaluate steaks for tenderness and juiciness using an eight point scale (1 = extremely tough, dry and 8 = extremely tender, juicy). Off-flavor (being defined as oxidative rancidity) and salty flavor were evaluated using a three point scale (1 = not detectable and 3 = strong). Overall acceptability was scored on a seven point scale (1 = extremely undesirable, 4 = acceptable and 7 = extremelydesirable). The panel was conducted twice a day for two days, and a maximum of 15 samples were served each session.

Odor panel analysis of uncooked steaks was also performed by trained panelists on a percentage of packages from each storage period, evenly

representing all packaging systems and treatment groups. Previously frozen steaks were allowed to temper for approximately 36 h at 4 °C. Packaging systems were individually opened with a knife, and the panel immediately evaluated the odor of the package. Panelists ranked each package using a six point scale (1 = odor not detectable and 6 = odor present, which is strong, overpowering, and intolerable and easily produces physiological effects). Steaks from odor panel were then cooked for WBSF measures.

Other Measures.

Previously frozen steaks were allowed to temper for 36 h at 4 °C before the following tests were performed. Objective lean color scores were measured, over all storage periods and representing treatment and packaging system, using a Hunter[®] colorimeter (Model 45/0-L). Color scores were expressed in CIE values, L*, a*, b*. L* values represent lightness of color (100 = white, 0 = black). The a* and b* values relate to the red/green and yellow/blue scales, respectively, with higher values tending toward red (100 = red, -100 = green) and yellow (100 = yellow, -100 = blue), respectively.

Packaged oxygen concentration was monitored in a percentage of randomly selected MAP and PVC packages for each storage period. Additionally, these identical steaks were used, along with a random selection of VP steaks, for purge loss and WBSF measures. Packaged oxygen percentages were measured using a PBI[®] Dansensor CheckMate head space analyzer. After packaged oxygen was measured, the same steaks were utilized for purge loss

measures. Packaged steaks were weighed, purge was removed, and the packaged steaks were reweighed. Package weight was taken into consideration and values were recorded as purge loss as a percentage of the "wet" steak weight.

Statistical Analysis

The data were analyzed using ordinary least squares (PROC GLM, SAS Institute, Cary, NC). The model included storage period, packaging system, treatment (enhancement) and, when applicable, subprimal type as main effects. Mean separation was accomplished using least significant difference (PDIFF, SAS Institute, Cary, NC).

Results and Discussion

WARNER-BRATZLER SHEAR FORCE

Generally, enhanced beef steaks possessed lower WBSF values, indicating greater tenderness, after 3 d of refrigeration and throughout the frozen storage periods than did non-enhanced steaks (Table 1). This is in accordance with past studies that found that enhancement with a phosphate/salt solution improved tenderness of fresh and/or pre-cooked beef (Vote et al., 2000; McGee et al., 2003; Robbins et al., 2003). Also, non-enhanced steaks, were generally in an intermediate tenderness category as described by Brooks et al. (2000)

The enhanced MAP and VP steaks appeared to become more tender over the frozen storage period up to d 60, while all non-enhanced steaks and the

enhanced PVC packaged steaks appeared to become more tender over the initial 30 d of frozen storage, and then increased in shear force values after d 30 (Figure 1). The enhanced VP steaks increased in tenderness up to 60 d but then decreased significantly in tenderness after that (Figure 1). The increase in tenderness of the beef cuts to some degree during frozen storage is in agreement with the work of Shanks and others (2002) who found that frozen storage decreased WBSF values and in partial agreement with the work of Smith and others (1969) who found that freezing for only 3 to 6 weeks had no significant affect on beef tenderness, but that longer periods could produce some tenderization effects.

LIPID OXIDATION

Using 0 d values as a reference, it became evident that non-enhanced steaks had higher TBARS than enhanced steaks (Figure 2). This agrees with several studies that found that steaks stored in chilled storage, when enhanced with a rosemary oleoresin, showed lower TBARS levels than their non-enhanced counterparts (Stoick et al., 1991; Sánchez-Escalante et al., 2001). Even for d 0 steaks, enhancement appeared to delay lipid oxidation in the period prior to packaging. All enhanced steaks, after 0 d, as well as non-enhanced VP steaks shared common letters, indicating similarity in oxidation levels (Figure 2). This is in accordance with the results of Payne et al. (2002) which stated that storage period and type presented no significant effect (P > 0.05) on TBARS values of enhanced MAP steaks. It was also found in this study that non-enhanced

Longissimus steaks possessed higher levels (P < 0.05) of lipid oxidation than non-enhanced *Semimembranosus* steaks over all storage periods and in all packaging systems (Figure 3).

Elevated levels of TBARS occurred for the non-enhanced steaks packaged in MAP and PVC (Table 2). On d 3 of refrigeration, the non-enhanced, MAP steaks possessed a significantly higher TBARS value than all other steaks (Table 2), which may be expected with the high level of oxygen in the packaging system the lack of antioxidant, and the refrigerated rather than frozen storage condition. During frozen storage, non-enhanced steaks packaged in oxygenated packaging systems (i.e., MAP and standard PVC over-wrap) displayed higher TBARS values (P < 0.05) compared to enhanced packaged or non-enhanced VP steaks (Table 2).

SENSORY ANALYSIS

Sensory Tenderness Ratings

MAP packaged steaks were considered to be toughest by a trained sensory panel after 3 d refrigeration and 15 d frozen storage and increased in perceived tenderness (P < 0.05) after 30 d frozen storage. The MAP steaks then became gradually tougher over the longer frozen storage periods (Figure 4). This is different from the reporting of Payne and others (2002) who showed that storage period had no affect on trained sensory panel tenderness scores for MAP packaged steaks. PVC packaged steaks followed a similar pattern, with the least tender steaks appearing after 3 d refrigeration (and 60 d frozen storage),

significant increase in tenderness after 15 d frozen storage and then a gradual decrease in tenderness afterward (Figure 4). Like the MAP steaks, the VP steaks received their least tender scores after 3 d refrigeration and 15 d frozen storage. However, the VP steaks became significantly more tender after further frozen storage and remained as tender through 90 d frozen storage (Figure 4).

Enhanced beef steaks were significantly more tender (P < 0.05) than nonenhanced steaks (Figure 5). This agrees with other studies that also found that beef steaks enhanced with a phosphate/salt solution were perceived by trained sensory panelists as being more tender than their non-enhanced counterparts (Vote et al., 2000; Robbins et al., 2003; Lawrence et al., 2004). Of the nonenhanced steaks, VP steaks were more tender (P < 0.05) than both the MAP and PVC packaged steaks (Figure 5), indicating that VP could intensify post-mortem aging in steaks under frozen storage conditions.

Sensory Juiciness Ratings

Generally, non-enhanced steaks were less juicy than the enhanced steaks (Figure 6), which is in agreement with the work of Robbins and others (2003) as well as Vote and others (2000) who both found that enhanced steaks pumped with a solution containing phosphate and salt showed increased juiciness scores according to trained sensory evaluation. One exception was that non-enhanced PVC packaged steaks maintained slightly higher levels of juiciness at 3 d of refrigeration and 15 d of freezing compared to other packaging system and enhancement combinations (Figure 6).

For the enhanced MAP steaks and the non-enhanced VP steaks, peak juiciness scores appeared after 30 d of frozen storage, followed by a maintained level of juiciness. For the VP steaks, both enhanced and non-enhanced, the lowest juiciness scores were received on d 15 of frozen storage (Figure 6).

Sensory Saltiness Ratings

As expected, all non-enhanced steaks possessed significantly lower saltiness scores than the enhanced steaks (P < 0.05) and were all statistically similar (P > 0.05) to each other (Figure 7). After 3 d of refrigeration, all enhanced steaks were scored similarly on saltiness, and saltiness score levels did not appear to change significantly over time, with the exception of d 60 enhanced, MAP and VP steaks exhibiting elevated saltiness scores (Figure 7). Also, throughout the frozen storage period, MAP steaks received significantly higher (P < 0.05) saltiness scores than VP steaks.

Sensory Off-flavor Ratings

During refrigerated storage, non-enhanced steaks showed significantly higher off-flavor scores than enhanced steaks (Figure 8). By looking at the TBARS values for steaks from d 3 (Figure 2), it appears that the elevated levels in the non-enhanced MAP steaks may have also caused off-flavors associated with oxidative rancidity and thus raised the overall off-flavor scores.

Over all frozen storage periods, off-flavor scores were higher (P < 0.05) for enhanced steaks rather than for non-enhanced steaks over all frozen storage periods (Figure 8). This was most likely not due to oxidative rancidity, but to a "soapy" flavor detected and mentioned, which has often been associated with the

addition of phosphate to an enhancement solution. From 15 d to 60 d frozen storage, off-flavor scores elevated significantly (P < 0.05) for both enhanced and non-enhanced treatments but then dropped off after 90 d frozen storage (Figure 8). This does not agree with the work of Payne et al. (2002) who found that frozen storage period did not affect uncharacteristic flavor.

MAP packaged steaks received significantly higher off-flavor scores than PVC steaks, which showed higher off-flavor scores (P < 0.05) than VP steaks (Figure 9). By looking at Table 2, one can see that the elevated off-flavor scores for the MAP and PVC steaks appear to be due to detectable levels of oxidative rancidity after 3 d refrigeration and 60 d frozen storage for the MAP steaks and 30 d frozen storage for the PVC steaks.

Sensory Overall Acceptability Ratings

After 3 d of refrigerated storage, the non-enhanced MAP and PVC packaged steaks were considered unacceptable by trained sensory panelists, and it appeared that non-enhanced steaks packaged in MAP were rated as being unacceptable as early as 15 d of frozen storage (Figure 10). Generally, enhanced steaks received acceptable overall sensory acceptability scores, and non-enhanced steaks received unacceptable overall sensory acceptability scores for all storage periods and types (Table 3). Also, for all packaging system and enhancement combinations, it appeared that overall acceptability ratings declined at 60 d of frozen storage (Figure 10).

ODOR ANALYSIS

All means appear to be within an acceptable odor range, from scores of "not detectable" to scores slightly above "odor present, which activates smell, is distinguishable, not necessarily objectionable in short periods" (Figure 11). Odor score means within this project are similar to those found by Payne et al. (2002).

Enhanced VP steaks received the highest odor scores (P < 0.05) after the refrigerated storage period (Figure 11). This was most likely due to discernment of common vacuum packaged odor. Generally, over time, odor increased for all packaging types and treatments, with the exception of steaks from the 90 d lot, when odor appeared to decrease slightly for all groups and significantly for the non-enhanced MAP steaks (P < 0.05).

Also, PVC packaged steaks possessed the lowest odor scores over all periods. Over all storage periods and considering all treatments, it was found that *Semimembranosus* steaks received significantly higher (P < 0.05) odor scores than did *Longissimus* steaks for all packaging systems (Figure 12).

OBJECTIVE COLOR SCORES

Longissimus steaks

From Figure 13, it can be seen that brightness (L*) values were highest for steaks submitted to the 3 d refrigeration period, even more so that the 0 d reference reading. All frozen storage periods showed mean brightness values lower than the 3 d refrigeration, but higher than the 0 d reference reading (Figure 13). This indicates that frozen storage is not detrimental to the brightness of steaks when compared to fresh, unpackaged steaks. Also, all steaks submitted

to a frozen storage period were similar in L* values (P > 0.05), indicating that if *Longissimus* steaks are home frozen immediately after purchase, there is no frozen storage period effect on brightness up to 90 d storage. These data differ slightly from the work of Payne et al. (2002) who found that, while frozen steaks generally had similar L* values throughout, frozen steaks were also similar to freshly fabricated steaks, rather than brighter.

From Figure 14, it can be seen that non-enhanced *Longissimus* steaks are significantly brighter in lean color score (P < 0.05) than enhanced *Longissimus* steaks over all time periods and types and considering all packaging systems. This is in agreement with Lawrence et al. (2004) who found that enhancement with a phosphate/salt/broth/rosemary solution significantly darkened the lean color of beef longissimus steaks.

Reference, or 0 d, a* values (redness) for both enhanced and nonenhanced *Longissimus* steaks are similar (Figure 15). After 3 d refrigeration, enhanced steaks displayed markedly increased redness values, while nonenhanced steaks exhibited lower (P < 0.05) redness values. Frozen storage, regardless of storage period, created lower redness values in enhanced *Longissimus* steaks when compared to 3 d refrigerated steaks (Figure 15). Also, non-enhanced *Longissimus* steaks however were not affected by long-term frozen storage, in that they remained the same throughout the investigation.

From Figure 16, it can be seen that after 3 d refrigeration, MAP steaks displayed a significant increase in a* lean color scores, which is in concurrence with the work of Payne et al. (2002), while VP steaks decreased in a* lean color

scores, as compared to d 0 reference samples. For frozen storage, MAP steaks exhibited higher, redder lean color values compared to PVC and especially VP steaks. This color advantage was slightly diminished as frozen storage time increased. Following each frozen storage period, VP steaks received the lowest, least red lean color scores compared to the remaining packaging types.

As can be seen from Figure 17, the effect of enhancement on a* values varied for each packaging type for *Longissimus* steaks. For VP steaks, no significant effect on a* values (i.e., redness) was observed between enhanced and non-enhanced steaks. However, for both the MAP and PVC packaged steaks, enhanced steaks were significantly redder in their respective lean color scores than their non-enhanced counterparts.

Reference, 0 d, b* values (yellowness) are similar for both enhanced and non-enhanced *Longissimus* steaks (Figure 18). After 3 d refrigerated storage, the enhanced steaks achieved significantly higher b* values than on d 0 and higher b* values (P < 0.05) than the non-enhanced steaks. Over time, enhanced steaks generally became slightly more yellow than the non-enhanced steaks. Within the frozen storage period, the yellowness of the steaks was not affected by storage period with the exception of the d 30 steaks which were significantly less yellow than steaks from all other storage periods. The reason for this phenomenon is unknown.

After 3 d refrigerated storage, MAP and PVC packaged steaks possessed significantly higher, more yellow, b* values than their previous 0 d values and than the 3 d VP steaks stored under the same conditions (Figure 19). As

compared to refrigeration stored steaks, similar findings were observed in that frozen steaks from all periods were significantly less yellow for the MAP and PVC packaged steaks compared to VP steaks (Figure 19).

Semimembranosus steaks

Reference, 0 d, lean color brightness scores for all *Semimembranosus* steaks were brought to an average of 31.84 (Figure 20). After 3 d refrigeration, and up to 60 d freezing, the L* values were generally higher (P < 0.05) than the initial L* value (Figure 20). However, after 90 d frozen storage, L* values were significantly lower (P < 0.05), indicating that storage up to 60 d does not greatly affect brightness of lean color in *Semimembranosus* steaks, but steaks stored any longer than that may have marked declines in brightness when compared to fresh steaks.

Looking at Figure 21, it can be seen that for *Semimembranosus* steaks, including all storage periods and temperatures and in both treatment groups, L* values were significantly lower in the VP steaks than all other packaged steaks (P < 0.05). The MAP and PVC packaged steaks were similar in brightness (P > 0.05).

After 3 d refrigeration compared to initial a* values, MAP steaks increased slightly in redness, PVC decreased slightly in redness, and VP steaks decreased significantly (P < 0.05) in redness, as was expected (Figure 22). Generally speaking, MAP steaks displayed significantly redder lean color scores compared to PVC steaks which were, in turn, redder than the VP steaks. Also, after 90 d frozen storage, it was noticed that all packaging systems increased significantly

in redness, when compared to all other storage periods and temperatures (Figure 22).

PACKAGED OXYGEN

By looking at Figure 23, it is observed that for all storage periods and temperatures and considering both subprimal types, the non-enhanced steaks packaged in MAP and PVC packages showed higher levels of packaged oxygen than enhanced steaks packaged in the same systems (P < 0.05). From a previous study performed by Payne and others (2002), it was found that for enhanced MAP steaks, packaged oxygen levels were significantly lower for 3 d refrigeration when compared to the frozen periods 15, 60 and 90 d. For the current study, there was no significant (P > 0.05) effect of storage temperature/period on packaged oxygen levels.

PURGE LOSS

Longissimus Steaks

Figure 24 shows that frozen storage over all periods increased purge loss significantly when compared to 3 d refrigeration (P < 0.05). Also, from period to period, enhanced and non-enhanced steaks were similar to one another, except for d 90 steaks where enhanced steaks had significantly more purge loss (P < 0.05).

Figure 25 and Table 4 show that purge loss was lowest for all packaging systems after 3 d refrigeration. For the frozen storage periods, VP cuts displayed

more purge loss compared to MAP and PVC over-wrapped steaks (Figure 25). For 15 and 30 d of frozen storage, VP steaks displayed the highest and PVC the lowest purge loss values (Table 4). This trend held consistent in that PVC packaged steaks displayed the least purge loss following 60 and 90 d of frozen storage (Table 4). Generally, MAP steaks experienced increased purge loss over time (Figure 25). This is in agreement with the results of Payne et al. (2002) who found that enhanced MAP *Longissimus* steaks possessed the least purge after 3 d refrigeration and gradually rose in purge volume over the frozen storage period.

Semimembranosus Steaks

After 3 d refrigeration period, *Semimembranosus* steaks produced significantly less purge loss (P < 0.05) when compared to all other frozen storage periods (Figure 26), and all frozen storage periods produced the same (P > 0.05) amount of purge loss from 15 to 90 d. For this experiment, purge amounts greater that 5.0% were considered excessive. It can be seen from Table 5 that all frozen period steaks displayed excessive purge loss.

Enhanced steaks reacted differently to different packaging systems when involving purge loss. To explain, enhanced steaks that were vacuum packaged produced significantly more (P < 0.05) purge loss than both enhanced MAP and PVC packaged steaks that were similar (Figure 27). However, non-enhanced steaks packaged in PVC packaging showed significantly more (P < 0.05) purge loss than non-enhanced vacuum packaged steaks, while MAP steaks were similar to both. From Table 6, it can be seen that unacceptable levels of purge

loss were found in the non-enhanced MAP and PVC steaks and in the enhanced VP steaks.

Implications

When considering modified atmosphere as a method of retail to freezer packaging, it is recommended that non-enhanced steaks be used quickly by the consumer to guarantee sensory acceptability and not stored frozen for more than one month to ensure undetectable levels of oxidative rancidity. Also, modified atmosphere packaged, enhanced steaks should not be stored in frozen conditions for more than one month to ensure an overall acceptable eating experience. For non-enhanced polyvinylchloride over-wrapped steaks, it is suggested that steaks not be frozen for longer than a period of fifteen days to ensure undetectable levels of oxidative rancidity and a pleasurable eating experience by the consumer.

Frozen storage of any length may be detrimental to vacuum packaged ribeye steaks from a purge loss standpoint, and extended frozen storage may cause excessive purge loss in modified atmosphere packaged ribeye steaks as well. Inside round steaks, due to leanness, should not be frozen if purge loss is of great concern, as excessive purge loss was found to be a problem in this project.

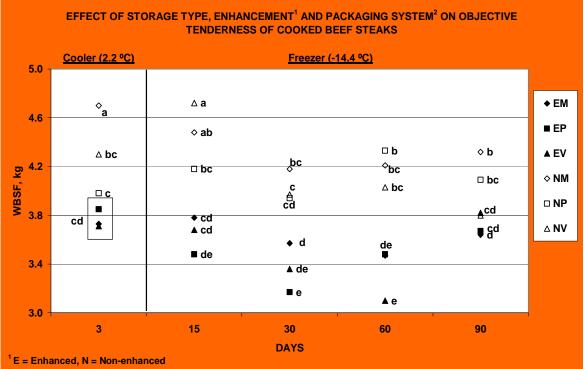
It is also recommended that if inside round steaks are to be sold nonenhanced, regardless of package or storage period and type, that all be tenderized in some fashion in order to guarantee tenderness

	2.2ºC	-14.4°C				
Treatment	3 d	15 d	30 d	60 d	90 d	Package
Enhanced	3.73 ^{cd}	3.78 ^{cd}	3.57 ^d	3.47 ^{de}	3.64 ^d	MAP
Enhanced	3.85 ^{cd}	3.48 ^{de}	3.17 ^e	3.48 ^{de}	3.67 ^{cd}	PVC
Enhanced	3.71 ^{cd}	3.68 ^{cd}	3.36 ^{de}	3.10 ^e	3.82 ^{cd}	VP
Non-enhanced	4.70 ^a	4.48 ^{ab}	4.18 ^{bc}	4.21 ^{bc}	4.32 ^b	MAP
Non-enhanced	3.98 ^c	4.18 ^{bc}	3.94 ^{cd}	4.33 ^b	4.09 ^{bc}	PVC
Non-enhanced	4.30 ^{bc}	4.72 ^a	3.97 ^c	4.03 ^{bc}	3.80 ^{cd}	VP

Table 1. Effect of storage type/ period, enhancement and packaging system on objective tenderness of cooked beef steaks (WBSF, kg).

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05). Shaded areas represent those enhancement and package system combinations with WBSF values greater than 3.9 kg (light shade) and 4.6 kg (dark shade).

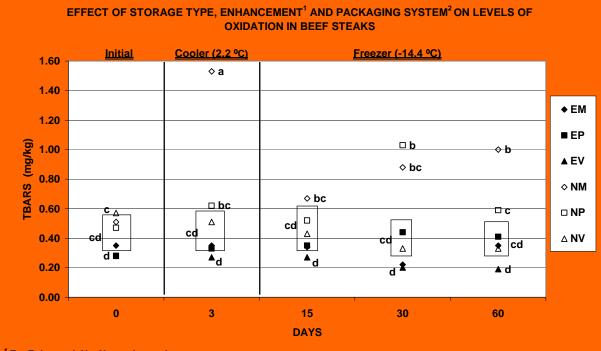
Figure 1.



² M = Modified atmosphere package, P = Polyvinyl chloride overwrap, V = Vacuum package

^{a,b,c,d,e} Means lacking a common letter differ (P < 0.05)

Figure 2.

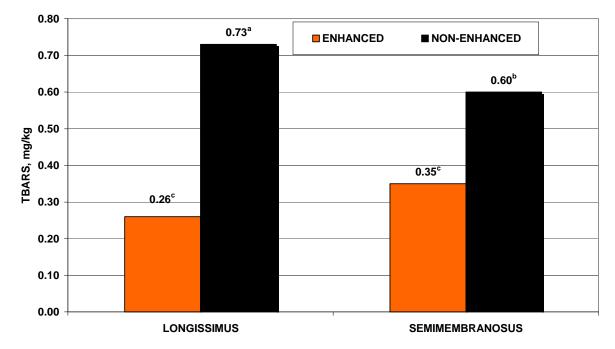


¹ E = Enhanced, N = Non-enhanced

² M = Modified atmosphere package, P = Polyvinyl chloride overwrap, V = Vacuum package

^{a,b,c,d} Means lacking a common letter differ (P < 0.05)

Figure 3.



EFFECT OF ENHANCEMENT AND SUBPRIMAL TYPE ON LEVELS OF OXIDATION IN BEEF STEAKS

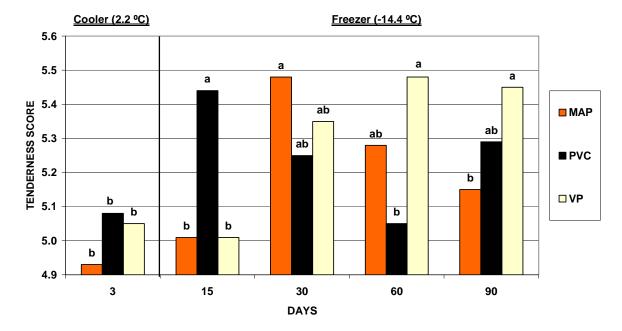
 $^{\rm a,b,c}$ Means lacking a common superscript letter differ (P < 0.05)

		2.2°C	-14.4°C			
Treatment	0 d	3 d	15 d	30 d	60 d	Package
Enhanced	0.35 ^{cd}	0.35 ^{cd}	0.34 ^{cd}	0.22 ^d	0.35 ^{cd}	MAP
Enhanced	0.28 ^d	0.33 ^{cd}	0.35 ^{cd}	0.44 ^{cd}	0.41 ^{cd}	PVC
Enhanced	0.28 ^d	0.27 ^d	0.27 ^d	0.20 ^d	0.19 ^d	VP
Non-enhanced	0.51 ^{cd}	1.53 ^a	0.67 ^{bc}	0.88 ^{bc}	1.00 ^b	MAP
Non-enhanced	0.47 ^{cd}	0.62 ^{bc}	0.52 ^{cd}	1.03 ^b	0.59 ^c	PVC
Non-enhanced	0.57 ^c	0.51 ^{cd}	0.43 ^{cd}	0.33 ^{cd}	0.33 ^{cd}	VP

Table 2. Effect of storage type/ period, enhancement and packaging system on levels of oxidation in beef steaks (TBARS, mg/kg).

^{a,b,c,d} Means lacking a common superscript letter differ (p<0.05). Shaded areas indicate lipid oxidation levels above the detectable threshold of offflavors defined as oxidative rancidity.

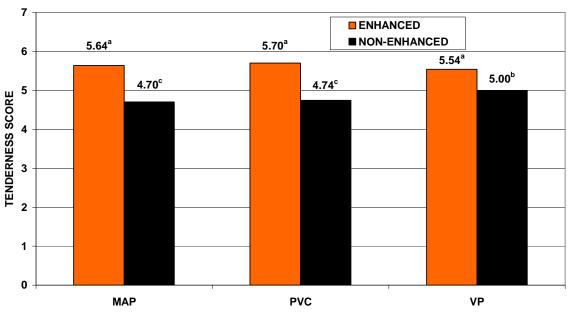
Figure 4.



EFFECT OF STORAGE TYPE/PERIOD AND PACKAGING SYSTEM ON SENSORY TENDERNESS¹ OF COOKED BEEF STEAKS

 1 Tenderness score: 1 = extremely tough, 8 = extremely tender $^{\rm a,b}$ Means lacking a common letter differ (P < 0.05)

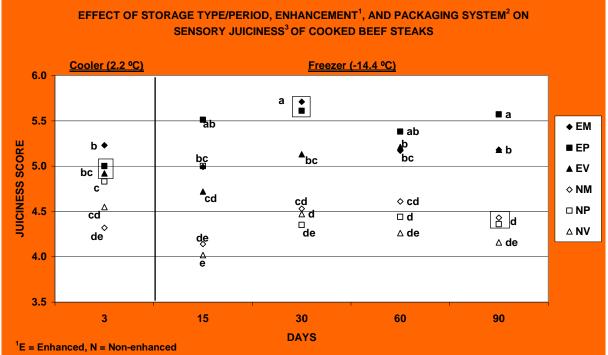
Figure 5.



EFFECT OF ENHANCEMENT AND PACKAGING SYSTEM ON SENSORY TENDERNESS¹ OF COOKED BEEF STEAKS

 1 Tenderness score: 1 = extremely tough, 8 = extremely tender $^{\rm a,b,c}$ Means lacking a common letter differ (P < 0.05)

Figure 6.

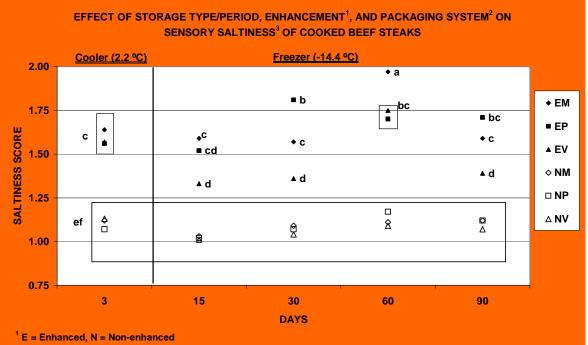


² M = Modified atmosphere package, P = Polyvinyl chloride overwrap, V = Vacuum package

³ Juiciness score: 1 = extremely dry, 8 = extremely juicy

^{a,b,c,d,e} Means lacking a common letter differ (P < 0.05)

Figure 7.

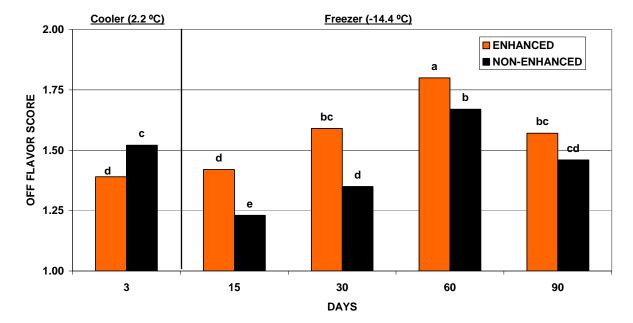


² M = Modified atmosphere package, P = Polyvinyl chloride overwrap, V = Vacuum package

³Saltiness score: 1 = not detectable and 3 = strong

^{a,b,c,d,e,f} Means lacking a common letter differ (P < 0.05)

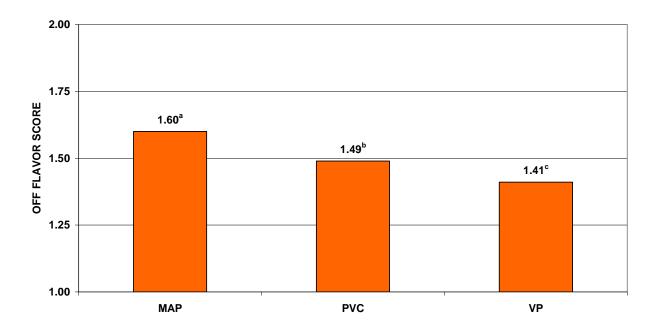
Figure 8.



EFFECT OF STORAGE TYPE/PERIOD AND ENHANCEMENT ON SENSORY OFF FLAVORS¹ OF COOKED BEEF STEAKS

 1 Off flavor score: 1 = not detectable and 3 = strong $^{\rm a,b,c,d,e}$ Means lacking a common letter differ (P < 0.05)

Figure 9.

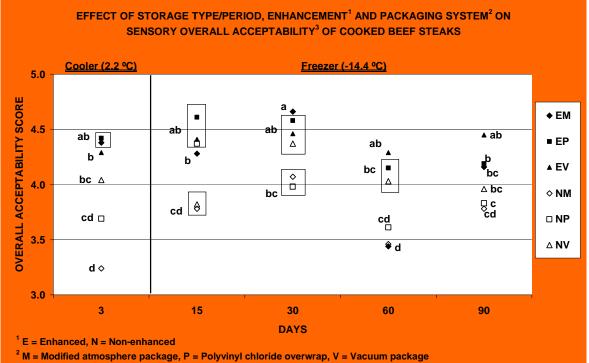


EFFECT OF PACKAGING SYSTEM ON SENSORY OFF FLAVORS¹ OF COOKED BEEF STEAKS

¹ Off flavor score: 1 = not detectable and 3 = strong

^{a,b,c} Means lacking a common superscript letter differ (P < 0.05)

Figure 10.



³ Overall acceptability score:1 = extremely undesirable, 4 = acceptable and 7 = extremely desirable

^{a,b,c,d} Means lacking a common letter differ (P < 0.05)

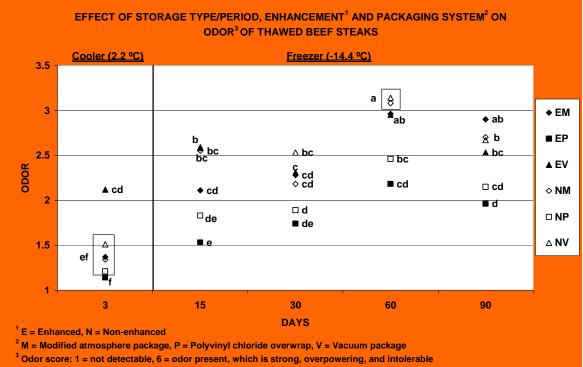
	2.2ºC	-14.4°C				
Treatment	3 d	15 d	30 d	60 d	90 d	Package
Enhanced	4.38 ^{ab}	4.28 ^b	4.66 ^a	3.44 ^d	4.16 ^{bc}	MAP
Enhanced	4.42 ^{ab}	4.61 ^{ab}	4.58 ^{ab}	4.15 ^{bc}	4.19 ^b	PVC
Enhanced	4.29 ^b	4.41 ^{ab}	4.46 ^{ab}	4.29 ^{ab}	4.45 ^{ab}	VP
Non-enhanced	3.24 ^d	3.78 ^{cd}	4.07 ^{bc}	3.46 ^d	3.78 ^{cd}	MAP
Non-enhanced	3.69 ^{cd}	4.37 ^{ab}	3.98 ^{bc}	3.61 ^{cd}	3.83 ^c	PVC
Non-enhanced	4.04 ^{bc}	3.82 ^{cd}	4.37 ^{ab}	4.03 ^{bc}	3.96 ^{bc}	VP

Table 3. Effect of storage type/period, enhancement and packaging system on sensory overall acceptability rating of cooked beef steaks.

¹ Overall acceptability score: 1 = extremely undesirable, 4 = acceptable and 7 = extremely desirable a,b,c,d Means lacking a common superscript letter differ (p<0.05). Shaded areas indicate unacceptable ratings (less than 4) for overall sensory

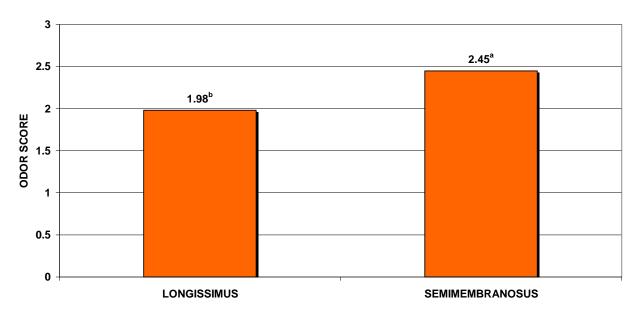
evaluation.

Figure 11.



and easily produce physiological effects a,b,c,d,e,f Means lacking a common letter differ (P < 0.05)

Figure 12.

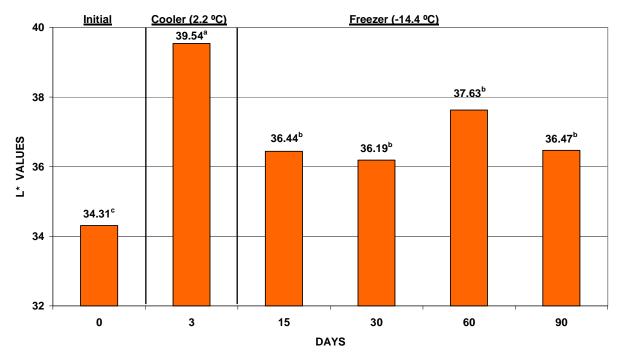


EFFECT OF SUBPRIMAL TYPE ON PERCEIVED ODOR¹ OF THAWED BEEF STEAKS

¹ Odor score: 1 = not detectable, 6 = odor present, which is strong, overpowering, and intolerable and easily produce physiological effects

 a,b Means lacking a common superscript letter differ (P < 0.05)

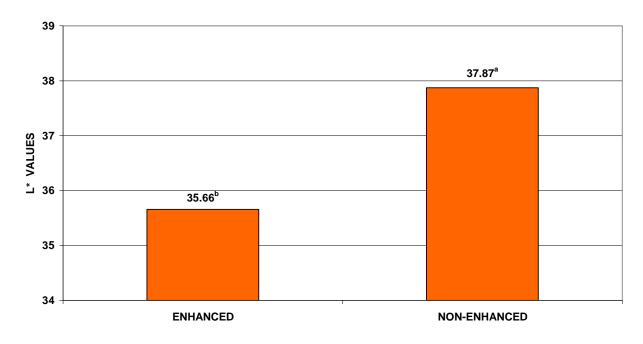
Figure 13.



EFFECT OF STORAGE TYPE/PERIOD ON L* VALUES OF THAWED LONGISSIMUS STEAKS

 $^{\rm a,b,c}$ Means lacking a common superscript letter differ (P < 0.05)

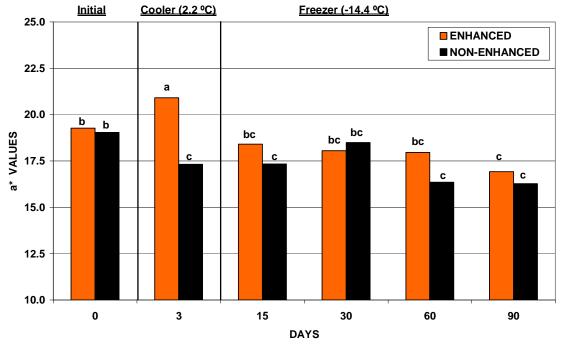
Figure 14.



EFFECT OF ENHANCEMENT ON L* VALUES OF THAWED LONGISSIMUS STEAKS

^{a,b} Means lacking a common superscript letter differ (P < 0.05)

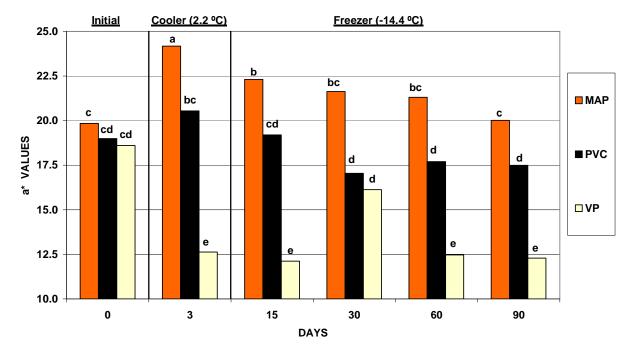
Figure 15.



EFFECT OF STORAGE TYPE/PERIOD AND ENHANCEMENT ON a* VALUES OF THAWED LONGISSIMUS STEAKS

^{a,b,c} Means lacking a common letter differ (P < 0.05)

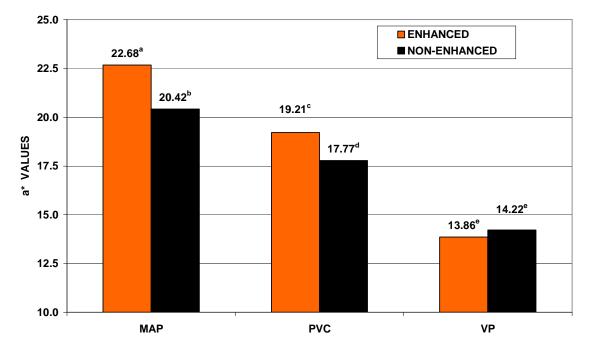
Figure 16.



EFFECT OF STORAGE TYPE/PERIOD AND PACKAGING SYSTEM ON a* VALUES OF THAWED LONGISSIMUS STEAKS

a,b,c,d,e Means lacking a common letter differ (P < 0.05)

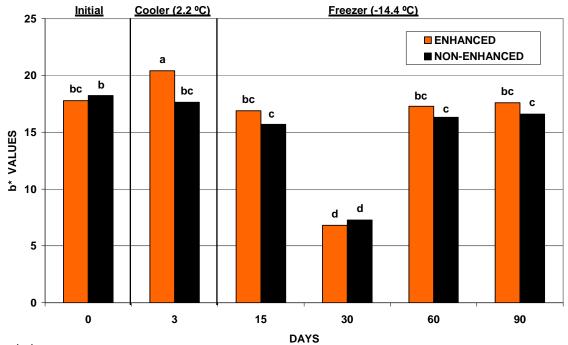
Figure 17.



EFFECT OF ENHANCEMENT AND PACKAGING SYSTEM ON a* VALUES OF THAWED LONGISSIMUS STEAKS

 a,b,c,d,e Means lacking a common superscript letter differ (P < 0.05)

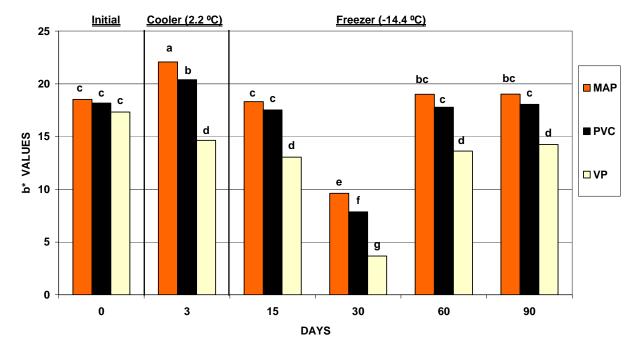
Figure 18.



EFFECT OF STORAGE TYPE/PERIOD AND ENHANCEMENT ON b* VALUES OF THAWED LONGISSIMUS STEAKS

 a,b,c,d Means lacking a common letter differ (P < 0.05)

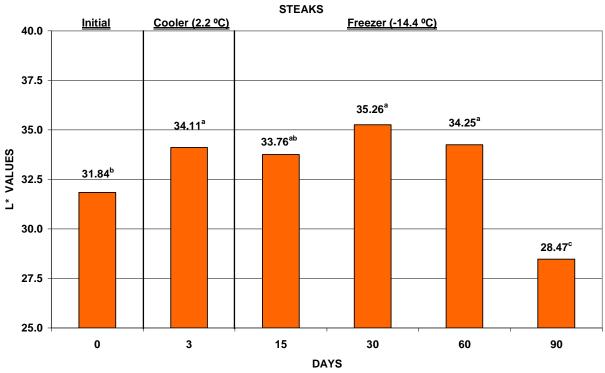
Figure 19.



EFFECT OF STORAGE TYPE/PERIOD AND PACKAGING SYSTEM ON b* VALUES OF THAWED LONGISSIMUS STEAKS

^{a,b,c,d,e,f,g} Means lacking a common letter differ (P < 0.05)

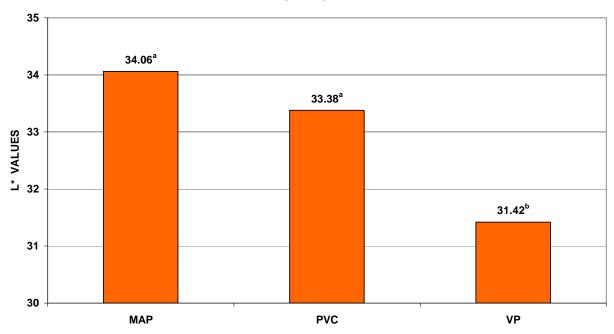
Figure 20.



EFFECT OF STORAGE TYPE/PERIOD ON L* VALUES OF THAWED SEMIMEMBRANOSUS

^{a,b,c} Means lacking a common superscript letter differ (P < 0.05)

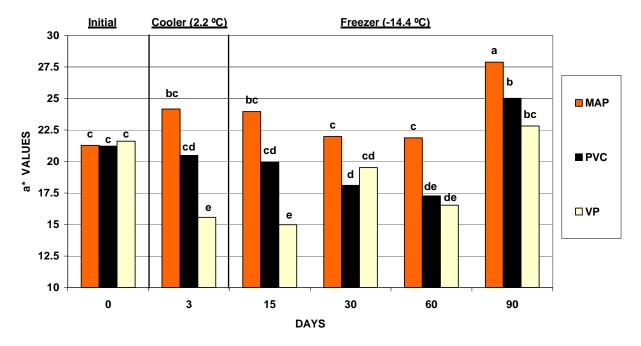
Figure 21.



EFFECT OF PACKAGING SYSTEM ON L* VALUES OF THAWED SEMIMEMBRANOSUS STEAKS

 $^{\rm a,b}$ Means lacking a common superscript letter differ (P < 0.05)

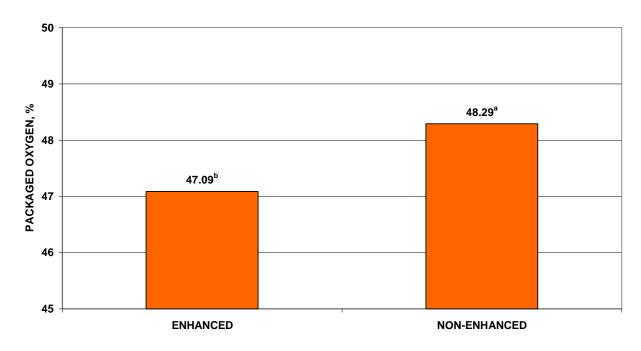
Figure 22.



EFFECT OF STORAGE TYPE/PERIOD AND PACKAGING SYSTEM ON a* VALUES OF THAWED SEMIMEMBRANOSUS STEAKS

 a,b,c,d,e Means lacking a common letter differ (P < 0.05)

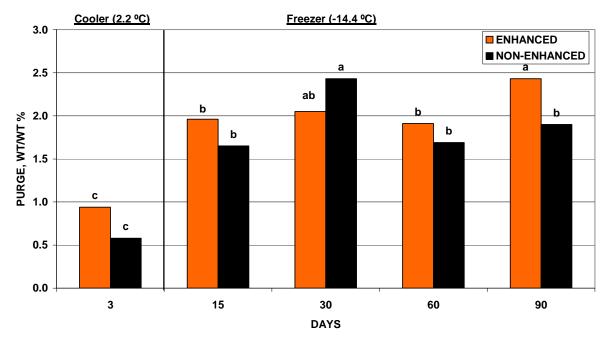
Figure 23.



EFFECT OF ENHANCEMENT ON PACKAGED OXYGEN CONCENTRATION OF THAWED BEEF STEAKS

 a,b Means lacking a common superscript letter differ (P < 0.05)

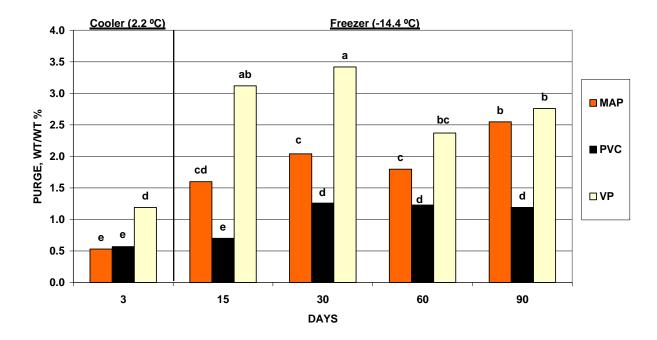
Figure 24.



EFFECT OF STORAGE TYPE/PERIOD AND ENHANCEMENT ON PURGE LOSS OF THAWED LONGISSIMUS STEAKS

^{a,b,c} Means lacking a common letter differ (P < 0.05)

Figure 25.



EFFECT OF STORAGE TYPE/PERIOD AND PACKAGING SYSTEM ON PURGE LOSS OF THAWED LONGISSIMUS STEAKS

^{a,b,c,d,e} Means lacking a common letter differ (P < 0.05)

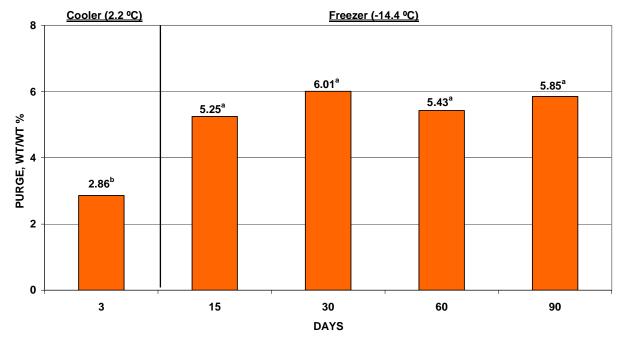
0	,	,					
	2.2ºC		-14.4°C				
Package	3 d	15 d	30 d	60 d	90 d		
MAP	0.53 ^e	1.60 ^{cd}	2.04 ^c	1.80 ^c	2.55 ^b		
PVC	0.57 ^e	0.70 ^e	1.26 ^d	1.23 ^d	1.19 ^d		
VP	1.19 ^d	3.12 ^{ab}	3.42 ^a	2.37 ^{bc}	2.76 ^b		

Table 4. Effect of storage type/period and packaging system on purge loss of thawed *Longissimus* steaks (wt/wt %).

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05).

Shaded areas designate purge loss over 2.5%, which, in this experiment, is considered the acceptability threshold.

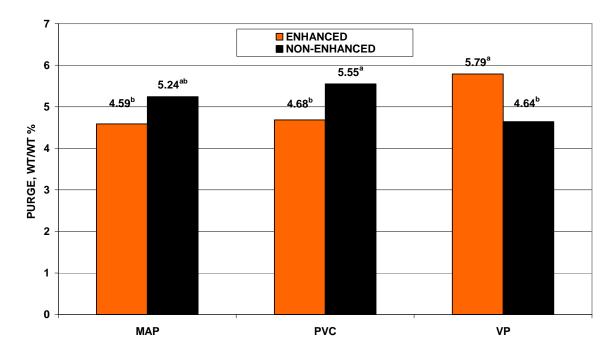
Figure 26.



EFFECT OF STORAGE TYPE/PERIOD ON PURGE LOSS OF THAWED SEMIMEMBRANOSUS STEAKS

^{a,b} Means lacking a common superscript letter differ (P < 0.05)

Figure 27.



EFFECT OF ENHANCEMENT AND PACKAGING SYSTEM ON PURGE LOSS OF THAWED SEMIMEMBRANOSUS STEAKS

 $^{\rm a,b}$ Means lacking a common superscript letter differ (P < 0.05)

Semimembranosus steaks (wt/wt %).							
	2.2°C	-14.4°C					
	3 d	15 d	30 d	60 d	90 d		
Semimembranosus	2.86 ^b	5.25 ^a	6.01 ^a	5.43 ^a	5.85 ^a		

Table 5. Effect of storage type/period on purge loss of thawed *Semimembranosus* steaks (wt/wt %).

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05). Shaded areas designate purge loss over 5.0%, which, in this experiment, is considered the acceptability threshold.

	Packaging system				
Treatment	MAP	PVC	VP		
Enhanced	4.59 ^b	4.68 ^b	5.79 ^a		
Non-enhanced	5.24 ^{ab}	5.55 ^a	4.64 ^b		

Table 6. Effect of enhancement and packaging system on purge loss of thawed Semimembranosus steaks (wt/wt %).

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05). Shaded areas designate purge loss over 5.0%, which, for this experiment, is considered the acceptability threshold.

CHAPTER IV

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APPENDIX

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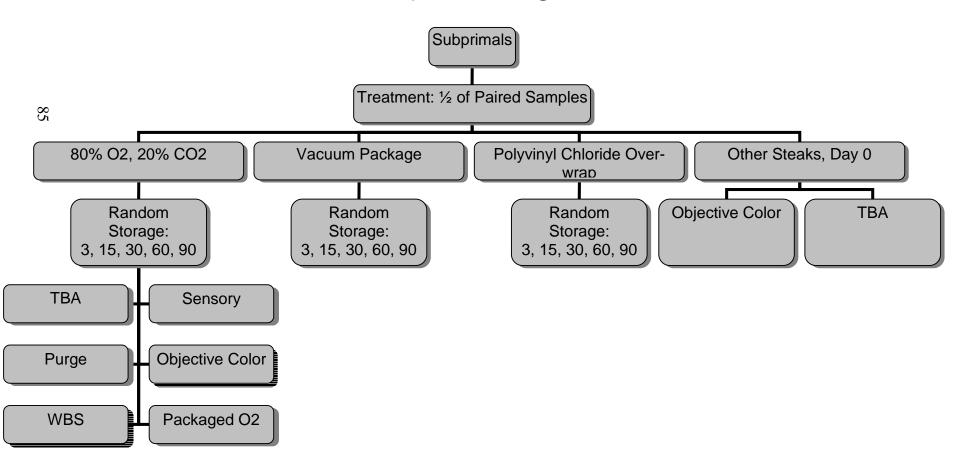
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Appendix A

Project Design



Appendix B Odor Panel Sheet

ID	Odor Acceptability	ID	Odor Acceptability
1		16	
2		17	
3		18	
4		19	
5		20	
6		21	
7		22	
8		23	
9		24	
10		25	
11		26	
12		27	
13		28	
14		29	
15		30	

Odor Panel Scale

- 1 Odor not detectable
- 2 Odor present, which activates smell but is not distinguishable
- 3 Odor present, which activates smell, is distinguishable, not necessarily objectionable in short periods
- 4 Odor present, which easily activates smell, is very distinct, and may be objectionable
- 5 Odor present, is objectionable, may cause a person to avoid completely, and could cause physiological effects
- 6 Odor present, which is strong, overpowering, and intolerable, and and easily produces physiological effects

Appendix C

Freeze Package Test Sensory Ballot

Booth #		Date:		Time:		Name:
Sample	Tenderness	Juiciness	Salty Flavor	Off- flavor	Comment	Overall Acceptability
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Tenderness

8 Extremely Tender
7 Very tender
6 Moderately Tender
5 Slightly Tender
4 Slightly Tough
3 Moderately Tough
2 Very Tough
1 Extremely Tough

Salty Flavor

3 Strong2 Slightly Detectable1 Not Detectable

Juiciness

8 Extremely Juicy
7 Very Juicy
6 Moderately Juicy
5 Slightly Juicy
4 Slightly Dry
3 Moderately Dry
2 Very Dry
1 Extremely Dry

Off-flavor

3 Strong 2 Slightly Detectable 1 Not Detectable

Overall Acceptability

7 Extremely Desirable

6 Desirable

- 5 Slightly Desirable
- 4 Acceptable
- 3 Slightly Undesirable
- 2 Undesirable
- 1 Extremely
- Undesirable

	2.2ºC		-14.4°C				
Package	3 d	15 d	30 d	60 d	90 d		
MAP	4.93 ^b	5.01 ^b	5.48 ^a	5.28 ^{ab}	5.15 ^b		
PVC	5.08 ^b	5.44 ^a	5.25 ^{ab}	5.05 ^b	5.29 ^{ab}		
VP	5.05 ^b	5.01 ^b	5.35 ^{ab}	5.48 ^a	5.45 ^a		

Table A. Effect of storage type/period and packaging system on sensory tenderness ratings¹ of cooked beef steaks.

¹Tenderness score: 1 = extremely tough, 8 = extremely tender ^{a,b} Means lacking a common superscript letter differ (p<0.05).

	2.2ºC		-14.4ºC			
Treatment	3 d	15 d	30 d	60 d	90 d	Package
Enhanced	5.23 ^b	4.99 ^{bc}	5.71 ^a	5.17 ^{bc}	5.18 ^b	MAP
Enhanced	5.00 ^{bc}	5.51 ^{ab}	5.61 ^a	5.38 ^{ab}	5.57 ^a	PVC
Enhanced	4.92 ^{bc}	4.72 ^{cd}	5.13 ^{bc}	5.21 ^b	5.18 ^b	VP
Non-enhanced	4.32 ^{de}	4.14 ^{de}	4.53 ^{cd}	4.61 ^{cd}	4.43 ^d	MAP
Non-enhanced	4.83 ^c	5.00 ^{bc}	4.35 ^{de}	4.44 ^d	4.36 ^d	PVC
Non-enhanced	4.55 ^{cd}	4.02 ^e	4.47 ^d	4.26 ^{de}	4.16 ^{de}	VP

Table B. Effect of storage type/period, enhancement and packaging system on sensory juiciness ratings¹ of cooked beef steaks.

¹ Juiciness score: 1 = extremely dry, 8 = extremely juicy a,b,c,d,e Means lacking a common superscript letter differ (p<0.05).

	2.2°C	-14.4ºC				
Treatment	3 d	15 d	30 d	60 d	90 d	Package
Enhanced	1.64 ^c	1.59 ^c	1.57 ^b	1.97 ^a	1.59 ^{bc}	MAP
Enhanced	1.56 ^c	1.52 ^{cd}	1.81 ^c	1.7 ^{bc}	1.71 ^c	PVC
Enhanced	1.57 ^c	1.33 ^d	1.36 ^d	1.75 ^{bc}	1.39 ^d	VP
Non-enhanced	1.12 ^{ef}	1.03 ^{ef}	1.09 ^{ef}	1.11 ^{ef}	1.12 ^{ef}	MAP
Non-enhanced	1.07 ^{ef}	1.02 ^{ef}	1.07 ^{ef}	1.17 ^{ef}	1.12 ^{ef}	PVC
Non-enhanced	1.13 ^{ef}	1.01 ^{ef}	1.04 ^{ef}	1.09 ^{ef}	1.07 ^{ef}	VP

Table C. Effect of storage type/period, enhancement and packaging system on sensory saltiness ratings¹ of cooked beef steaks.

¹ Saltiness score: 1 = not detectable and 3 = strong a,b,c,d,e,f Means lacking a common superscript letter differ (p<0.05).

Tallinge et eeentea s								
	2.2°C		-14.4°C					
Treatment	3 d	15 d	30 d	60 d	90 d			
Enhanced	1.39 ^d	1.42 ^d	1.59 ^{bc}	1.80 ^a	1.57 ^{bc}			
Non-enhanced	1.52 ^c	1.23 ^e	1.35 ^d	1.67 ^b	1.46 ^{cd}			

Table D. Effect of storage type/period and enhancement on sensory off-flavor ratings¹ of cooked beef steaks.

¹ Off-flavor score: 1 = not detectable and 3 = strong a,b,c,d,e Means lacking a common superscript letter differ (p<0.05).

	2.2°C		-14.4ºC			
Treatment	3 d	15 d	30 d	60 d	90 d	Package
Enhanced	1.37 ^{ef}	2.11 ^{cd}	2.28 ^{cd}	2.96 ^{ab}	2.90 ^{ab}	MAP
Enhanced	1.14 ^f	1.53 ^e	1.74 ^{de}	2.18 ^{cd}	1.96 ^d	PVC
Enhanced	2.12 ^{cd}	2.58 ^{bc}	2.31 ^c	2.95 ^{ab}	2.53 ^{bc}	VP
Non-enhanced	1.34 ^{ef}	2.55 ^{bc}	2.18 ^{cd}	3.08 ^a	2.70 ^b	MAP
Non-enhanced	1.21 ^{ef}	1.83 ^{de}	1.89 ^d	2.46 ^{bc}	2.15 ^{cd}	PVC
Non-enhanced	1.51 ^{ef}	2.59 ^b	2.53 ^{bc}	3.14 ^a	2.67 ^b	VP

Table E. Effect of storage type/period, enhancement and packaging system on odor scores¹ of thawed beef steaks.

¹ Odor score: 1 = not detectable, 6 = odor present, which is strong, overpowering, and intolerable and easily produce physiological effects a,b,c,d,e,f Means lacking a common superscript letter differ (p<0.05).

		2.2ºC	-14.4°C			
Treatment	0 d	3 d	15 d	30 d	60 d	90 d
Enhanced	19.27 ^b	20.91 ^a	18.41 ^{bc}	18.05 ^{bc}	17.96 ^{bc}	16.92 ^c
Non-enhanced	19.03 ^b	17.32 ^c	17.33 ^c	18.49 ^{bc}	16.36 ^c	16.27 ^c

Table F. Effect of storage type/period and enhancement on a* values of thawed *Longissimus* steaks.

^{a,b,c} Means lacking a common superscript letter differ (p<0.05).

		2.2ºC	-14.4°C			
Package	0 d	3 d	15 d	30 d	60 d	90 d
MAP	19.85 ^c	24.18 ^a	22.31 ^b	21.63 ^{bc}	21.31 ^{bc}	20.02 ^c
PVC	18.98 ^{cd}	20.54 ^{bc}	19.19 ^{cd}	17.04 ^d	17.70 ^d	17.49 ^d
VP	18.61 ^{cd}	12.63 ^e	12.11 ^e	16.13 ^d	12.47 ^e	12.28 ^e

Table G. Effect of storage type/period and packaging system on a* values of thawed *Longissimus* steaks.

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05).

		2.2ºC	-14.4ºC			
Treatment	0 d	3 d	15 d	30 d	60 d	90 d
Enhanced	17.78 ^{bc}	20.42 ^a	16.90 ^{bc}	6.82 ^d	17.29 ^{bc}	17.61 ^{bc}
Non-enhanced	18.23 ^b	17.65 ^{bc}	15.69 ^c	7.28 ^d	16.32 ^c	16.61 ^c

Table H. Effect of storage type/period and enhancement on b* values of thawed *Longissimus* steaks.

^{a,b,c} Means lacking a common superscript letter differ (p<0.05).

		2.2ºC	-14.4ºC			
Package	0 d	3 d	15 d	30 d	60 d	90 d
MAP	18.53 ^c	22.08 ^a	18.31 ^c	9.62 ^e	19.01 ^{bc}	19.03 ^{bc}
PVC	18.17 ^c	20.39 ^b	17.54 ^c	7.86 ^f	17.77 ^c	18.05 ^c
VP	17.32 ^c	14.64 ^d	13.04 ^d	3.68 ^g	13.64 ^d	14.26 ^d

Table I. Effect of storage type/period and packaging system on b* values of thawed *Longissimus* steaks.

^{a,b,c,d,e,t,g} Means lacking a common superscript letter differ (p<0.05).

	_	2.2⁰C	-14.4°C			
Package	0 d	3 d	15 d	30 d	60 d	90 d
MAP	21.28 ^c	24.16 ^{bc}	23.95 ^{bc}	21.98 ^c	21.88 ^c	27.88 ^a
PVC	21.22 ^c	20.48 ^{cd}	19.97 ^{cd}	18.10 ^d	17.28 ^{de}	25.02 ^b
VP	21.61 ^c	15.57 ^e	14.99 ^e	19.52 ^{cd}	16.53 ^{de}	22.82 ^{bc}

Table J. Effect of storage type/period and packaging system on a* values of thawed *Semimembranosus steaks*.

^{a,b,c,d,e} Means lacking a common superscript letter differ (p<0.05).

Longissimus steaks (wuwi %).								
	2.2ºC		-1					
Treatment	3 d	15 d	30 d	60 d	90 d			
Enhanced	0.94 ^c	1.96 ^b	2.05 ^{ab}	1.91 ^b	2.43 ^a			
Non-enhanced	0.58 ^c	1.65 ^b	2.43 ^b	1.69 ^b	1.9 ^b			

Table K. Effect of storage type/period and enhancement on purge loss of thawed *Longissimus* steaks (wt/wt %).

^{a,b,c} Means lacking a common superscript letter differ (p<0.05).

VITA

Marcia Anne Dalton

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF FRESH AND FROZEN STORAGE ON PALATABILITY, OXIDATIVE RANCIDITY AND COLOR OF PACKAGED BEEF STEAKS

Major Field: Animal Science

Biographical:

- Personal Data: Born in Grand Island, Nebraska on November 13, 1980, the daughter of Jackie Leierer and Maurice Dalton.
- Education: Graduated from Oklahoma School of Science and Mathematics, Oklahoma City, Oklahoma in May 1999; received a bachelor of Science degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma in August 2003; Completed the requirements for the Master of Science degree with a major in Animal Science at Oklahoma State University in May, 2005.
- Experience: Raised in Ringwood, Oklahoma while surrounded by an extended loving family; employed by Oklahoma State University, Food and Agricultural Product Center as an undergraduate, 2002; interned at Tyson Fresh Meats, Emporia, Kansas in the summer of 2002; and currently, Oklahoma State University graduate research assistant from 2003 to present.

Professional Memberships: American Meat Science Association Institute of Food Technologists