EFFECT OF INJECTION AND FROZEN STORAGE ON THE QUALITY ATTRIBUTES OF FULLY COOKED BONE-IN HAMS

A Thesis

by

DENISE GAIL PHILLIPS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Wesley Osburn
Committee Members,	Jimmy Keeton
	Rhonda Miller
Head of Department,	Gary Acuff

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ABSTRACT

Effect of Injection and Frozen Storage on the Quality Attributes of Fully Cooked Bone-in Hams. (December 2009)

Denise Gail Phillips, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Wesley N. Osburn

This study determined the effects of sucrose and sorbitol as cryoprotectant (CR) on the quality and sensory attributes of bone-in hams (N=90) injected (20% of weight) with following brine treatments (BT): control (2% sucrose; CNT), 2% CR (1% sucrose, 1% sorbitol), 4% CR (2% sucrose, 2% sorbitol). Hams that were frozen and injected with CNT after thawing (FZ I) were used as a negative control. After reaching the designated ST all bone-in hams were thawed under refrigeration (4^{o}C) and FZ I hams were then injected with CNT (n=8). Hams were cooked to 70^{o}C, chilled (7^{o}C), sliced, vacuum packaged and analyzed for lipid oxidation, color, protein solubility and purge at 0, 28, and 56 of refrigerated storage while sensory evaluation and shear force determinations were conducted at 28 d.

Color, pH, and lipid oxidation values tended to remain similar or decrease as frozen storage time increased. Water holding capacity (percent bound water) and protein solubility increased as frozen storage increased. The 4% CR BT exhibited the lowest shear force value (4.04 N/g) but was not statistically different than CNT or FZ I on ham knuckle muscles. Trained sensory panelists found hammy and salty were the

strongest flavor and basic taste attributes. The results of this study confirm that quality attributes and protein functionality were maintained but not significantly improved by injecting a brine solution with cryoprotectants prior to freezing.

DEDICATION

To Mom, Dad, and Denny Ray

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The term "meat packing" describes the method used by farmers to preserve meat by salting and then packing the meat in wooden barrels for long term storage. In the early stages of meat packing, local butchers slaughtered and fabricated animals simply for local farmers; however, as the demand for meat increased, meat packing plants were established to meet consumer demands, and the preservation of meat became a common practice. In 1906, Upton Sinclair wrote The Jungle, which explicitly exposed Chicago meat packing plants of their harsh working and unsanitary conditions which resulted in government regulations to improve the wholesomeness of meat products. Additionally, this exposure created innovations in technology which industrialized the meat packing industry through automation which increased production efficiency, creations of packaging and preservation techniques which increase shelf life, and transportation systems to deliver meat closer to the point of purchase. Today's modern methods of packaging technologies. The development of frozen foods in the form of meals and entrees for the consumer soared to new heights in the late 1980's (Erickson & Hung, 1997).

This thesis follows the style of *Meat Science.*

The frozen-foods industry quickly responded to the American consumer who began utilizing frozen foods for their convenience. Meat processors continue to fine tune tactics and approaches to satisfy consumer demands.

Freezing systems for meat preservation, such as air, contact, or immersion methods effectively freeze meat products for long-term storage. Quality attributes, such as water holding capacity, color, tenderness, and protein extraction, are affected by ice crystal formation, freezing rate and storage conditions. Rapid freezing rates are more effective in maintaining quality attributes of meat products compared to slower freezing rates. Storage conditions such as holding temperatures, air speed and air velocity are factors that impact frozen product quality attributes. If storage conditions are not properly monitored and controlled, ice crystals can melt and recrystallize into larger ice crystals causing damage to muscles resulting in increased fluid loss during thawing and a less palatable product after cooking. New packaging technologies, such as the development of multilayer plastics, have assisted in maintaining the quality of frozen meat products during frozen storage, warehousing, transportation and delivery.

Although meat freezing is generally conceded to cause tissue damage and some quality loss, it remains the preferred method of long-term storage (Lind, Harrison, & Krop, 1971). Recently, new advancements in technology have been developed to maintain quality attributes in frozen meat products. Cryoprotectants, such as nucleotides, surfactants, or carbohydrates, are added to meat products prior to freezing that assist in maintaining quality characteristics and extending the shelf life of frozen meat products. Furthermore, cryoprotectants retard ice crystal growth and the migration

of water from structural proteins, thus stabilizing the protein in its native form during frozen storage (Matsumota, 1992).

In ham manufacturing, the use of previously frozen raw materials has resulted in products with poor texture and an inability to retain water and flavoring constituents. In addition, decreased water holding capacity negatively impacts the overall cooking yields, which reduces product value. Wilson, Dickson, and Holmes (1994) studied the quality characteristics of fully-cooked hams that were brine injected prior to freezing. Some hams were kept frozen for 90 days and others without any frozen storage. It was observed that hams with frozen storage had greater oval weight loss and total moisture, but had higher color values. Little differences were found in shear values or lipid oxidation. A sensory panel found the frozen hams to be less firm and paler in color as compared to non-frozen hams. In this study, the brine treatments did not contain cryoprotectants (Wilson et al., 1991).

This study was performed at a local ham manufacturing plant, Columbia Packing Company in Dallas, TX. Currently, ham manufactures purchases raw materials, bone in hams, throughout the year and these materials are frozen and remain in frozen storage until production is scheduled. This study was designed to investigate the effectiveness of injecting solutions that contain cryoprotectants into bone in hams prior to frozen storage. This study will help determine if cryoprotectants can minimize the negative effects of freezing by assessing their impact on cooked product yields, color, lipid oxidation, water holding capacity, protein solubility and sensory attributes.

1.2 The Freezing Process

Freezing, also identified as rapid heat transfer, is one of the most effective methods of meat preservation. Frozen storage has proven to be an important long-term storage method for muscle foods as it prevents microbial spoilage; however, it is associated with deterioration of meat protein functionality such as emulsifying capacity, binding ability, and protein extraction (Park, Lanier, Keeton & Hamann, 1987). Meat is composed of soluble and structural proteins, fats, and electrolytes that when combined create product properties that are more complicated than single-phased water-based system when undergoing freezing (Devine, Bell, Lovatt, & Chrystall, 1996). The quality of frozen meat is influenced by ice crystal formation, freezing rate, frozen storage temperature conditions, length of storage, and packaging materials.

1.2.1 Ice Crystal Formation

Ice crystal formation occurs in two distinct steps: the formation of nuclei and the later growth of the nuclei to a specific crystal size. Nucleation is defined as the formation of stable nuclei as a sequence of bimolecular processes, whereby atoms in the liquid phase join a growing cluster of nuclei, known as an embryo (Reid, 1983). Nucleation is the initial process of freezing which upon activation is driven by supercooling. The removal of heat below 0^{o}C without a phase change is known as supercooling. Supercooling results in a thermodynamic unstable state that initiates the formation of submicroscopic water aggregates leading to a suitable interface necessary for a liquid to solid transformation (Reid, 1983). The greater the supercooling, the greater the number of nuclei formed, which is dependent on the volume of the meat

sample (Fennema, 1973). Water molecules within the meat tissue add to the nuclei already formed, requiring minimal subsequent supercooling (Fennema, 1973). The freezing process begins when the surface of the product reaches freezing temperatures. Pure water becomes ice at 0^{o}C and solutions do not freeze until temperatures are below 0^{o}C while muscle tissue freezes at -2^{o}C due to salts in the sarcoplasm (Buchmuller, 1987). A continuous freezing front moves through the object, exterior to interior, until the entire object is frozen. Extracellular components of the object freeze faster due to lower ionic and solute concentrations (Devine et al., 1996). Freezing can be further described when food comes into contact with a refrigerating medium, such as cooler air, and the area of direct contact with the medium is where ice nucleation occurs. Within the inner regions of the product, nucleation may not be achieved because of the thermal gradients and the lack of supercooling which results in the growth of large ice crystals (Bevilacaqua, Zaritzky & Calvelo, 1980).

1.2.2 Freezing Rate

The size and location of ice crystal formation is closely related to the rate of freezing that further effects quality attributes such as color, texture, tenderness, and water holding capacity (Martino, Oter, Sanz & Zaritzky, 1998; Woinet, Andrieu, Laurent & Min, 1998; Ngapo, Babare, Reynolds & Mawson, 1999a; Ngapo, Babare, Reynolds & Mawson, 1999b) . The original composition and quality of meat products can be maintained with more rapid freezing rates (Buchmuller, 1987). With slower freezing rates, the surface temperature initially passes below the freezing point to instigate nucleation. Consequently, temperatures fluctuate due to changes in ambient freezing

temperatures and remain near the freezing point for an extended amount of time allowing for a continuous freezing boundary throughout the product (Aberle, Forrest, Gerrard & Mills, 2001). Additionally, large ice crystals form due to the low rate of nucleation and the absence of nuclei (Martino et al., 1998).

Slow freezing rates (0.1-0.2 cm/h) cause large ice crystals to form within the muscle cells, causing cell wall damage. Conversely, rapid freezing rates (5.0 cm/h) form fine ice crystals causing little damage to meat tissue (Buchmuller, 1987). Volume changes of water due to the translocation of water molecules from intracellular areas to extracellular areas and the period of crystallization within the meat are shorter when utilizing rapid freezing. This also produces a filament like ice crystal entrapping solutes and minimizing ion concentration effects which assists in maintaining product quality. Rapid freezing causes small ice crystals to form in the intra and extra cellular spaces of meat myofibrils at the same rate. These crystals do not increase in size and reflect more light from meat surfaces, resulting in lighter color, as compared to slower freezing rates.

Rapid freezing results in discontinuous freezing boundaries, because a continuous freezing front forms and moves through meat products from the exterior to the interior (Devine et. al, 1996). This continuous freezing front assists in maintaining meat quality such as protein solubility, water holding capacity, and texture. Rapid freezing rates have ice crystals formed with in the cell, which simply “loosens” the protein structure creating more protein charges, allowing for an increase in water reabsorbtion during thawing (Deatherage & Hamm, 1960). Rapid freezing rates are obtained with fast air movement or direct contact with a heat transfer medium and

extremely low temperatures (-40^{o}C) (Aberle et al., 2001). In 1993, Reid found that smaller ice crystals were observed with rapid freezing. This was also observed in rapidly frozen ground beef patties compared to patties with slower freezing rates (Nusbaum, Sebranek, Topel & Rust, 1983). In 1993,

Petrovic et al. conducted an experiment examining different freezing rates of beef *Longissimus dorsi* muscle. The slowest rate was defined as 0.22 – 0.29 cm/h compared to more rapid rates of 3.33 – 5.55 cm/h. Frozen storage temperatures were set to achieve freezing rates and to allow freezing to occur from the exterior edges of the product to the interior areas of the product. Petrovic found the freezing rates of 4.92 – 5.55 cm/h to be an acceptable rapid freezing rate of beef *Longissimus dorsi* muscle, and also found that the freezing rate of 3.33 – 3.95 cm/h had the least influence on meat quality, such as weight loss during freezing, thawing, and cooking, water-binding capacity, and sensory attributes (Petrovic et al., 1993). Petrovic concludes that optimum freezing rates should average 2 – 5 cm/h from the exterior to the center of meat products.

1.2.3 Frozen Storage Conditions

Holding temperature, air speed and velocity must be properly maintained during frozen storage to sustain meat quality attributes such as color, water holding capacity, protein solubility, and tenderness. Environmental changes, such as holding temperature variation, occur over time and meat surface tissue qualities, like color, alter more rapidly compared to the deeper-lying internal meat tissues. Numerous chemical changes such as protein solubility, tenderness, and water holding capacity, can be eliminated by reducing temperatures to -80^{o}C; however these conditions are not economical and unrealistic to

attain by industrial storage facilities (Aberle et al., 2001). Commercial and home freezer units are recommend to be maintained at -18^{o}C to reduce the growth of pathogenic or spoilage microorganisms and most enzymatic reactions. By maintaining frozen meat products at this temperature, storage life of meat products can be extended.

Recrystallization is temperature dependent and occurs while meat products are frozen and internal temperatures fluctuate resulting in an increase in diameter size of ice crystals. Ice crystals thaw and become water molecules as internal product temperature increase. Once freezing temperatures are achieved these water molecules migrate for nucleation and larger ice crystals are formed (Bevilacaqua et. al, 1980).

Recrystallization occurs in the intracellular space after rapid freezing; however, after slow freezing, it occurs in the extracellular space which leads to larger crystals within the muscle fiber (Bevilacaqua et al., 1980). To minimize ice crystal growth during frozen storage, temperature fluctuations should be avoided. If fluctuations do occur, water molecules migrate and recrystallize to form larger ice crystals, leading to further damage to muscle fibers. In 1988, the storage of frozen beef was monitored for ice crystal size modifications over five months at different freezing temperatures (-5^{o}C – 20^{o}C). This and other studies have concluded that the protein solubility and liquid exudates are effected by recrystallization due to its relationship with water, which in turn produces greater amounts of liquid exudates after thawing (Awad, Powrie, & Fennema, 1968; Martino el. al, 1988; Farouk, Wieliczko & Merts, 2003).

1.2.4 Packaging

Packaging materials for frozen meat products can assist in maintaining acceptable product quality over time. Moisture and oxygen impermeable wrapping materials are needed to prevent product dehydration and flavor changes (Bratzler, 1955). Advancements in packaging materials began with the development of multilayer plastics which created new food preservation techniques that have revolutionized food packaging materials. Polypropylene, polystyrene, polyvinylidine chloride, and polyvinyl chloride are some important plastics and films used for packaging (Ott, 1988). New packaging technology includes modified atmosphere and vacuum packaging.

Modified atmosphere packaging (MAP) is defined as the enclosure of food products in gas-barrier material, polyvinyl chloride or polyvinylidene chloride, while providing a gaseous environment that has been changed or modified from a normal atmosphere to an atmosphere with a specific composition of nitrogen, oxygen, and carbon dioxide (Young, Reviere, & Cole, 1998). These modifications result in lower oxygen content of the air within the package headspace and increased levels of nitrogen and carbon dioxide (Smith, Ramaswamy, & Simpson, 1990). Nitrogen is utilized as a filter to prevent package collapse with products that absorb carbon dioxide or to displace oxygen and inhibit the action of spoilage agents and retard oxidation (Young et al., 1998). MAP assists in maintaining product quality, such as shape and color, and to reduce microbiological growth and enzymatic spoilage to increase shelf life (Smith el al., 1990).

Vacuum packaging entails the product being placed in a film of low oxygen permeability, removing air from the package, and applying a hermetic seal (Smith et al., 1990). Under good vacuum packaging conditions, oxygen levels can be reduced to less than 1% which has been shown to be effective in inhibiting bacterial growth at low temperatures. Vapor-proof material with low temperature stability can retain moisture and exclude oxygen penetration to frozen products (Aberle et al., 2001). Tight fitting bags which limit voids are recommended because they inhibit freezer burn or oxidative rancidity. A study found that after long term frozen storage of ground beef patties, oxygen-impermeable packaging maintained color and minimized surface discoloration (Bhattacharya, Hanna, & Mandigo, 1988).

Using MAP packaging, the quality of fresh or frozen foods can be maintained even if temperature fluctuation occurs during transport, storage, and frozen storage (Erickson et al., 1997). Further developments in packaging and packaging technologies continue to influence improvements in frozen-food packages which adhere to consumer's needs for convenience and increased storage time.

1.2.5 Thawing and Tempering

Thawing is considered to be the reverse process of freezing, with the exception of differences in thermal properties of water and ice (Delgado & Sun, 2001). Freezing has also been characterized as a dehydration process in which water is removed from its original locations within myofibrils. During thawing, water may or may not be reabsorbed into its original location within the myofibrils (Pham & Mawson, 1997). Thawing is generally performed by microwave or convective heating by water or air, but

studies have shown that optimal thawing conditions include low temperatures (15°C) and high humidity (90%) air (Mannapperuma, 1988).

Research has shown that frozen pork normally regains its original structure upon thawing; however, the drip loss of frozen pork following thawing depends on the freezing rate with a lower drip loss for rapidly frozen pork (Ngapo et al. 1999a; Ngapo et al. 1999b). Temperatures used during thawing are of great importance because the external surfaces of frozen products will increase in temperature more rapidly and will be exposed longer compared to the interior portions of the product. This scenario can result in microbial growth on the surface of partially thawed products (Golden & Arroyo-Gallyoun, 1997). Whole and minced buffalo muscle was plate or blast frozen until an internal temperature of -30°C, and stored for 3 mth at -15°C. Samples were thawed by running water (27°C) for 50 min, at room temperature (26°C) 180 min, at chill temperature (4°C) 14 h, or in a microwave oven (210 W) for 10 min. This study concluded that samples that were plate frozen and thawed at chilled temperatures showed less drip loss compared to other methods of thawing (Mahendraker, Rao, & Amla, 1993).

1.3 Freezing Systems

Environments required to maintain temperature and boundary conditions at product surfaces are primary factors that establish the effectiveness of freezing systems. Freezing systems are classified as either direct or indirect contact. Direct contact systems use direct contact between the refrigerating medium and the product surface.

Refrigerating mediums include low temperature air moving over product surfaces at high air speeds or liquid immersion (Heldman & Lund, 1992). This process is highly effective because any barriers with respect to heat transfer are significantly reduced. When products are separated by a barrier (i.e., packaging material) during the freezing process, it is considered an indirect contact freezing systems which include plate and blast freezing systems. Achieving a frozen state in meat products can be achieved through air, contact, or immersion freezing systems.

1.3.1 Air Freezers

Still- air freezing or sharp freezing, where air is the heat transfer medium, is a very slow method of freezing. These freezers are held between -10^{o}C and -30^{o}C, and little or no control of air flow or temperature stability can be maintained; these freezers are commonly used for storage of previously frozen product (George , 1997). When large amounts of unfrozen products are placed in still-air freezers, the freezing rate is greatly reduced because the product is separated from the freezing medium by barriers, such as packaging materials (Heldman, 1992). One of the most common freezing methods used for commercial freezing is known as blast or quick freezing. Freezing is accomplished with high air velocities and low temperatures in rooms or tunnels, equipped with fans that provided rapid air movement around the product (Heldman et al., 1992). With the rapid air movement, the heat transfer rate is increased which provides a processor greater opportunity to increase production volumes (George, 1997). Air velocities can range from 30 - 1000 m/min while temperatures range from -10^{o}C - -40^{o}C. With blast freezing, the spacing of products is very important to maintain the

freezing rate. The air flow must be able to move between boxes and product to avoid any uneven freezing. In tunnel blast freezers, the conveyor speed is maintained in order to achieve frozen product and it offers a continuous means of production (George, 1997).

1.3.2 Contact Freezers

Plate or direct contact freezing systems are usually limited to meat products such as steaks, chops, patties or regular shaped products with flat surfaces. Heat transfer mechanism is achieved when surfaces of produce come into contact with a metal plate acting as the heat transfer medium that is cooled by a mechanical refrigeration system. Automation of the plate movement to facilitate a semi continuous operation is possible (George, 1997). The metal plate brings the heat transfer medium into contact with maximum amount of the product's surface (Heldman, 1992). These plates are maintained at -10^{o}C - -30^{o}C and freezing rates are slightly faster than still air freezing rates. This method of freezing is not usually used commercial freezing; however, adjustments can be made to incorporate circulating cold air over products to increase the freezing rate (Aberle et al., 2001).

1.3.3 Immersion

Immersion freezing systems expose products to liquid refrigerants such as nitrogen, carbon dioxide, or Freon, which undergo a phase change during the freezing process (Heldman, 1992). Liquids must be nontoxic and have a low freezing point with high heat conductivity such as glycerols or sodium chloride brines. Corrosion of tanks and equipment are negative effects from these brines. Products are placed in plastic bags and stored on racks or pallets which are immersed in freezing liquid or moved through a

liquid by conveyers. Afterwards, products still in packages are sprayed to remove the liquid and placed in cooling rooms for the completion of freezing. A negative effect with this type of freezing are holes in packaging or seepage of liquids through packages are known to occur (Aberle et al., 2001). Freezing products with liquid immersion or liquid sprays is the most widely used method of freezing for poultry.

Cryogenic freezing occurs when products are immersed into very low temperatures using vapor or liquefied gases, such as nitrogen or carbon dioxide. Small portions or pieces of a product are immersed into liquid nitrogen compared to larger portions (i.e., pork chop) of product due to the great extent of shattering and cracking of the product. A great cooling effect is achieved when liquid nitrogen evaporates into nitrogen gas. Other types of cryogenic freezing include using liquid nitrogen spray or liquid carbon dioxide released as snow, to freeze small pieces of meat such as fillets or patties. This type of freezing system assists in maintaining original meat qualities (George, 1997).

1.4 Freezing Effects on Meat Quality

Frozen storage is an important preservation method, but deteriorations in muscle protein texture, flavor, and color due to biochemical and functional changes that are known to occur. Protein denaturation and the reduction of water binding capacity in meat during frozen storage have been identified due to changes in physiochemical properties of myofibrillar proteins (Park, Lanier & Pilkington, 1993). Animal muscle proteins are less susceptible to freeze denaturation compared to plant-derived proteins

and fish muscle. Ice occupies a greater volume than water within muscle protein and the exclusion of solutes from ice crystals causes an increase in the ionic strength of unfrozen water. Together these phenomena cause a loss in tissue structure and a partial denaturation of some muscle proteins which further effects meat quality such as water holding capacity (Tomaniak, Tyszkiewicz & Komosa, 1998).

1.4.1 Mechanical Damage

Mechanical damage to cellular structures resulting from volume changes and chemical damage caused by concentration of solutes, such as salts and sugars. Woinet et al. (1998) found that the addition of sodium chloride caused an increase in ice crystal size. Volume changes of extracellular ice crystals formed from slow freezing rates cause mechanical damage due to the expansion of large ice crystals formations and the shrinkage of muscle fibers. The formation of pure ice crystals and the increased concentration of solutes in unfrozen solutions are favored due to a lack of nucleation sites necessary for ice crystal formation in the intracellular solutions. The point at which solute concentration increases and freezing point decreases is known as the eutectic point. At this point, solutes begin to crystallize simultaneously with ice crystal formations causing additional damage to muscle fibers. The crystallization of solutes also forces water out of muscle fibers which contributes to the formation of large extracellular ice crystals (Aberle et al. 2001).

The formation of ice crystals from either intracellular or extracellular water can result in mechanical damage caused by irregular ice crystals protruding through and disrupting cell walls. The size and location of ice crystals is influenced by freezing rate,

storage time, and temperature fluctuations (Xiong, 1997). It was observed that rapid freezing rates assist in maintaining protein structure and functionality compared to an increase of denaturation in myofibril proteins from slow freezing rates and long frozen storage (Hansen, Trinderup, Hviid, Darre & Skibsted, 2003). Slower freezing rates cause more extracellular water freezing than intracellular water freezing due to lower solute concentration and formation of large ice crystals between muscle fibers (Deatherage & Hamm, 1960). During frozen storage, if temperatures fluctuate crystallization occurs causing small ice crystals to melt and recrystallize onto existing larger crystals. This change in shape caused by the movement of ice crystals causes tissue damage and accelerates protein denaturation (Xiong, 1997).

1.4.2 Moisture Loss

Freezing can have a negative impact on the quality of meat products due to loss of moisture. The excessive loss of moisture on meat surfaces can leave areas of dehydration and discoloration, know as freezer burn, which results in unattractive packages, loss of nutritional value, and dryness in cooked meats. Studies have shown freezing rates to be directly related to amount of drip loss of muscle proteins. Faster freezing rates have shown to have a decrease in drip loss compared to greater drip loss by slower freezing rates (Deatherage et al., 1960; Bevilacaqua et al., 1980; Miller, Ackerman & Palumbo, 1980; Ngapo et al, 1999; Petrovic et al., 1993; Martino et al., 1998). Greater drip loss in slowly frozen meat may have resulted from greater structural damage from intercellular ice crystals (Farouk et al., 2003). Drip loss obtained from fast freezing rates of pork *Biceps Femoris* m. were found to not be significantly different

compared to the drip loss of fresh pork samples. Significant differences in drip loss were observed from samples frozen with slower freezing rates (Ngapo et al., 1999). Awad (1968) observed the volume of drip per 100 g of frozen bovine muscle after 8 wk of storage at -4^{o}C to increase up to 24 ml, as compared to unfrozen muscle resulting in 7.3 ml of drip loss. Conversely, it was observed that the effect of freezing rate on drip losses of pork chops, steaks, and small beef joints show no significant differences (Bailey, 1972.)

1.4.3 Water Holding Capacity

Water holding capacity is negatively impacted as a result of freezing due to the weakening of protein functionality. Awad (1968) froze portions of bovine muscle at -4^{o} C up to 8 wk. It was concluded that water holding capacity was negatively impacted by frozen storage which increased drip loss. Water holding capacity decreased with slower freezing rates compared to rapid freezing rates of minced beef (Deatherage et al., 1960). Samples of bovine muscle were frozen at different rates (.22 cm/h to 5.66 cm/h) and significant ($P < 0.05$) differences in water binding capacity were observed. Samples frozen at the slowest rate resulted in the lowest water binding capacity in contrast to samples frozen at a faster rate. This study found samples frozen at 3.95 cm/h had the highest water binding capacity compared to samples frozen at 5.66 cm/h (Petrovic et al., 1993).

Beef and pork raw materials for frankfurters were frozen and held at -17.8 ^{o}C from 0 – 37 wk. Water holding capacity of these raw materials was observed to decrease with increased frozen storage time (Miller et al., 1980). A gradual decrease was

observed in water holding capacity of thawed beef muscle up to 9 mth of frozen storage and a more dramatic decrease afterwards (Farouk et al., 2003).

1.4.4 Extractable Protein

Studies have indicated that the denaturation of muscle proteins play a dominate role in determining the functionality and quality of frozen meat products. Extractable proteins, from the sarcoplasmic and actomyosin decrease with increased frozen storage time. The total extractable protein (TEP) in beef muscle over an 8 wk storage period at -4^{o}C changed from 90.99% TEP to 50.84% TEP (Awad, 1968). Total extractable nitrogen of chicken breast muscle stored at -4^{o}C decreased from 88% to 56% over a 50 wk period (Khan, Van der Berg & Lentaz, 1963). The solubility of myofibrillar proteins extracted from frozen beef increased with faster freezing rates (Petrovic et al., 1993). Other investigators have found that total and myofibrillar protein solubility decreases over frozen storage time. Storage temperature alone affected solubility of myofibrillar and sarcoplasmic protein of beef stored at -75^{o}C (Farouk et al., 2003). Total extractable protein of 72% pork: 60% beef mixture for frankfurters decreased over frozen storage to 28% pork: 22% beef (Miller et al., 1980).

Emulsion systems, finely comminuted or homogenized meat products, have muscle proteins that act as emulsifiers to lower the surface tension at the water-oil interface. Salt soluble myofibrillar proteins are a main contributor to emulsion characteristics and stability of finely chopped meat systems. The ability of myofibrillar proteins to stabilize fat particles in a meat system is decreased by freezing and prolonged storage below the freezing point (Xiong, 1997). A decrease in emulsifying capacity was

also found with increased frozen storage time, due to a decrease in protein solubility (Miller et al., 1980).

1.4.5 Color

Color is impacted by freezing rate and storage time. Beef steaks frozen at lower temperatures (-34^{o}C) had the most desirable color compared to steaks frozen at -9^{o}C which had darker color (Guenther & Henrickson, 1962). Beef slices (1.5 cm) derived from the *Longissimus dorsi* muscle were frozen at slow and rapid freezing rates. Paler and lighter color was observed in the rapid frozen slices compared to higher redness values in slow frozen slices (Jaksobsson & Bengtsson, 1973). Beef *Semimembranosus* muscle was sliced (70 mm) and frozen at slow (0.56 mm/h) or rapid (12.04 mm/h) freezing rates. Samples were individually sealed in water impermeable bags and remained in frozen storage (-18, -35, -75^{o}C) over time (0, 3, 6, 9, 12 myh). Samples were thawed in water at 10^{o}C prior to analysis. It was observed that samples slowly frozen were lighter (higher L* values) in color as compared to rapidly frozen samples. This was explained by higher amounts of drip loss which increased the light reflection of the thawed meat from slow freezing rates. Thawed beef samples became redder or less brown (hue angle decreased) with frozen storage time due to low storage temperatures (Farouk et al., 2003). Beef patties were frozen by cryogenic tunnel at -80^{o}C, air-blast at -30^{o}C, air-blast at -15^{o}C, or still-air at -10^{o}C and stored at -30^{o}C. Darkening of surface color was observed on beef patties that were slowly frozen (still-air -10^{o}C) due to the presence of large ice crystals and surface deterioration (Nusbaum et al., 1983).

The color stability of pork *Longissimus dorsi* muscle was evaluated after 30 min of frozen storage (-20^{o}C). *Longissimus dorsi* muscle was frozen as an intact muscle, and thawed for 24 h at 5^{o}C. Afterwards, the muscle was cut into 2 cm chops, individually placed on a plastic tray, wrapped in polyethylene and placed in an illuminated chill (4^{o}C) cabinet for up to six days. Chops were analyzed randomly after 0, 1, 4, and 6 d of chilled storage for color measurements. The color stability of these frozen and thawed chops were found to have significantly ($P < 0.01$) lower redness (a* values) and significantly ($P < 0.001$) higher yellowness (b* values) than found in fresh chops. No significant differences were found in lightness (L* values) of frozen pork samples compared to fresh samples (Hansen, Juncher, Henckel, Karlsson, Berelsen, & Skibsted, 2004).

1.4.6 Tenderness

In 1962, it was found that freezing beef steaks at -17.7^{o}C and below were slightly more tender than unfrozen, or fresh, steaks. Furthermore, freezing beef steaks at -12.2^{o}C and 9.4^{o}C had an adverse effect on tenderness and was more detrimental as the duration of frozen storage was increased (Guenther, 1962). Beef steaks with longer frozen storage times were found to have significantly lower tenderness scores (Jakobsson et al., 1973). Beef Longisimus dorsi m was aged for 4 or 14 d prior to freezing and frozen by three different rates (13, 3, or 0.04 cm/h). Samples were held in frozen storage (-20^{o}C) for 1 or 9 mth and thawed at 10^{o}C prior to analysis. Tenderness scores were higher on samples with rapid freezing rates compared to slower freezing rates. It was also

concluded that the freezing of meat over a wide range of conditions (i.e. freezing rates or storage temperatures) has little effect on eating quality (Dransfield, 1974).

The sensory evaluation of slowly frozen (0.22 cm/h and 0.39 cm/h) beef samples was shown to be less tender than fresh samples, while quick frozen (5.66 cm/h) beef samples were found to be tender (Petrovic et al., 1993). Meat aged after freezing had lower shear force values compared to meat aged prior to freezing (Crouse & Koohmaraie, 1990). A decrease in shear force values was also found with an increase in frozen storage time (Miller et al., 1980; Farouk et al., 2003). These outcomes have been explained by the breakdown of muscle structure caused by enzyme activity and or ice crystal formation during freezing. Pork chops were individually wrapped in a double layer of polyethylene-coated freezer paper and frozen at -18^{o}C for 1, 21, or 42 days. Once removed from frozen storage, chops were oven-broiled to an internal temperature of 77^{o}C. Tenderness of fresh and frozen pork chops were evaluated utilizing the averages of Warner-Bratzler shear values from nine core samples. Tenderness values could also be related to the physiological age of the animal. It was concluded that neither freezing nor the length of frozen storage had any significant effect on tenderness (Berry, Smith, Spencer, & Kroening, 1971).

1.4.7 Lipid Oxidation

The oxidative process can contribute to protein denaturation and quality deteriorations during frozen storage that may lead to rancidity. Proteins exposed to oxidizing environments are susceptible to chemical modifications, initiated by the natural oxidizing lipids in muscle. Oxidizing agents, such as enzymes, heme iron, or

transition metals, can directly or indirectly react with proteins causing physical and chemical modifications (Xiong, 1997). Lipid degradation products, like malondialdehyde, are capable of cross-linking polypeptides and causing proteins to become insoluble (Buttkus, 1970). Lipid-protein interactions alter the functional properties of meat systems and may cause unwanted changes in final product quality. The decline in flavor and odor acceptance from frozen products is largely due to lipid oxidation. During frozen storage, fatty acids are broken down due to lipid oxidation.

Research has shown that only minor quantities of fatty acids need to be destroyed to generate detectable levels of rancid volatiles (Erickson, 1990). Peroxide values on end products of lipid oxidation, have been measured in pork and have shown to increase with frozen storage time. However, it has also been noted that peroxide values decrease at 25-37 wk of frozen storage at -17^{o}C (Awad et al., 1968; Miller et al., 1980). Lipid oxidation values of beef remain similar to pork, but after 13 wk of frozen storage, beef values increased significantly (Miller et al., 1980).

Mechanically and hand deboned turkey were evaluated for freezing effects and lipid oxidation effect on protein functionality. Samples were frozen at -20^{o}C and analyzed after 26 wk of frozen storage. Conclusions of this study reported that lipid oxidation, freezing, and frozen storage caused myofibrillar denaturation and decreased functionality in both mechanically and hand deboned turkey (Smith, 1987). In 2004, Hansen evaluated the effects of lipid oxidation after the long term frozen storage (-20^{o}C for 30 mth) of pork *Longissimus dorsi* muscle. It was observed that chops with low pH (5.5) had a rapid increase in lipid oxidation during chill storage (4^{o}C for 6 d). It was also

concluded that individual variation between the pigs used for this experiment produced a large standard deviation, and frozen chops were not found to have significantly higher lipid oxidation values compared to fresh chops (Hansen el al., 2004).

1.4.8 Impact of Freezing on Sensory Attributes

Frozen storage may be useful in relation to varying market demands, and experience has shown that long-term frozen storage of meat products may have quality problems such as inferior color and rancid odors and tastes. Beef *Longissimus dorsi* muscle was evaluated for the effect of freezing on sensory attributes (meat taste, juiciness, and tenderness) by trained and consumer panels (Lagerstedt, Enfalt, Johansson, & Lundstrom, 2008). Frozen beef samples evaluated by a trained panel were found to have lower intensity of meat taste, juiciness, and tenderness compared to fresh beef samples. However, consumer panel evaluations of the same attributes showed no significant ($P < 0.05$) differences between fresh and frozen beef samples. Buffalo meat samples were vacuum packaged and frozen at -18^{o}C for 5 days and subsequently thawed at refrigerated temperatures (4^{o}C) for one day. This cycle was continued for a total of 4 cycles. A trained panel evaluated samples for color and odor using a 5 point scale, where 5 was extremely desirable and 1 extremely poor. Panelists found that the freeze-thaw cycles did cause significant degradation in color and odor of meat samples (Sen & Sharma, 1999).

Beef and pork raw materials for frankfurters were frozen and held at -17.8 ^{o}C from 0 – 37 wk. A panel of 10 experienced tasters determined significant differences between control (fresh raw materials) and treated (frozen raw materials) frankfurters by

a triangle sensory test. Panelists detected differences at 7 wk of frozen storage at -17.8°C and continued throughout the course of storage (Miller et al., 1980). In 1993, panelist detected tougher samples of beef *Longissimus dorsi* m that were frozen slowly (0.22 cm/h) compared to rapidly (5.66 cm/h) frozen samples. Panelists also identified rapidly frozen samples to be juicier in contrast to slowly frozen samples that were determined to be the driest samples (Petrovic et al., 1993). Pork chops were individually wrapped in a double layer of polyethylene-coated freezer paper and kept fresh, frozen at -18°C and cooked frozen, frozen at -18°C and cooked thawed, or frozen at -196°C and cooked thawed. Once removed from frozen storage, chops were oven-broiled to an internal temperature of 77°C. A three member trained panel evaluated samples for flavor, juiciness, tenderness, and overall satisfaction using a nine-point hedonic scale (9 = extremely liked, 1 = disliked extremely). No significant differences in tenderness ratings between treatments were noted. However, fresh pork chops had significantly (P <0.05) higher flavor ratings compared to frozen treatments, except samples frozen at -18°C and cooked frozen (Berry, Smith, Spencer, Kroening, 1971).

1.4.9 Use of Scan Electron Microscopy to Determine the Impact of Freezing on Muscle Proteins

The proteins of muscle, for example actin and myosin, can be affected by freezing and a primary tool used to observe these changes is the microscope. Many light and electron microscope techniques are available for observing these structures, however for extensive evaluation, electron microscopy techniques should be employed (Mallikarjunan & Hung, 1997). Two types of scanning electron microscopes may be

utilized: transmission electron microscopes (TEM) and scanning electron microscopes (SEM). In TEM, the electrons penetrate the sample at a voltage of 20-100 kV. An enlarged shadow of the sample is produced and viewed on a fluorescent screen for focusing and a point of interest can be selected and photographed (Kalab, 1983). In SEM, a specific position on the sample is scanned by an electron beam, and on impact the primary beam produces secondary and back-scattered electrons from the sample surface. These secondary or reflected electrons are processed to create an image that can be photographed. Common resolutions for scanning electron microscopes are in the order of 10 nm.

SEM is commonly used to study the internal structures of foods such as myofibrils (Mallikarjunan et al., 1997). Most foods contain water, which will produce volatile vapors under vacuum inside the electron microscope, thus all water from the sample must be removed. To minimize unwanted structural changes in the sample, special procedures such as fixation, dehydration, and specific drying techniques must be utilized to maintain the composition of the structures. Afterwards, the sample is mounted on metal stubs, rendered conductive by a coating of carbon and gold prior to examination in the electron microscope (Kalab, 1993).

The ultrastructure of meat samples has been evaluated using SEM to investigate damage caused by freezing. Studies have concluded that meat tissues that utilized fast freezing rates produced smaller ice crystals and more severe damage was observed from freeze-thaw cycling (Bello, Luft, & Pigott, 1982). Changes in electron micrographs of beef *Longissimus dorsi* muscle frozen at several different temperatures (-10, -22, -33, -

78, and -115^{o}C) was evaluated (Rahelic & Puac, 1984). . It was concluded that the ultrastructure of muscles frozen at various temperatures changed in relation to the freezing temperature. Observations of the effect of freezing on meat samples frozen at -22^{o}C found that water was frozen intracellularly, but specifically only in I-band regions. It was also observed that samples frozen at -33^{o}C had ice crystal formation from water found throughout the entire sarcomere (A and I-band regions). Samples frozen at -78 and -115^{o}C indicated that ice crystals were formed intracellularly. Beef *Semitendenous* muscle frozen under controlled conditions which simulate the operation of an industrial freezer and the areas of ice crystal formation were measured from micrographs by SEM. This study concluded that the diameter of intracellular and extracellular ice crystals was highly correlated with the freezing time (Bevilacaqua, Zaritzky, & Calvelo, 1979).

Cryo-scanning electron microscopy, similar to SEM without a chemical fixation of the sample, was used to study the ultrastructure of porcine *Biceps femoris* muscle with six freezing rates, two storage times, and three thawing rates. Large cavities in the frozen state caused great distortion of the muscle cell structures; however, upon thawing structures were observed to almost completely recover from its previous unfrozen state. Trends were apparent, but significant freezing rate effects were not observed, where as significant storage time effects were found (Ngapo et al., 1999b).

In 2003, Hansen evaluated the following meat structures: (1) fresh meat, (2) meat frozen and stored for 3 days, and (3) meat frozen, thawed and with three days of storage to identify any possible relation to the size of cavities that became visible during dehydration, fixation and microscopy evaluation. It was concluded that cavities in fresh

meat correlate to the extracellular fluids that occupy this space, and the frozen meat samples correlate to the size of the ice crystals developed during freezing. The area of cavities was measured by calculating the diameter of the assumed circular cavity. It was found that air-frozen (diameter of 39.7±5.0 μm) and cryogen-frozen (diameter of 31.3±0.7 μm) meat samples had larger extracellular cavities compared to fresh meat samples (diameter of 11.2±0.7 μm) (Hansen et al., 2003).

1.5 Use of Cryoprotectants to Minimize the Negative Impact of Freezing on Meat Quality

Cryoprotectants are compounds that assist in the long term stability of muscle protein properties, such as gel-forming ability, texture, and water binding properties, that are damaged by freezing (Fennema, 1973). Cryoprotectants have also been described as preventative compounds that stabilize muscle proteins at ambient temperatures, because most of these compounds will equally exhibit the same effectiveness during frozen storage (MacDonald & Lanier, 1997). Red meat proteins, such as pork or beef, suffer less deterioration during frozen storage compared to surimi, a refined myofibrillar component of fish muscle (MacDonald et al., 1997). The onset of ice crystal formation can induce changes in the protein environment, such as dehydration or mechanical damage, which results in poor protein functionality. Maximum cryoprotection occurs when extracted protein molecules create an intimate association with the cryoprotectant. Cryoprotectants are found more often in minced or comminuted products due to the intense interaction between protein molecules and the cryoprotectant. Various

cryoprotectants are incorporated with proteins during processing or product formulation before freezing to minimize physicochemical changes and to prevent functionality losses (MacDonald et al., 1991).

1.5.1 Cryoprotective Substances

Surimi's myofibrils are more unstable compared to beef or pork myofibrils; thus, the manufacturing of surimi requires an addition of a cryoprotective compound to stabilize its functional properties, such as gel-forming and water holding ability (Connell, 1961). Many compounds have been evaluated for this role and a model system was developed by Noguchi to predict the ability of compounds to cryoprotect the functionality of surimi during extended frozen storage (Noguchi, 1974). The following compounds have been evaluated for cryoprotection:

1.5.1.1 Nucleotides and Triglycerides

The changes in quantity and composition of adenosine nucleotides and their relation to frozen fish muscle during frozen storage (-20^{o}C) was studied (Jiang, Hwang, & Tsao, 1987). Protein denaturation was evaluated by measuring phosphate levels (pK value) of actomyosin (AM), Ca-ATPase and Mg (EGTA)-ATPase activities of AM. This study concluded that protein denaturation increased at room temperature compared at frozen storage. This study stressed the importance of maintaining proper temperatures during handling, transportation, storage, and processing of fish muscle. Triglycerides have been found to have a cryoprotective effect on muscle proteins. Free fatty acids released through hydrolysis of phospholipids were thought to denature proteins, instead they react with triglycerides protecting proteins from damage during freezing (Wessels, Simmonds,

Seamn, Avery, 1981). Loomis studied the cryoprotective capacity of products of anaerobic metabolism in stabilizing membranes and labile enzymes. It was concluded lactate, as well as other end-products of anaerobic metabolism, "have properties similar to other, well-characterized cryoprotectants" (Loomis et al., 1989).

1.5.1.2 Surfactants

Surfactants or surface acting agents are soluble compounds with or with out a charge that can be added to products during processing. Two non-ionic surfactants Tween 20 (polyoxyethylene sorbitan monolaurate) and Triton X-100 (sucrose fatty acid ester, 95% monoester) were used to evaluate the effects of denaturation of myofibrillar proteins of rabbit under various freezing and thawing conditions. A myosin solution (0.2 mg/ml myosin, 0.5 M KCl, and 7mM potassium phosphate, pH 7.0) with or without 2% surfactant was put in a polypropylene tube and frozen in liquid nitrogen for 3 minutes. The tube was transferred into a bath (-5^{o}C) for one hour, then thawed in a bath (25^{o}C), and stored in an ice-water bath until assayed. Denaturation of proteins was detected by changes in the Ca^{2+} -ATPase activity in myosin and its solubility in a 0.5M KCl solution. This study concluded that Tween 20, as a surfactant, completely protected the myosin from denaturation during freezing and thawing. Triton X-100, as a surfactant, was less effective and was concluded not to be a suitable protective reagent for myosin (Watanabe, Kitabatake, & Doi, 1988).

1.5.1.3 Salt

Salt has been used to preserved meat for many years and the effects of this salt addition to meat products has been evaluated. Previous studies have shown that as the concentration of salt in meat was increased, water holding capacity and drip loss decreased (DiMarco, 1970). Oxidative rancidity increased with the addition of salt in meat products; however, studies have shown that incorporating less than 5% salt in a formulation will actually inhibit rancidity whereas concentrations above 15% accelerate the development of rancid products (Chang & Watts, 1950).

Phosphates are allowed in meat products at a level not to exceed 0.5% (USDA, 1975) and have been shown to increase water holding capacity, color development in cured products, and inhibit the development of rancidity (Rahelic et al., 1966). The combined effects of salt and phosphate have been studied and were found to increase water holding capacity beyond levels detected by using the two ingredients separately (Flesch and Bauer, 1975). Flaked, cured pork was manufactured using 25 combinations of salt (NaCl) and sodium tripolyphosphate (STP) to evaluate the effects of frozen storage at 3 week intervals of a period of 18 weeks. As salt and/or STP concentrations were increased, smokehouse and cooking yields increased and that cryoprotection of proteins was achieved through these ingredients. Salt was found to increase rancidity while STP retarded its development and ultimately, all products initially rated as acceptable were still scored acceptable after 18 w of frozen storage (Neer & Mandigo, 1977).

The addition of a relatively high salt level (4% NaCl) to minced beef prior to freezing was observed to increase destabilization of muscle proteins and assisted in maintaining functional properties such as water holding capacity (Park et al., 1987). Other salts, such as polyphosphates, which are commonly used in the red meat industry have shown to reduce freeze damage in minced fish (surimi) (Krivchenia & Fennema, 1988). Park and Lanier (1987) found that 0.25 and 0.5% tripolyphosphate or a mixture of sodium acid and tetra-sodium pyrophosphate reduced freeze-induced aggregation in stored fish myofibrils.

Sodium lactate is another compound that cryoprotects and stabilizes muscle proteins by inducing conditions that thermodynamically favor the protein state. Sodium lactate has been found to be an effective cryoprotectant during freeze-thaw and a stabilizer during heat-denaturation of tilapia actomyosin (MacDonald & Lanier, 1994).

1.5.1.4 Carbohydrates

The most effective cryoprotectants for myofibrillar proteins are carbohydrates, such as sucrose, sorbitol, maltodextrins, and polydextrose (Tomaniak, 1998). A study analyzed the use of cryoprotectants for the stabilization of functional properties, such as water holding capacity, gel-forming ability, and protein solubility, of prerigor beef and post rigor beef (Park, Lanier, Keeton, & Hamann, 1987). Beef *Semimembranosus* m. was excised from freshly slaughter beef carcasses and remained whole throughout the rigor process or was immediately comminuted. Polydextrose or 1:1 mixture of sucrose and sorbitol, was added as a cryoprotectant at a 5.6% level to a meat sample along with 4% sodium chloride and 14% water. Samples were stored at -28^{o}C for six months. This

study found that the addition of cryoprotectants (polydextrose or a mixture of 1:1 sucrose and sorbitol) significantly improved the maintenance of water holding capacity, gel-forming ability and protein solubility properties of both salted prerigor and post rigor meats.

Park and Lanier (1987) studied the effects of phosphates and sugar on the protein stabilization of fish myofibrils. Muscle tissue was excised from Jumping Mullet (*Mugil cephalus*) and was comminuted to isolate myofibrils. Phosphates were added individually or in combination with a cryoprotectant (8% polydextrose or sucrose/sorbitol mixture) to myofibril samples. The samples were stored for 5 wk at -20^{o}C and tested on weeks 1, 3, and 5. The study concluded that the combination of phosphates and sugar was most effective in stabilizing functional properties, such as protein suspendability and relative viscosity.

Dziomdziora and Krala (2000) studied the effects of selected cryoprotectants (polydextrose, sorbitol, and Abise S90 (containing carrageens) on functional properties of frozen (-25^{o}C) minced pork during 120 days of storage. Protein solubility, emulsifying capacity, thermal stability of emulsions, water holding capacity and the amount of drip loss were analyzed. They found that polydextrose and sorbitol decreased the amount of drip loss and did not affect water holding capacity. Sorbitol and Abise S90 stabilized the solubility of proteins, but polydextrose did not. It was concluded that none of the tested cryoprotectants protected all the properties of frozen minced pork at the same time.

1.6 Summary of the Literature Review

To improve functionality of frozen meats, the use of ingredients in processed meat products are a means of preventing or inhibiting freeze-induced denaturation and/or aggregation. Cryoprotection of food is growing and developing with many new applications. Cryoprotection of red-meats was stimulated by the successful measures applied to the cryoprotection of surimi in the fish industry. Research has shown that the cryoprotection of red meat could potentially improve the functionality of meat proteins (Park et al., 1987). Many compounds have been identified as cryoprotectants, however the concept of cryoprotection has not gained wide acceptance in the food industry.

With the addition of cryoprotectants the optimal freeze/thaw conditions could increase industry's ability to deliver high quality frozen foods to consumers. Factors such as cost, additional processing, and quality effects must be considered for industrial use when incorporating new technologies. Industrial practice requires the purchase of raw materials when available at lower costs and these materials are subsequently frozen in order to save money, synchronize raw material inventory to increase the availability of product, and to minimize the control of production overtime. However, by freezing these raw materials prior to usage, there is a resulting decrease in product quality. With the injection of cryoprotectants prior to freezing these frozen raw materials could maintain a higher level of protein integrity, resulting in improved protein extractability, binding ability, water holding capacity, and texture qualities, more representative of fresh raw material attributes. With these positive impacts, producers will have the

flexibility to purchase and freeze raw materials while maintaining the economical benefits and quality attributes of fresh raw materials.

2. MATERIALS AND METHODS

This section provides detailed descriptions of procedures and processes utilized during this research project.

2.1 Experimental Design and Statistical Analysis

A 1:1 mixture of sucrose and sorbitol was chosen as a cryoprotectant for this project. A preliminary study was conducted to determine the concentration of cryoprotectants to be used. The preliminary study evaluated concentrations of cryoprotectants (sucrose: sorbitol) and based on its conclusions, concentrations of 2.0% and 4.0% sucrose: sorbitol was selected for this study.

Two bone-in hams were randomly assigned to one of four brine treatments (BT): control (CNT), 2% cryoprotectant (CR) (1% sucrose, 1% sorbitol), and 4% CR (2% sucrose, 2% sorbitol). An additional two bone-in hams were frozen, removed at specified ST days, thawed, injected (FZ I) with the CNT brine. Each pair of bone-in hams from each brine treatment were randomly assigned to a frozen storage (-23^{o}C) treatment (ST): 0, 60, 120, and 180 days as shown in Table 1. The experiment was replicated three times.

Table 1.
Number of hams in experimental design for one replication (N=30).

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	2	2	2	2
2% CR	2	2	2	2
4% CR	2	2	2	2
FZ I	0	2	2	2

Data were analyzed using the Proc GLM or Proc Mixed procedure of the Statistical Analysis System (SAS version 9.1, SAS Institute Inc., Cary, NC) with an alpha < 0.05. As the design was not a complete factorial arrangement, the data were analyzed in two steps. For Warner-Bratzer shear force, drip loss, and cook yield, an experimental unit was defined as a ham. In the first step, the full model included brine treatment, days of storage after freezing, and their interaction. Replicate was defined as a random effect. If the interaction was not significant ($P > 0.05$), then the interaction was excluded from the model and a final model was calculated. Least squares means were calculated and if differences were defined in the analysis of variance, least squares means were separated using the pdiff function. For water activity, pH, TBARS, protein solubility, water holding capacity, CIE L*, a* and b* color space values, data were analyzed with Proc GLM where main effects of brine treatment, days of frozen storage and days of storage after freezing and their two-way interactions were included in the model. Replication was included as a random effect. A final model was determined that included only significant ($P < 0.05$) interactions and all main effects. Least squares means were

calculated and differences were determined using the pdiff function. For this analyses, interaction means were combined into one variable to account for missing cells. For L* color space values for *Semimembranosus* m., all two-way interactions were significant. Due to missing cells, these effects were accounted for by combining all three variables into one variable so that least squares means could be calculated. These least squares means were used to determine two-way interaction means. Tukey's mean separation tests were calculated to separate these means. Purge was analyzed using Proc Mixed as data was only collected on days 28 and 56 of days of storage after freezing. Frozen storage days and brine treatment were combined into one variable. The effect of the combined variable and days of storage and their interaction were included in the model. Replicate was defined as a random effect and storage days after freezing were defined as a repeated effect. Least squares means were calculated and differences were determined as previously discussed.

Trained sensory panel data was statistically analyzed using general linear models procedure and least squares means were generated and separated ($P<0.05$) using the PDIFF procedure in the Statistical Analysis System (Version 9.1, SAS Institute, Inc., Cary, NC). The model for all dependant variables included the block effect of brine treatment, frozen storage day and replication. All variables were analyzed for a significant interaction between brine treatment, frozen storage treatment, and panelist before being pooled across all panelists.

2.2 Product Manufacturing

2.2.1 Raw Material Preparation

Pork bone-in hams (IMPS 401 N= 90 hams) were provided by a regional ham processor. Hams were received fresh in 907 kg combo bins and were stored at 4^{o}C until trimming. Each ham was trimmed by removing the pork leg (fresh ham) hock (IMPS 417A), skinned, and trimmed to approximately 0.62 cm external fat. All trimmed bone-in hams were sorted within a 0.45 – 0.68 kg range to minimize pumping variation. Each ham weighed between 7.22 – 8.12 kg, with an average weight of 7.67 kg. All hams were randomly selected for each brine and frozen storage treatments.

2.2.2 Ham Manufacturing Procedures

Brine treatments were formulated with water, salt (High grade evaporated salt, Cargill Incorporated, Minneapolis, MN), sucrose (Granulated sugar, Imperial-Savannah LP, Sugarland, TX), sorbitol (Crystalline Sorbitol, Archer Daniels Midland Comp., Decatur, IL), sodium lactate (Purasal S, PURAC, Lincolnshire, IL), sodium tripolyphosphate (Brifisol® 512, BK Giulini Corp., Simi Valley, CA), curing salt (Prague Powder, Griffith Laboratories USA Inc, Alsip, IL), and sodium erythorbate (Clifco Spice Sales, Burleson, TX) in the amounts shown in Table 2. Each brine was mixed in a 208 L stainless steel barrel with a removable electric mixer (3/4 HP Agitator, Leeson Electric Corp., Grafton, WS) with a stainless steel propeller. Ingredients were weighed prior to mixing and added to water in the following order: sodium tripolyphosphate, salt, sugar, sorbitol, sodium erythorbate, sodium nitrite, and sodium lactate.

Table 2
Brine ingredients for different cryoprotectant treatments formulated for 43.45 kg of brine.

	Brine Formulation		
Ingredient	CNT	2%CR	4%CR
Water	27.5	27.5	27.5
Sodium Tripolyphosphate	1.13	1.13	1.13
Salt	4.54	4.54	4.54
Sucrose	4.54	2.27	4.54
Sorbitol	0.00	2.27	4.54
Sodium Erythorbate	0.12	0.12	0.12
Prague Powder (6.25%)	0.71	0.71	0.71
Sodium Lactate	6.80	6.80	6.80

Each ham was injected using an automatic needle injector (Famco FGM 26SC M2 injector, Food Machinery Company, Sandvacsej, Denmark) at a belt speed of 43, pump pressure setting of 3.33, and with 24 5 mm needles. Each bone-in ham was injected to 22% of its raw weight. After injection, each ham was allowed 10 min to rest and achieve a target pump weight of 20%. All bone-in hams were netted in polypropylene netting (Vers-A-Nets, Trenton Mills, LLC, Treton, TN) and clipped on each end with a clipper (SZ3214 Double Clipper, Tippertie, Apex, NC). Each netted ham was placed in a 46 X 76 cm poly bag (BH 620 bag, Cryovac Sealed Air Corp, Duncan, SC) with an oxygen transmission 15-30, cc @ a 23^{o}C , sealed with no vacuum,

and placed in an additional poly bag, sealed without vacuum. Hams were stored in this manner to undergo the harshest conditions possible during freezing. Two hams of each BT/ST were placed in a cardboard box (51 X 41 X15 cm box, Georgia Pacific, Wachahaxie, TX) with a poly bag liner (74 X 48 X 76 cm poly bag, J&M Plastic Packaging, Eureka, KS), folded shut, and tie-wrapped with two 0.95 cm polypropylene straps (RUS-FLEX, Rusco Packaging, Dallas, TX). All boxes were placed on a pallet, 5 boxes per tier with a pallet spacer between each tier for proper air flow during freezing. The pallet was placed in a commercial blast freezer (-29^{o}C) for 48 h. After 48 h, all boxes were removed from the blast freezer and placed in frozen storage for designated frozen storage periods (-23^{o}C) of 0, 60, 120, and 180 d.

2.2.3 Tempering Process

Boxes were removed from frozen storage at each frozen storage time and placed in a holding cooler (4^{o}C) for approximately 4 d to allow for controlled tempering to minimize brine loss. On day 4 of thawing, all FR-I treatment hams (frozen, thawed, injected) were injected with the CNT brine following same procedures as previously described. All processing occurred at a regional ham manufacturing plant. After tempering hams were transferred to the Rosenthal Meat Science and Technology Center at Texas A&M University via refrigerated transport.

2.2.4 Smoking

On day 5 of tempering, each ham was weighed to determine drip loss. All treated hams from each replication for a specific frozen storage day (60, 120, and 180 d) were assigned to one of three Alkar single truck smokehouses (Alkar Model 1000, Lodi,

WI) so all three replications were thermally processed at the same time. Each ham was randomly assigned to a smoke truck area; the smoke truck of bone-in hams was then placed in the smoke house and smoked/cooked following a designated smoke schedule (Table 3). Once an internal temperature of 70^{o} C was reached, each smoke truck was removed from the smokehouses and placed in a common holding area for 90 min. Once completed, the smoke trucks were placed in a refrigerated cooler and chilled until the internal product temperature reached 7^{o}C within 15 h.

Table 3.
Thermal processing schedule for bone in hams frozen (-23^{o}C) for 0, 60, 120, 180 d before cooking.

Time (h)	Dry Bulb ^{o}C	Wet Bulb ^{o}C	Dampers	Smoke
1.5	71	43	OFF	None
1.5	77	57	Closed	Smoke
1.5	82	66	Closed	Smoke
6.0	88	72	Closed	Smoke

2.2.5 Slicing/Packaging

Once the hams reached targeted temperature (7^{o}C), each ham was removed from netting and weighed to determine cook yields. The butt portion of each ham was removed by cutting perpendicular to the femur bone from the posterior end of the aitch bone. Each ham was subsequently cut into approximately 5, 1.27 cm slices and 1, 2.54 cm thick slices on an upright band saw (Biro Meat Saw model# 44, Biro Mfg. Co.

Marblehead, OH), vacuum packaged, labeled, placed in cardboard boxes and stored at 4^{o}C until analyzed. The first slice was 1.27 cm in thickness and was analyzed for proximate composition. The second slice was 2.54 cm in thickness, vacuum packaged and analyzed for shear force determinations (analyzed on day 28). The third slice was 1.27 cm in thickness, vacuum packaged and was analyzed for sensory evaluation (analyzed on day 28). Slices 3 – 5 were sliced 1.27 cm in thickness, vacuum packaged and were designated for refrigerated storage at 0, 28 and 56 days and analyzed for lipid oxidation, color, protein solubility and purge. All slices remained in refrigerated storage (4^{o}C) to determine the impact of each BT/ST until analyzed.

2.3 Processing and Compositional Analyses

2.3.1 Weight Loss/Cooking Loss/Purge Determinations

Weight loss was monitored throughout processing, including total weight loss (raw weight to final smoked weight). Drip loss was calculated as:

Drip loss % = (Pumped weight – tempered weight) X 100

Cooking yield was calculated as:

Cooking yield % = (weight of cooked ham / weight of tempered ham) X 100

Total overall yield was calculated as:

Overall yield % = (Weight of cooked ham / initial weight of ham) X 100

Purge was monitored on slices designated for the shelf life study. Each package of ham slice was weighed before it was opened and the fluid drained. Excess fluid was blotted dry with paper towels and then the package was reweighed. Purge loss was calculated as:

Purge loss % = ((package weight before liquid draining – package weight after liquid drying) / package weight before draining) X 100

2.3.2 Color Measurements

Color was analyzed on each ham slice designated for refrigerated storage days 0, 28, and 56. The slice was removed from the vacuum package and exposed to oxygen for approximately 10 min prior to measuring. Samples were obtained from each ham slice and color measurement was taken in triplicate on the *Semimembranosus* m., duplicate on the *Semitendenous* m. and *Biceps Femoris* m, and once from each ham knuckle muscle (*Vastus Laterials* m., *Vatus medialis* m., and *Rectus Femoris* m.). Color was obtained on the surface using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards. CIE L*, a*, and b* color space values were calculated using illuminant A and a 10^{o} observer.

2.3.3 pH Determination

The pH of each slice was determined using a pH Meter (IQ Model IQ150 IQ Scientific Instruments, Inc. Reston VA) calibrated with buffers 4.01 and 7.0. The pH was determined by inserting the pH probe in homogenized sample (100 g) day 0, 28, and 56 of refrigerated storage.

2.3.4 Residual Nitrite

Nitrite was analyzed on each ham slice designated for days 0, 28, and 56 of refrigerated storage using the nitrite analysis determination method (AOAC, 2000). A homogenized meat sample (6 g) and 40 ml distilled water were heated to 80^{o}C in a 100 ml glass beaker. The heated solution was transferred to a 500 ml flask by quantitatively washing the beaker with successive portions of hot distilled water and adding all washings to the flask (approximately 300 ml). The flask was placed in a water bath (100^{o}C) and shaken intermittently for 1 h. After cooling to room temperature, the solutions were filtered through two Whatman No. 2 filter papers into a 500 ml volumetric flask, bringing the volume to 500 ml with distilled water. The filtrate (25 ml) was transferred into a 50 ml volumetric flask, and 2.5 ml sulfanilamide reagent was mixed thoroughly. After 5 minutes, 2.5 ml N-(1-naphthyl) ethylene diamine (NED) reagent was added and the solution was brought to volume with distilled water and set for another 15 min to let the color develop. A standard curve was prepared by adding 10, 20, 30 and 40 ml of nitrite working solution to individual 50 ml volumetric flasks. The absorbance of the standard solutions and samples were measured at 540 nm against a blank of 45 ml distilled water + 2.5 ml sulfanilamide reagent + 2.5 ml NED reagent

using a UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Sugarland, TX). Concentrations were read directly off of the spectrophotometer and reported in ppm.

2.3.5 Lipid Oxidation

The degree of lipid oxidation was determined on each ham slice designated for days 0, 28, and 56 of refrigerated storage using the 2-thiobarbituric acid (TBA) test of Tarladgis et al. (1960) as modified by Rhee (1978). Homogenized meat samples (30 g) were blended in a Warning blender (Model 700S, Torrington, CT) with 45 ml of distilled water and 15 ml 0.5% Propyl gallate and 0.5% ethylenediamine tetraacetic acid, an antioxidant solution. The blended sample (30 g) was collected and combined with an additional 77.5 ml of distilled water and 2.5 ml of 4 N HCl in a Kjeldahl flask. The acidified sample was distilled and 50 ml of distillate collected. Following distillation, 5 ml of distillate was added to 5 ml of 0.02 M TBA reagent then heated in boiling water for 35 min to fully develop the color reaction. The solution (2 ml) was added to cuvettes. Absorbance was measured at 530 nm using a UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Sugarland, TX). Results were reported as mg of malonaldehyde per kg of meat.

2.3.6 Water Holding Capacity

Water holding capacity was evaluated on the *Semitendenous* m. on days 0, 28, and 56 of refrigerated storage using the Carver Press Method. Whole muscle samples were obtained and cut into cubes and the weight (~500mg) was recorded. Two sheets of Whatman #1 filter paper were removed from a desiccator containing a saturated solution

of KCl (RH = 80%). The meat sample was placed between two pieces of filter paper, which was placed between to metal plates. The plates were pressed at 500 psi for 1 min. The outer edge of the pressed meat sample and the outer edge of the pressed moisture was traced with a pencil. Each inner (meat film) and outer (pressed moisture) circles were measured by taking the average of 6 diameters of each circle (cm) to calculate an area. Free and bound water was calculated using the following equation:

$$\% \text{ Free Water} = \frac{[(\text{Total Surface Area} - \text{Meat Film Area})\ 61.60 \times 100]}{\text{Total Moisture (mg) of meat sample}}$$

% Bound Water = 100 = % Free Water

Total moisture of the meat sample using the AOAC Air Drying Oven Method

2.3.7 Protein Solubility

The protein solubility of each ham sample was determined using the Bradford protein solubility determination on days 0, 28, and 56 d of refrigerated storage. The first standard dilution, known as Stock BSA, was composed of 40 µg Bovine Serum Albumin in 100 µL of distilled water. The second standard dilution, known as Serial Stock Solutions were composed (Table 4).

Table 4.
Formulation for serial stock solutions of distilled water and Bovine Serum Albumin.

	0 µg/50 µL	**2 µg/50 µL**	**5 µg/50 µL**	**10 µg/50 µL**	**20 µg/50 µL**
Stock BSA (40 µg/100 µL)	0 µL	5 µL	12.5 µL	25 µL	50 µL
dd-water	50 µL	45 µL	37.5 µL	25 µL	0 µL
TOTAL	50 µL	50 µL	50 µL	50 µL	50 µL

Homogenized meat samples (6 g) were blended in a Waring Blender (Model 700S, Torrington, CT) with 30 ml of deionized water for 30 seconds "on," 30 seconds "off," and 30 seconds "on." The blended meat sample (30 – 35 g) was pored into a 50 ml polycarbonate centrifuge tube and centrifuged using centrifuge rotor at 20,000 x g for 25 min at 2^{o}C. Supernatant (1 ml) was collected by placing the pipette tip below the fat layer and above the pellet) and placed in a micro centrifuge tube as a reserve. A test sample (50 µL of reserve sample) was stored in a 2 ml plastic micro centrifuge tube with 1 ml of Coomassie Plus Reagent at room temperature for 30 min. The Coomassie Plus Reagent (1 ml) was added to each Serial Stock Solution. The absorbance of each Serial Stock Solution and samples was measured at 595 nm using a UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Sugarland, TX). The Serial Stock Solutions created a standard curve for sample evaluation. Absorbance of each testing sample was measured as µg of per µL of sample.

2.3.8 Water Activity

The water activity of each ham sample was determined using a Chilled Mirror Dewpoint Machine (Aqua Lab Model Series 3 Decagon Devices Inc., Pullman, WA) on days 0, 28, and 56 of refrigerated storage. A verification standard of a specially prepared salt solution (0.5m KCl) with specific molality and water activity (0.983 ± 0.001) was used for calibration of the machine. Homogeneous samples were placed in a disposable sample cup, completely covering the bottom of the cup and filling the cup half full. The cup was placed into the drawer and securely closed. After 3 min, sample measurements of water activity were calculated by the machine and recorded.

2.3.9 Shear Value Determinations

Shear force values were determined using a Warner Bratzler apparatus attached to the Instron Universal Testing Machine (Model 1001, Canton, MA) on day 28 of refrigerated storage, concurrent with the week of sensory evaluation. Cores (1.27 cm diameter) were excised from a 2.54 cm thick slice. Three cores were removed from the *Biceps Femoris*, *Semimembranosus* m., *Semitendenous* m., and one core from *Vastus Lateralis* m., *Vastus Medialis* m., and *Rectus Femoris* m. All samples were evaluated at room temperature (19 - 22^{o}C). Cores from each sample were removed running parallel with muscle fiber direction and cores were weighed. All cores were sheared once, perpendicular to the muscle fiber using a 20 kg load cell with a crosshead speed of 200 mm/min. Shear values were reported in Newtons/gram.

2.3.10 Proximate Composition

One ham slice from each ham was designated for proximate composition analyses. All subcutaneous fat and the femur bone cross section were removed from each slice. The slice was chopped, frozen in liquid nitrogen and thoroughly powdered using a Waring Blender (Model 700S, Torrington, CT) for 5 min. Approximately 250 g of each powdered sample was placed in a plastic Whirl Pac® bag and placed in a -23^{o} C freezer until analyzed. Total percent moisture was determined using modified AOAC (2000) air-dry oven methods. Powdered sample (~2.5g) was placed in pre-weighed, previously dried paper thimbles (Whatman #2 filter paper) and the thimble plus sample weights were recorded. Samples were dried for 16 h at 100^{o}C, cooled to room temperature in a desiccator, and the dried thimble plus sample weight was recorded. Percent moisture was calculated as:

% Moisture = ((wet sample weight - dried sample weight) / sample weight) X 100

Percent protein was determined using Leco FP-528 (Leco Corporation, St. Joseph, MI) nitrogen analyzer which vaporized powdered samples of 0.15 g to release total nitrogen. Percent protein was calculated as 6.25 times the percent nitrogen.

2.3.11 Scan Electron Microscopy

Cubes from each ham sample were acquired from the *Biceps femoris* m. on day 28 of refrigerated storage. The center section of the muscle was removed and small 0.5 cm cubes were cut. Cubes were placed in a vial containing 2% gluteraldhyde solution

buffered with 0.1 M sodium phosphate pH 7.0 (Trump's Solution). Fixation of the samples was achieved by submerging sample in 1% osmium tetroxide solution. Samples were rinsed with deionized water and dehydrated by removing water with methanol in 5% increments using the methanol/water combinations. Samples were mounted on stubs and coated with layer of gold in an ion-sputter coater (Hummer Sputtering System). The microstructures of prepared samples were examined on a scanning microscope (JEOL, JSM-6400 Microscope) at a 10 mm working distance using an accelerating voltage of 12 KV at a magnification of 1000x at the Texas A&M University Microscopy Center. Samples were prepared in duplicate and each sample was examined.

2.3.12 Trained Sensory Panel

Sensory evaluations were determined by a six member descriptive attribute panel at Texas A&M University during the week of day 28 of refrigerated storage. The panel was trained according to AMSA (1995) and Civille & Lyon (1996). Each sample was evaluated for surface wetness, springiness, juiciness, hardness, ham flavor intensity, cured fat flavor intensity, mature animal flavor intensity, canned meat flavor intensity, smoke flavor intensity, cardboard flavor intensity, salt flavor intensity, sweet flavor intensity, after taste cured lean flavor intensity, after taste salt flavor intensity, and after taste sweet flavor intensity using the Spectrum^{TM} Universal scale, where 0=absence and 15=extremely intense flavor and aromatic/smell. Panelist attended a training session prior to evaluation days due to excessive length in time between sample evaluations.

Testing took place in climate controlled, partitioned booths. The S*emimembranosus* m. was excised from the designated sensory slice, and 1.27 cm cubes

were excised. A glass custard dish with 3 cubes of each sample and a watch glass lid were placed in a Hatco Cook& Hold Oven at 60 ^{o}C for 45 min. Each sample was served to panelists through breadbox style domes that separate the food preparation area from the sensory testing area. Cool incandescent lights with red filters were used to disguise visual differences between samples. Panelists handled sample cubes with an approved odorless plastic spoon, and evaluated for 15 attributes. Expectorant cups were provided to prevent taste fatigue along with distilled deionized water, unsalted soda crackers, and whole ricotta cheese was used to clean the palate between samples. The panelist evaluated one warm up sample and discussed the results prior to evaluating 8 samples per session. A maximum of two sessions was held per day with approximately 8 min between each sample and a 15 min break in between sessions. The serving order of samples was randomized by treatment on each sensory day.

3. RESULTS AND DISCUSSION

3.1 Injection – Percent Pump and Retention

There was not a significant interaction for the main effects of brine treatment and frozen storage for percent pump and drip loss. Table 5 shows the significant effect ($P < 0.0001$) of brine treatment for percent pump prior to freezing and drip loss after thawing; however, there was no significant effects of frozen storage day. The 4% CR had the lowest percent pump (13.60 %) prior to freezing, compared to CNT (15.82%) and 2% CR (15.49 %). Drip loss occurs due to irreversible tissue damage caused during freezing which is determined after thawing. This was confirmed in 1994 (Wilson et al.) who found a significant difference ($P < 0.05$) in brine-injected bone-in hams that were frozen and stored frozen (-20^{o}C) for 90 d. In Wilson's study, frozen and stored hams had greater drip loss compared to hams that were frozen and not stored which provides evidence that extended frozen storage has an effect on drip loss. Tissue damage occurred due to the length of frozen storage. Cryoprotectants incorporated in the brine addition prior to freezing were ineffective in preventing tissue damage caused by freezing since there were no significant differences in drip loss percentage. These results were different compared to a previous study which found that addition of cryoprotectants (a mixture of 1:1 sucrose and sorbitol) in excised *Semimembranosus* m. from freshly slaughter beef carcasses stored at -28^{o}C for six months had significant improvement in water holding capacity, with decreased drip loss (Park et al., 1987).

Table 5.
Least square means for percent pump (%) and drip loss (%) from bone-in hams frozen (-23°C) for 60, 120, 180 d before cooking.

Brine Treatment	Percent pump[e]	Drip Loss[f] (%)
CNT	15.82[a]	1.45[a]
2% CR	15.40[a]	1.39[a]
4% CR	13.60[b]	1.23[b]
FZ-I	15.18[a]	1.46[a]
P value	*0.0001*	*0.0011*
SEM[d]	0.64	0.26

[a-b] Means with the same letter within a column are not significantly different (P > 0.05).
[d] SEM = Standard error of mean.
[e] Percent pump = 100 – ((weight after injection – initial weight)*100)
[f] Drip loss = 100 – ((weight after injection – weight after thawing) *100)

3.2 Cook Loss and Overall Yield

Length of frozen storage had a significant effect (P< 0.05) on cook loss (Table 6) with the lowest loss at 60 d (11.44%) of frozen storage, followed by 180 d (12.6%) of frozen storage, and the greatest loss at 120 d (13.03%). As length of frozen storage increased, cooking loss increased in comparison to 0 d.

Overall yields were not significantly affected by brine treatment; however, overall yields were significantly affected by length of frozen storage. The highest yield (100.58%) was found after 60 d frozen storage, where as the lowest overall yield (97.12 %) was found after 0 d frozen storage. The overall yields tended to remain similar after 120 d of frozen storage. Our results differed compared to the results of a previous study, Miller et al. (1980), that evaluated beef and pork raw materials for frankfurters that were frozen and held at -17.8°C up to 37 wk. Water holding capacity for these raw materials decreased with increased frozen storage time (Miller et al., 1980). A gradual

decrease in water holding capacity was observed in thawed beef muscles that were frozen up to 9 mth and a more dramatic decrease after 9 mth of frozen storage (Farouk et al., 2003).

Table 6.
Least square means for cook loss (%) and overall yield (%) from bone-in hams frozen (-23°C) for 60, 120, 180 d before cooking.

Days of Frozen Storage	Cook Loss (%)	Overall Yield (%)
0	14.82[a]	97.12[b]
60	11.44[c]	100.58[a]
120	13.03[b]	98.80[b]
180	12.60[b]	99.79[ab]
P value	*0.0001*	*0.0001*
SEM[d]	0.55	0.70

[a-c] Means with the same letter within a column are not significantly different (P > 0.05).
[d] SEM = Standard error of mean.

3.3 Purge Loss

There was a significant interaction (P <0.05) between length of frozen storage, brine treatment, and length of refrigeration on purge (Figures 1 & 2). Purge values after 28 d of refrigeration were very similar across brine treatments up to 60 d of frozen storage. After 120 d of frozen storage 2% CR had more purge compared to the other brine treatments, however, after 180 d of frozen storage purge values tended to be similar across all brine treatments. The increase in purge for 2% CR may be contributed to tissue damage from freezing. Overall, after 28 d of refrigeration purge values tended to increase as the length of frozen storage increased, ice crystal formation generated larger

crystals that cut and/or ruptured more muscle tissue, ultimately having a negative effect on meat quality (Figure 1).

After 56 d of refrigeration (Figure 2) purge values were very similar across brine treatments at 0 d frozen storage. However, as frozen storage increased purge values tended to increase across brine treatments. The highest purge value after 60 d of frozen storage was the CNT brine treatment, after 120 d of frozen storage was the 4% CR brine treatment, and after 180 d of frozen storage was the CNT brine treatment. Purge values tended to be similar among all brine treatments across all frozen storage with the exception of 4% CR. After 120 d of frozen storage, 4% CR purge value was the highest value (9.40%) throughout the entire study, however the purge value decreased after 180 d of frozen storage. Interestingly, the FZ I brine treatment tended to have similar purge values compared to other brine treatments throughout the entire study, with the exception of the highest purge value at 180 d of frozen storage and 56 d of refrigeration. Overall, as the length of frozen and refrigerated storage increased, purge values tended to increase due to damage from ice crystal formation and the loss of protein functionality.

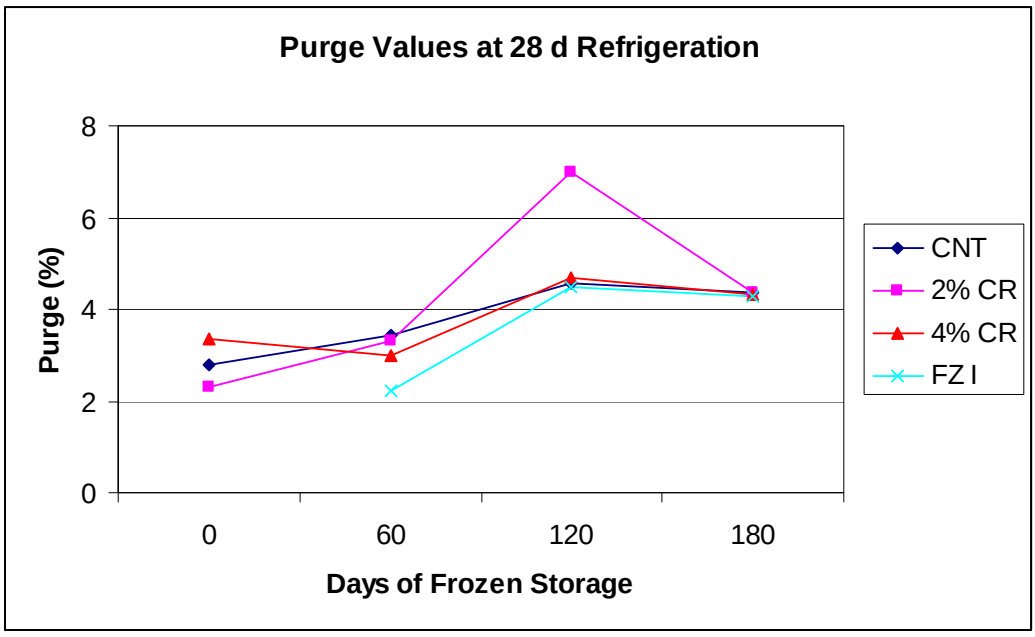


Figure 1. Two-way interaction for purge (%) after 28 d of refrigeration from bone-in hams frozen (-23°C) for 0, 60, 120, 180 d before cooking.

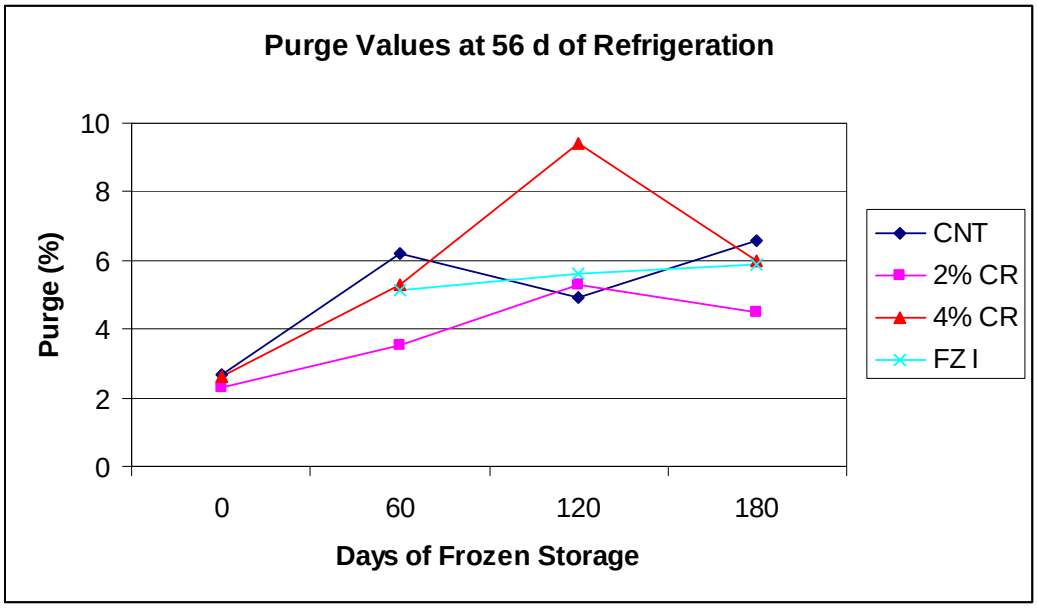


Figure 2. Two-way interaction for purge (%) after 56 d of refrigeration from bone-in hams frozen (-23°C) for 0, 60, 120, 180 d before cooking.

3.4 Color Measurements

3.4.1 Biceps Femoris Muscle

There was not a significant interaction for the main effects of brine treatment and length of frozen storage; however, there was a significant interaction of frozen storage and refrigerated storage as shown in Table 7. L^* (lightness) values were variable between 0 and 56 d of refrigeration over frozen storage compared to values that were lower at 28 d of refrigeration. Yellowness (b^*) values tended not to be different across refrigerated storage within each frozen storage treatment. Redness (a^*) values from 0, 60, and 120 d of frozen storage were lowest at 0 d of refrigeration compared to increasing values after 28 and 56 d of refrigeration. After 180 d of frozen storage, a^* values tended to remain the same and did not increase with any length of refrigeration.

Table 7.
Least square means of *L**, *a**, and *b** values for *Biceps Femoris* m. (SEM) for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, 56 d of refrigerated (4°C) storage[h].

		Days of Refrigerated Storage		
Days of Frozen Storage		0	28	56
0				
	*L**	64.17[ab] (0.40)[i]	57.00[f] (0.40)	58.88[e] (0.40)
	*a**	13.30[f] (0.23)	16.57[b] (0.20)	15.86[c] (0.20)
	*b**	8.31[b] (0.51)	10.88[a] (0.44)	9.32[b] (0.44)
60				
	*L**	62.15[c] (0.46)	59.83[e] (0.40)	63.37[b] (0.41)
	*a**	13.63[f] (0.20)	15.25[d] (0.20)	14.61[c] (0.21)
	*b**	8.20[b] (0.44)	8.94[b] (0.44)	8.95[b] (0.45)
120				
	*L**	64.98[a] (0.43)	39.81[h] (0.40)	60.96[d] (0.43)
	*a**	13.60[f] (0.20)	17.58[a] (0.20)	15.34[d] (0.20)
	*b**	10.27[ab] (0.4)	10.62[ab] (0.44)	9.98[ab] (0.44)
180				
	*L**	52.79[g] (0.43)	62.85[bc] (0.40)	60.69[de] (0.43)
	*a**	16.30[bc] (0.20)	14.48[e] (0.20)	15.84[c] (0.20)
	*b**	9.65[ab] (0.43)	8.88[b] (0.42)	9.99[ab] (0.44)

[a-g] Means with the same letter within a color measurement are not significantly different (P > 0.05).
[h] Refrigerated (4°C) ham slices were vacuum packaged.
[i] SEM = Standard error of the mean.

3.4.2 Ham Knuckle Muscles (Vastus Laterials m., *Vatus Medialis* m., and *Rectus Femoris* m)

Frozen storage day x refrigerated storage day interaction had a significant ($P < 0.05$) effect on *L*, a*,* and *b** values (Table 8). *L** (Lightness) values varied across frozen and refrigerated storage with a range from 52.83 to 63.26, with the exception of 39.46 which was found after 120 d of frozen storage and 28 d of refrigeration. Decreases in L* values can be attributed to the decrease in pigmentation in the myoglobin. Redness (*a**) and yellowness (*b**) values tended to remain the same or increase as refrigeration increased. Table 9 shows the significant interaction of brine treatment and frozen storage of *b** (yellowness) values. These values remained very similar as frozen storage increased throughout all brine treatments with the highest value of 10.02. Slight differences in b* values can be attributed to slight differences in raw materials.

Table 8.
Least square means of *L**, *a**, and *b** values (SEM) for ham knuckle muscles which consist of *Vastus Laterials* m., *Vastus Medialis* m., and *Rectus Femoris* m., for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, 56 d of refrigerated (4°C) storage[d].

		Days of Refrigerated Storage		
Days of Frozen Storage		0	28	56
0				
	*L**	63.09[a] (0.34)[e]	55.59[e] (0.34)	53.43[f] (0.34)
	*a**	14.55[f] (0.21)	16.74[c] (0.21)	17.34[b] (0.21)
	*b**	8.71[bc] (0.24)	10.20[a] (0.24)	10.12[a] (0.24)
60				
	*L**	60.66[c] (0.30)	59.20[d] (0.29)	62.66[ab] (0.31)
	*a**	14.45[f] (0.18)	16.45[cd] (0.18)	14.14[f] (0.19)
	*b**	7.74[c] (0.21)	8.88[b] (0.21)	8.24[c] (0.21)
120				
	*L**	63.26[a] (0.30)	39.46[g] (0.30)	60.12[cd] (0.30)
	*a**	13.47[g] (0.18)	18.00[a] (0.18)	15.52[de] (0.18)
	*b**	8.02[c] (0.21)	9.72[a] (0.21)	8.80[bc] (0.21)
180				
	*L**	52.83[f] (0.30)	61.87[b] (0.30)	59.84[d] (0.30)
	*a**	16.22[cd] (0.18)	15.32[e] (0.18)	16.03[d] (0.18
	*b**	8.94[b] (0.21)	8.23[c] (0.21)	8.82[b] (0.21)

[a-c] Means with the same letter within a color measurement are not significantly different (P > 0.05)
[d] Refrigerated (4°C) ham slices were vacuum packaged.
[e] SEM = Standard error of the mean.

Table 9.
Least square means of *b** values (SEM) for ham knuckle muscles which consist of *Vastus Laterials* m., *Vastus Medialis* m., and *Rectus Femoris* m., m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, or 180 d before cooking.

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	10.04[a]	8.01[c]	8.52[bc]	8.47[bc]
	(0.27)[d]	(0.27)	(0.27)	(0.27)
2% CR	9.62[ab]	8.74[bc]	8.99[b]	8.87[bc]
	(0.27)	(0.27)	(0.27)	(0.27)
4% CR	9.37[ab]	8.40[bc]	9.61[ab]	8.69[bc]
	(0.27)	(0.27)	(0.27)	(0.27)
FZ I	-na-	7.98[c]	8.26[c]	8.60[bc]
		(0.27)	(0.27)	(0.27)

[a-c] Means with the same letter within color measurement are not significantly different (P > 0.05).
[d] SEM = Standard error of the mean.
-na- Not available

3.4.3 Semimembranosus Muscle

There were three significant interactions (brine treatment x frozen storage, frozen storage x refrigerated storage, and brine treatment x refrigerated storage) on *L** (lightness) values (Tables 10, 11, 12). Redness (*a**) values were significantly affected by the interaction of brine treatment x frozen storage (Table 13). There were no significant interactions on *b** (yellowness) values.

*L** (Lightness) values showed similar trends in all brine treatments as frozen storage increased (Table 10). Values remained the same up to 60 d of frozen; however, a significant decrease was observed after 120 d of frozen storage, but values increased after 180 d of frozen storage. As refrigerated storage time increased, values decreased after 120 d of frozen storage compared to increased values after 180 d of frozen storage

(Table 11). There is no explanation for the much lower *L** values observed for ham slices from hams frozen for 120 d and analyzed at 28 d of refrigerated vacuum packaged storage. It was also found that *L** values among brine treatments tended to decrease after 28 d of refrigeration then increased after 56 d of refrigeration (Table 12). Decreases in *L** values can be attributed to increase in myoglobin concentration due perhaps to moisture lost as purge. A decrease in *L** values during frozen storage was also observed in a study by Hansen et al (2004). An increase in *L** values may be due to reabsorption of moisture during extended vacuum packaged storage. Redness (*a**) values among brine treatments tended to remain the same or increase as frozen storage increased (Table 13). It was observed that *a** values for frozen storage tended to remain the same or slightly increase as refrigeration increased (Table 14). Overall *Semimembranosus* m. *a** values, although statistically different were similar values across all frozen storage and refrigeratied storage treatments.

Table 10.
Least square means of *L** values (SEM) for *Semimembranosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, or 180 d before cooking.

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	62.00[ab] (0.67)[d]	61.61[ab] (0.74)	54.77[c] (0.67)	59.62[b] (0.67)
2% CR	61.24[ab] (0.67)	62.29[ab] (0.67)	55.56[c] (0.74)	59.26[b] (0.67)
4% CR	62.32[ab] (0.67)	62.77[a] (0.67)	55.85[c] (0.67)	59.06[b] (0.67)
FZ I	-na-	60.74[ab] (0.67)	54.79[c] (0.67)	59.82[b] (0.67)

[a-c] Means with the same letter are not significantly different (P > 0.05).
[d] SEM = Standard error of the mean.
-na- Not available

Table 11.
Least square means of *L** values (SEM) for *Semimembranosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, 56 d of refrigerated (4°C) storage[e].

	Days of Refrigerated Storage		
Days of Frozen Storage	0	28	56
0	65.21[a] (0.67)[f]	56.37[c] (0.67)	63.98[a] (0.67)
60	62.61[ab] (0.67)	60.08[b] (0.67)	62.86[ab] (0.74)
120	64.02[a] (0.67)	40.86[d] (0.67)	60.84[b] (0.74)
180	54.51[c] (0.67)	63.33[ab] (0.67)	60.49[b] (0.67)

[a-d] Means with the same letter are not significantly different (P > 0.05)
[e] Refrigerated (4°C) ham slices were vacuum packaged.
[f] SEM = Standard error of the mean.

Table 12.
Least square means of *L** values (SEM) for *Semimembranosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, 56 d of refrigerated (4°C) storage[d].

	Days of Refrigerated Storage		
Brine Treatment	0	28	56
CNT	61.69[ab] (0.67)[e]	55.13[c] (0.67)	61.67[ab] (0.74)
2% CR	62.01[a] (0.67)	55.17[c] (0.67)	61.58[ab] (0.74)
4% CR	62.02[a] (0.67)	55.44[c] (0.67)	62.55[a] (0.67)
FZ I	59.11[b] (0.67)	54.41[c] (0.67)	61.84[a] (0.67)

[a-c] Means with the same letter are not significantly different (P > 0.05)
[d] Refrigerated (4°C) ham slices were vacuum packaged.
[e] SEM = Standard error of the mean.

Table 13.
Least square means of *a** values (SEM) for *Semimembranosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, or 180 d before cooking.

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	13.57[bc] (0.32)[d]	13.52[c] (0.33)	14.59[ab] (0.32)	13.81[bc] (0.32)
2% CR	13.92[bc] (0.32)	13.71[bc] (0.32)	14.34[b] (0.32)	14.11[bc] (0.32)
4% CR	13.79[bc] (0.32)	13.31[c] (0.32)	14.44[b] (0.32)	14.44[b] (0.32)
FZ I	-na-	13.00[c] (0.32)	13.82[bc] (0.32)	15.39[a] (0.32)

[a-c] Means with the same letter are not significantly different (P > 0.05).
[d] SEM = Standard error of the mean.
-na- Not available

Table 14.
Least square means of *a** values (SEM) for *Semimembranosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, 56 d of refrigerated (4°C) storage[f].

	Days of Refrigerated Storage		
Days of Frozen Storage	0	28	56
0	12.61[e] (0.23)[g]	15.05[b] (0.23)	13.62[de] (0.23)
60	13.28[de] (0.20)	13.79[d] (0.20)	13.05[e] (0.20)
120	12.69[e] (0.20)	16.12[a] (0.20)	14.08[cd] (0.20)
180	15.43[b] (0.20)	13.52[de] (0.20)	14.36[c] (0.20)

[a-e] Means with the same letter are not significantly different (P > 0.05)
[f] Refrigerated (4°C) ham slices were vacuum packaged.
[g] SEM = Standard error of the mean.

3.4.4 Semitendinosus Muscle

Frozen storage day x refrigerated storage day interaction was significant (P < 0.05) for *L**, *a**, and *b** values (Table 16). *L** (Lightness) values ranged from 63.40 to 54.70 with one value at 40.55. *L** values were inconsistent across refrigerated storage within each frozen storage treatment. Redness (*a**) values tended to increase as refrigeration increased when compared to 0 d. Yellowness (*b**) values increased across all frozen storage treatments as refrigeration increased except after 180 d of frozen storage. Yellowness (b*) values tended to remain similar across all brine treatments except FZ I as frozen storage increased (Table 15). Differences in the *L** values among these four muscles (*Biceps femoris* m., *Semimembranosus* m., *Semitendinosus* m., and ham knuckle muscles) consistently decreased as frozen storage time increased. In 2004,

Hansen et al. measured lightness (*L** values) in frozen and fresh pork chops and found no significant effect due to freezing. Our study did not compare lightness to fresh pork samples, but differences in lightness due to length of frozen storage time were observed. *L** values remained consistent across frozen storage within each refrigeration period. Redness (*a**) values were variable with no identified trend across frozen and refrigerated storage due to moisture loss and increase in pigment concentration with in each muscle. These results are not similar to Hansen et al. (2004) who found a decrease in redness due to increased frozen storage. Yellowness (*b**) values increased as frozen storage increased, which was also reported by Hansen et al (2004).

Table 15.
Least square means of *b** values (SEM) for *Semitendinosus* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, or 180 d before cooking.

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	9.52^{b}	8.95^{bc}	9.44^{bc}	10.11^{ab}
	$(0.37)^{d}$	(0.37)	(0.37)	(0.37)
2% CR	9.78^{ab}	9.72^{ab}	9.98^{ab}	10.70^{a}
	(0.37)	(0.37)	(0.37)	(0.37)
4% CR	10.07^{ab}	9.90^{ab}	10.46^{ab}	9.59^{b}
	(0.37)	(0.37)	(0.37)	(0.37)
FZ I	-na-	8.46^{c}	8.58^{bc}	9.82^{ab}
		(0.37)	(0.37)	(0.34)

[a-c] Means with the same letter within color measurement are not significantly different (P > 0.05).
[d] SEM = Standard error of the mean.
-na- Not Available

Table 16.
Least square means of *L**, *a**, and *b** values (SEM) for *Semitendinosous* m. for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[f].

		Days of Refrigrated Storage		
Days of Frozen Storage		0	28	56
0				
	*L**	63.86[a] (0.33)[g]	56.09[d] (0.33)	61.18[c] (0.33)
	*a**	11.67[e] (0.26)	14.64[bc] (0.22)	14.89[bc] (0.22)
	*b**	8.55[c] (0.30)	10.36[ab] (0.30)	10.46[ab] (0.30)
60				
	*L**	61.26[c] (0.33)	60.51[c] (0.33)	63.41[ab] (0.33)
	*a**	12.93[d] (0.26)	14.40[c] (0.26)	13.92[c] (0.26)
	*b**	8.28[cd] (0.27)	10.59[a] (0.27)	8.89[c] (0.27)
120				
	*L**	63.17[ab] (0.33)	40.55[f] (0.33)	60.83[c] (0.33)
	*a**	12.34[d] (0.26)	16.65[a] (0.22)	14.77[bc] (0.23)
	*b**	7.65[d] (0.27)	10.88[a] (0.27)	10.34[ab] (0.27)
180				
	*L**	54.70[e] (0.33)	62.87[b] (0.33)	60.54[c] (0.33)
	*a**	15.19[b] (0.22)	14.06[c] (0.23)	15.26[b] (0.22)
	*b**	9.86[b] (0.26)	9.74[b] (0.26)	10.56[ab] (0.26)

[a-e] Means with the same letter within a color measurement are not significantly different (P > 0.05)
[f] Refrigerated (4°C) ham slices were vacuum packaged.
[g] SEM = Standard error of the mean.

3.5 pH Determinations

There was a significant main effect interaction on pH as shown in Table 17. Frozen storage day x refrigerated storage day interaction had a significant ($P < 0.05$) effect on pH (Table 18).

Respectively, only slight decreases in pH values were observed as frozen storage time increased across all brine treatments as shown in Table 17. The highest pH value (6.33) was observed in the control brine treatment at 60 d of frozen storage, while the lowest pH value (6.05) was observed in the frozen-injected brine treatment at 180 d of frozen storage. The control brine treatment had similar values ranging from 6.18 to 6.33 in pH from 0 d of frozen storage to 120 d, but decreased to a value of 6.06 at 180 d of frozen storage.

As refrigerated storage time increased, pH values were significantly affected by frozen storage days (Table 18). The pH values tended to decrease as refrigeration increased pH due to lactic acid bacteria present in an anaerobic condition (vacuum package).

Table 17.
Least square means for pH for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking.

	Days of Frozen Storage				
Brine Treatment	0	60	120	180	SEM[c]
CNT	6.18[ab]	6.33[a]	6.29[ab]	6.06[b]	0.05
CR 2%	6.21[ab]	6.07[b]	6.23[ab]	6.13[b]	0.05
CR 4%	6.25[ab]	6.07[b]	6.18[ab]	6.07[b]	0.05
FZ I	-na-	6.13[b]	6.14[b]	6.05[b]	0.05

[a-b] Means with the same letter are not significantly different (P > 0.05)
[c] SEM = Standard error of the mean.
-na- Not available

Table 18.
Least square means of pH for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[f].

	Days of Refrigerated Storage			
Days of Frozen Storage	0	28	56	SEM[g]
0	6.18[c]	6.32[b]	6.13[c]	0.04
60	6.46[a]	6.15[c]	5.84[e]	0.04
120	6.30[b]	6.32[b]	6.00[d]	0.04
180	6.19[c]	6.12[c]	5.93[de]	0.04

[a-e] Means with the same letter are not significantly different (P > 0.05)
[f] Refrigerated (4°C) ham samples were stored in cryovac bags.
[g] SEM = Standard error of the mean.

3.6 Residual Nitrite

Residual nitrite was analyzed to ensure the correct concentrations of the sulfanilamide reagent used during TBA evaluations. The sulfanilamide reagent forms a diazonium salt with nitrite and prevent any interference. All values ranged from 0.24 to 10 ppm of residual nitrite per sample. These low values were expected due to minimal amounts of sodium nitrite allowed by USDA regulations. The further incorporation of sodium erythorbate in brine formulation may have assisted in keeping these levels low.

3.7 Lipid Oxidation

There was a significant interaction ($P < 0.05$) of frozen storage day x refrigerated storage for TBA values, presented in Table 19. TBA values at 120 d frozen storage and 0 d refrigeration were not included due to incorrect data collections. TBA values slightly decreased or remained the same as refrigerated storage increase. A similar effect was seen in 2004, Hansen et al. evaluated the effects of lipid oxidation from the long term frozen storage (-20^{o}C for 30 m) of pork *Longissimus dorsi* muscle. They found chops with low pH (5.5) had a rapid increase in lipid oxidation during chill storage (4^{o}C for 6 d), but frozen chops were not found to have significantly higher lipid oxidation values compared to fresh chops.

Table 19.
Least square means for TBA values for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[f].

	Refrigerated Storage Days			
Days of Frozen Storage	0	28	56	SEM[g]
0	0.24[de]	0.54[b]	0.11[e]	0.04
60	0.13[e]	0.15[e]	0.20[de]	0.03
120	-na-	0.20[de]	0.24[d]	0.03
180	0.75[a]	0.38[c]	0.40[c]	0.03

[a-e] Means with the same letter are not significantly different (P > 0.05).
[f] Refrigerated (4°C) ham samples were stored in cryovac bags.
[g] SEM = Standard error of the mean.
-na- Not available

TBA values ranged from 0.13 to 0.75 for all frozen storage treatments across all refrigerated storage days. None of the treatments reached the threshold TBA value of 1, except on 120 d of frozen storage and 0 d of refrigeration which was not included due to incorrect data collection. The oxidative stability over time can be attributed to the effect of phosphates, sodium erythorbate, and sodium nitrite which were incorporated to each treatment. Previous study found salt to increase rancidity while sodium tripoly phosphate retarded its developments and ultimately, all products initially rated as acceptable were still scored acceptable after 18 weeks of frozen storage (Neer & Mandigo, 1977).

3.8 Water Holding Capacity

There was a significant interaction ($P < 0.05$) of brine treatment x frozen storage day on bound water as shown in Table 20. Frozen storage day x refrigerated storage day interaction had a significant ($P < 0.05$) effect on bound water (Table 21).

The percentage of bound water for the CNT brine treatment increased significantly after 60 d of frozen storage, however a decrease was found at 120 and 180 d frozen storage. Bound water percentages for the remaining brine treatments tended to remain the same throughout all frozen storage, but the FZ I brine treatment had significantly lower values after 60 and 120 d of frozen storage. No changes in percentage of bound water were found after 0 d of frozen storage and 56 d of refrigeration. After 120 d of frozen storage the percentage of bound water increased as the length of refrigeration increased. It was also found that after 60 d of frozen storage at 56 d of refrigeration, bound water values increased. These results differ from studies which reported a decrease in water holding capacity as frozen storage time increased (Miller et el., 1980). A gradual decrease was observed in water holding capacity of thawed beef muscle up to 9 mth of frozen storage and a more dramatic decrease afterwards (Farouk et al., 2003). Our study has shown an increase in bound water with an increase in frozen storage, therefore water holding capacity was not negatively affected.

Table 20.
Least square means for bound water (%) for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking.

Brine Treatment	Days of Frozen Storage				SEM[d]
	0	60	120	180	
CNT	44.29[c]	56.68[a]	49.59[bc]	54.17[ab]	2.13
CR 2%	50.76[b]	51.88[ab]	49.95[bc]	54.71[ab]	2.13
CR 4%	51.39[ab]	53.97[ab]	52.31[ab]	53.47[ab]	2.13
FZ I	-na-	42.16[c]	40.01[c]	48.95[bc]	2.13

[a-c] Means with the same letter are not significantly different (P > 0.05)
[d] SEM = Standard error of the mean.
-na- Not available

Table 21.
Least square means for bound water (%) (SEM) for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[e].

Days of Frozen Storage	Days of Refrigerated Storage		
	0	28	56
0	47.83[c] (2.00)[f]	45.99[c] (2.00)	52.61[bc] (2.00)
60	47.98[c] (1.73)	52.52[bc] (1.73)	53.00[b] (1.73)
120	39.01[d] (1.73)	47.19[c] (1.77)	57.58[ab] (1.73)
180	46.36[c] (1.73)	52.95[b] (1.73)	59.15[a] (1.73)

[a-d] Means with the same letter are not significantly different (P > 0.05)
[e] Refrigerated (4°C) ham samples were stored in cryovac bags.
[f] SEM = Standard error of the mean.

3.9 Protein Solubility

There was a significant interaction ($P < 0.05$) of brine treatment x frozen storage day for protein solubility as shown in Table 22 and frozen storage day x refrigerated storage day interaction had a significant ($P < 0.05$) effect on protein solubility (Table 23).

Protein solubility of each brine treatment increased after 0 d frozen storage, but was not appreciably different from 60 d to 180 d in each respective treatment. FZ I brine treatment caused a decline in protein solubility only at 120 d of frozen storage (Table 22). As frozen storage progressed from 0 to 28 d (Table 23), protein solubility values increased on each respective refrigerated storage day. Previous research found that amount of extractable protein decreases with an increase in frozen storage time. Awad (1968) found total extractable protein (TEP) in beef muscle over an 8 wk storage period at -4^{o}C changed from 90.99% TEP to 50.84% TEP. Another study observed total extractable protein of 72% pork: 60% beef mixture for frankfurters decreased over frozen storage to 28% pork: 22% beef (Miller et al., 1980). Our study found protein solubility levels of each brine treatment tended to remain the same with in the same frozen storage period. However, protein solubility typically declined from 0 to 180 d of storage at each refrigerated storage day. This was similar to results from other studies that have shown a decrease in protein solubility with and increase in frozen storage time.

Table 22.
Least square means for protein solubility (SEM) of vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, 180 d before cooking.

	Days of Frozen Storage			
Brine Treatment	0	60	120	180
CNT	8.85[c] (0.52)[d]	12.91[a] (0.52)	11.71[ab] (0.52)	11.43[ab] (0.52)
CR2%	8.78[c] (0.52)	11.09[b] (0.52)	11.46[ab] (0.52)	12.00[ab] (0.52)
CR 4%	8.50[c] (0.52)	11.39[ab] (0.52)	10.15[bc] (0.52)	12.08[ab] (0.52)
FZ I	-na-	12.08[ab] (0.47)	9.43[c] (0.52)	11.11[b] (0.53)

[a-c] Means with the same letter are not significantly different (P > 0.05).
[d] SEM = Standard error of the mean.
-na- Not available

Table 23.
Least square means for protein solubility of vacuum packaged cooked ham slices cut from bone-in hams frozen (-23°C) for 0, 60, 120, 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[f].

	Days of Refrigerated Storage			
Days of Frozen Storage	0	28	56	SEM[g]
0	10.10[d]	8.23[e]	7.89[e]	0.35
60	11.01[c]	17.02[a]	7.56[e]	0.30
120	11.09[c]	10.46[cd]	10.51[cd]	0.30
180	12.28[b]	12.57[b]	9.94[d]	0.30

[a-e] Means with the same letter are not significantly different (P > 0.05).
[f] Refrigerated (4°C) ham samples were stored in cryovac bags.
[g] SEM = Standard error of the mean.

3.10 Water Activity

There was a significant interaction of frozen and refrigerated storage for water activity as shown in Table 24. Values for water activity ranged from 0.97 to 0.99 and appeared to vary slightly without a consistent trend. Overall, values tended to remain similar within a narrow range during 180 d of frozen storage and 56 d of refrigeration. Awad (1968) found in a study that froze portions of bovine muscle at -4° C up to 8 wk and concluded that water holding capacity was negatively impacted by frozen storage which increased drip loss.

Table 24.
Least square means for water activity for vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking and subsequently stored at 0, 28, and 56 d of refrigerated (4°C) storage[h].

	Days of Refrigerated Storage			
Days of Frozen Storage	0	28	56	SEM[i]
0	0.972[e]	0.973[e]	0.978[cd]	0.001
60	0.979[c]	0.986[b]	0.999[b]	0.001
120	0.991[a]	0.972[e]	0.958[g]	0.001
180	0.976[d]	0.971[e]	0.967[f]	0.001

[a-g] Means with the same letter are not significantly different (P > 0.05)
[h] Refrigerated (4°C) ham samples were stored in cryovac bags.
[i] SEM = Standard error of the mean.

3.11 Shear Value Determinations

There was not a significant main effects of brine treatment and frozen storage for shear value across all muscles. Shear force values for each muscle are as follows: *Semitendenosous* m. ranged from 4.35 to 5.41 N/g; *Semimembranosus* m. ranged from 4.09 to 4.77 N/g; *Biceps femoris* m. ranged from 3.75 to 4.29 N/g; ham knuckle muscles *(Vastus Laterials* m., *Vatus medialis* m., and *Rectus Femoris* m) ranged from 4.04 to 4.76 N/g. These results are similar to a study which evaluated the tenderness of fresh and frozen pork chops utilizing the averages of Warner-Bratzler shear values from nine core samples. It was concluded that neither freezing nor the length of frozen storage had any significant effect on tenderness (Berry, Smith, Spencer, & Kroening, 1971).

Table 25 shows the significant main effects ($P < 0.05$) of brine treatment and frozen storage day for ham knuckle muscles *(Vastus Laterials* m., *Vatus medialis* m., and *Rectus Femoris* m) of shear value (N/g). The ham knuckle muscles displayed the toughest (least tender) values (4.7099) at 180 d of frozen storage compared to the most tender values (4.0428) found at 0 d of frozen storage. These results differ from previous studies that found a decrease in shear force value with an increase in frozen storage time (Miller et al., 1980; Farouk et al., 2003). The ham knuckle muscles displayed the most resistance in the 2% CR brine treatment compared to all other brine treatments.

Table 25.
Least squares means for shear values (N/g) of ham knuckle (KN) muscle which consist of *Vastus Laterials* m., *Vastus medialis* m., and *Rectus Femoris* m. from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking.

Brine Treatment	Shear Force Value
CNT	4.17[b]
2% CR	4.76[a]
4% CR	4.04[b]
FZ I	4.38[ab]
P value	*0.0418*
SEM[c]	0.27

Frozen Storage Day	Shear Force Value
0	4.04[b]
60	4.53[ab]
120	4.07[b]
180	4.71[a]
P value	*0.0398*
SEM[c]	0.22

[a-b] Means with the same letter within each main effect are not significantly different (P > 0.05)
[c] SEM = Standard error of mean

3.12 Proximate Composition

Moisture values were not affected by an interaction of the main effects or individual main effects of brine treatment and frozen storage. Moisture values were determined from a homogenous ham slice and values ranged from 67.71% to 68.83%. There was not a significant interaction for the main effects of brine treatment and frozen storage for protein values. Data showed significant effects (P < 0.05) of brine treatment and frozen storage day on protein percentages (Table 26). 4% CR brine treatment maintained higher protein percentages compared to CNT and FZ I brine treatments. Protein values

were similar after 60 -180 d of frozen storage and was highest (21.71) at 0 d frozen storage.

Table 26.
Least square means main effect of brine treatment and frozen storage for protein from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking.

Brine Treatment	Percent Protein
CNT	20.46[b]
2% CR	20.85[ab]
4% CR	21.18[a]
FZ I	20.43[b]
P value	*0.0457*
SEM[c]	0.22

Frozen Storage Day	Percent Protein
0	21.71[a]
60	20.29[b]
120	20.45[b]
180	20.46[b]
P value	*0.0001*
SEM[c]	0.22

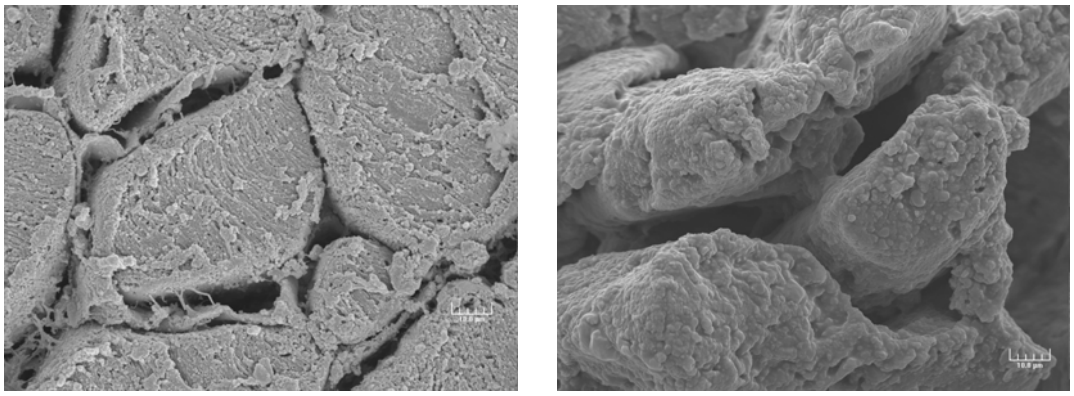
[a-b] Means with the same letter within a main effect are not significantly different (P > 0.05)
[c] SEM = Standard error of mean

3.13 Scanning Electron Microscopy

It was observed during the evaluation of samples, that frozen storage time had an effected on muscle cell structures of the ham samples as seen in Figures 3 & 4. In both figures, differences in muscle structure and cavity area are evident when comparing samples at 60 d of frozen storage and 180 d of frozen storage. At 60 d of frozen storage, structures are intact because myofibers exhibit organized muscle structure and cavities between fibers are minimal. The lack of organized muscle cell structure can be observed

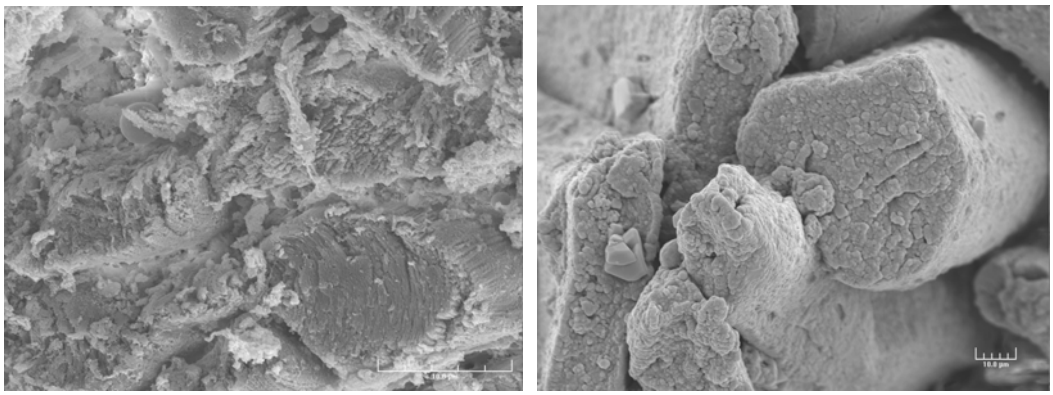
at 180 d of frozen storage due to increase in ice crystal formation with extended lengths of frozen storage. These results are similar to Ngapo et al., 1999 as significant storage time effects were observed in the ultrastructure of pork samples.

Different quality characteristics obtained in frozen meats has been evaluated and different sizes of ice crystals formed are generally considered to be a main factor responsible for these quality changes (Ngapo et al., 1999).



(a) **(b)**

Figure 3. CNT brine treatment samples from bone-in hams frozen (-23^{o}C) for 60, 120, or 180 d with before cooking and subsequently stored at 28 d of refrigerated (4^{o}C) storage. Electron micrograph of ham slices after (a) 60 d frozen (b) 180 d frozen storage (Scale bar = 100 μm).



(a) **(b)**

Figure 4. 4% CR brine treatment samples from bone-in hams frozen (-23^{o}C) for 60, 120, or 180 d with before cooking and subsequently stored at 28 d of refrigerated (4^{o}C) storage. Electron micrograph of ham slices after (a) 60 d frozen (b) 180 d frozen storage (Scale bar = 100 μm).

3.14 Sensory Analysis

Main effects for length of frozen storage were significant ($P<0.05$) for springiness and juiciness descriptive sensory attributes as seen in Table 27. Additionally, two way interaction was significant ($P<0.05$) for taste of salt and sweet, after tastes of cured lean, salt, and sweet, flavors of hammy, cured fat, canned meat, smokey, and cardboardy, and textures of hardness and surface wetness (Table 28 & 29). Overall, hammy and salty were the strongest flavor and basic taste attributes, respectively.

Table 27.
Least square means of frozen storage for springiness and juiciness of vacuum packaged ham slices from bone-in hams frozen (-23°C) for 60, 120, or 180 d before cooking.

Frozen Storage	Springiness	Juiciness
0	5.0[ab]	4.3[c]
60	5.2[a]	5.0[a]
120	4.7[b]	4.7[b]
180	4.8[b]	4.7[b]
P value	*0.0083*	*0.0003*
SEM[d]	0.10	0.10

[a-c]Means with the same letter within a column are not significantly different (P > 0.05).
[d] SEM = Standard error of mean.

Table 28.
Least square means for tastes, after tastes, and textures of vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, 180 d before cooking.

Attribute	Brine Treatment	Days of Frozen Storage 0	60	120	180	SEM[g]
Tastes						
Salt						
	CNT	9.8[ab]	7.9[bc]	8.9[b]	10.3[ab]	0.45
	2% CR	9.8[ab]	8.1[bc]	9.1[b]	10.9[a]	
	4% CR	9.0[a]	7.1[c]	7.5[c]	9.8[ab]	
	FZ I	-na-	7.5[b]	8.5[a]	5.3[cd]	
Sweet						
	CNT	2.7[a]	1.7[cd]	1.8[cd]	0.8[d]	0.19
	2% CR	2.6[a]	1.7[cd]	1.6[cd]	1.0[d]	
	4% CR	2.7[a]	2.0[b]	2.7[a]	1.2[d]	
	FZ I	-na-	1.8[c]	1.8[c]	1.3[d]	
After Tastes						
Cured Lean						
	CNT	2.8[a]	1.9[bc]	2.2[b]	1.8[c]	0.13
	2% CR	2.8[a]	1.9[bc]	2.1[bc]	2.0[bc]	
	4% CR	2.8[a]	2.0[bc]	2.2[bc]	1.8[c]	
	FZ I	-na-	2.0[bc]	2.2[c]	1.8[c]	
Salt						
	CNT	3.5[a]	2.8[bc]	2.6[bc]	2.7[bc]	0.16
	2% CR	3.5[a]	2.7[bc]	2.9[b]	2.8[b]	
	4% CR	2.9[b]	2.5[bc]	2.3[c]	2.4[c]	
	FZ I	-na-	2.8[bc]	2.5[bc]	2.2[c]	
Sweet						
	CNT	2.0[a]	1.0[c]	0.8[cd]	0.5[c]	0.12
	2% CR	1.8[a]	1.1[bc]	0.7[d]	0.6[d]	
	4% CR	2.0[a]	1.3[bc]	1.4[b]	0.7[cd]	
	FZ I	-na-	1.3[bc]	1.0[cd]	0.8[cd]	
Textures						
Hardness						
	CNT	5.9[a]	5.9[a]	5.0[b]	5.2[b]	0.21
	2% CR	5.7[ab]	6.0[a]	4.7[b]	5.0[b]	
	4% CR	5.9[a]	5.5[ab]	4.8[b]	4.7[b]	
	FZ I	-na-	5.9[a]	5.2[b]	4.9[b]	
Surface Wetness						
	CNT	5.9[c]	7.5[b]	8.7[a]	5.4[cd]	0.26
	2% CR	5.8[c]	7.0[b]	8.6[a]	5.0[d]	
	4% CR	5.8[cd]	7.3[b]	8.4[a]	5.1[d]	
	FZ I	-na-	7.5[b]	8.5[a]	5.3[cd]	

[a-d] Means in an attribute with different superscripts are significantly different ($P < 0.05$).
[g] SEM = Standard error of the mean.
-na- Not available

Table 29.
Least square means for flavors of vacuum packaged ham slices cut from bone-in hams frozen (-23°C) for 60, 120, 180 d before cooking.

Attributes	Brine Treatment	Days of Frozen Storage 0	60	120	180	SEM[g]
Flavors						
Hammy						
	CNT	10.0[a]	8.7[bc]	9.5[ab]	8.3[c]	0.23
	2% CR	9.6[ab]	8.8[bc]	9.7[ab]	8.8[bc]	
	4% CR	9.6[ab]	9.2[b]	8.9[bc]	8.9[bc]	
	FZ I	-na-	9.0[bc]	9.2[b]	8.4[c]	
Cured Fat						
	CNT	2.0[ab]	2.1[a]	1.9[ab]	1.9[b]	0.06
	2% CR	2.1[a]	2.0[ab]	1.9[b]	2.0[ab]	
	4% CR	2.0[ab]	2.0[ab]	1.6[c]	2.0[ab]	
	FZ I	-na-	1.9[ab]	1.9[b]	1.9[b]	
Canned Meat						
	CNT	0.0[c]	0.2[bc]	0.0[c]	0.2[bc]	0.12
	2% CR	0.0[c]	0.3[bc]	0.0[c]	0.4[b]	
	4% CR	0.0[c]	0.1[c]	0.1[c]	0.5[ab]	
	FZ I	-na-	0.2[bc]	0.1[c]	0.8[a]	
Smokey						
	CNT	2.5[a]	1.6[b]	1.5[b]	0.4[c]	0.13
	2% CR	2.2[a]	1.5[b]	1.6[b]	0.6[c]	
	4% CR	2.4[a]	1.7[b]	1.7[b]	0.5[c]	
	FZ I	-na-	1.7[b]	1.4[b]	0.4[c]	
Cardboardy						
	CNT	0.0[c]	0.0[c]	0.0[c]	0.3[b]	0.07
	2% CR	0.0[c]	0.0[c]	0.0[c]	0.3[b]	
	4% CR	0.0[c]	0.0[c]	0.0[c]	0.6[a]	
	FZ I	-na-	0.0[c]	0.0[c]	0.7[a]	

[a-c] Means in same attribute with different superscripts are significantly different ($P < 0.05$).
[g] SEM = Standard error of the mean.
-na- Not available.

On 0 d of frozen storage, hams did not differ in hammy, cured fat, canned meat, smokey or cardboardy flavor aromatics, sweet basic tastes, after tastes, hardness and surface wetness, across treatments. However, on 0 d of frozen storage hams treated with 4% CR had less salt after taste and tended to be lower in salt basic taste. With an increase in frozen storage, hammy and smokey flavor aromatics, sweet basic taste, and cured lean, salt and sweet after-tastes decreased. It was noted that hammy flavor slightly increased from 60 d to 120 d of frozen storage, but in general hammy decreased with frozen storage. Higher levels of hammy, cured fat, and smokey would be considered positive favor attributes, where as increased levels of canned meat and cardboardy would be considered negative flavor attributes. It would be expected that some flavor aromatics would decrease or increase with frozen storage. Increased frozen storage has been associated with increased levels of cardboardy flavor aromatic as cardboardy flavor aromatic is associated with increased levels of lipid oxidation (Civille et al., 1996). Cryoprotectants were added to treatments to decreases damage caused by freezing and to hopefully stabilize flavor, basic tastes, and textures of ham cured storage. While cryoprotectant treatments did not affect initial flavor sensory attributes at 0 d of frozen storage, after 180 d of frozen storage slightly higher levels of canned meat flavor aromatic was reported. The addition of 4% CR decreased salt basic taste after 60 d of frozen storage and cardboardy flavor aromatic increased in hams containing 4% CR which was similar in cardboardy flavor to FZ I hams with 180 d frozen storage. Hams containing 2% and 4% CR were less springy and at 0 d of frozen storage, hams across treatments had similar hardness and surface wetness values. As storage time increased,

hams were less springy and juicier, within treatments, as storage time increased hardness slightly decreased similarly, across treatments. Surface wetness increased in hams stored from 0 d to 120 d of frozen storage, but after 180 d of frozen storage surface wetness was lower than the surface wetness of hams at 0 d of frozen storage.

4. CONCLUSIONS

Quality attributes and protein functionality of bone-in hams injected with a brine solution containing sucrose and sorbitol at 2% and 4% with cryoprotectants (CR) prior to freezing were maintained but not significantly improved. Overall yields for cooked hams were not affected by brine treatment (BT). However, the greatest yields were observed with hams frozen for 60 d.

A three way interaction existed (brine treatment x frozen storage x refrigerated storage) for purge. Graphical representation of purge plotted against BT and frozen storage at 28 d of refrigerated vacuum packaged storage indicated that purge values for all BT were similar at both 60 and 180 d of frozen storage. At 56 d of refrigerated vacuum packaged storage the same general trends were observed with greater percent purge observed at each frozen storage day. The 2% CR tended to have the lowest purge values among BT.

Differences in lightness (*L**) values among four muscles (*Biceps femoris* m., *Semimembranosus* m., *Semitendinosus* m., and ham knuckle muscles) were variable with no identifiable trends in L* value differences observed as frozen storage time increased. Our study did not compare lightness to fresh pork samples, but differences in lightness due to length of frozen storage time were observed. Redness (*a**) values were variable with no identified trend across frozen and refrigerated storage due to moisture loss and an increase in pigment concentration within each muscle.

A BT x frozen storage interaction existed for pH with values ranging from 6.05 to 6.33, as well as a frozen x refrigerated storage interaction with pH values ranging

from 5.84 to 6.46. The lowest pH values were observed at 56 d of vacuum packaged storage due to the growth of lactic acid bacteria. Residual nitrite values ranged from 0.24 to 10 ppm.

A frozen storage x refrigerated storage interaction existed for lipid oxidation values, which tended to remain similar or slightly increase ($P < 0.05$) as refrigerated storage increased but no values were found above 0.75 mg malonaldehyde/kg sample. Water holding capacity was affected by a BT x frozen storage day interaction and at frozen storage x refrigerated storage day interaction. At 180 d of frozen storage, the 2% CR BT, although not different than any other BT had a numerically higher (54.71) percent bound water.

Although not statistically different, protein solubility for 2% CR and 4% CR were greatest at 180 d compared to the other BT. Protein solubility typically declined at each refrigerated storage day across all frozen storage treatments with the exception of hams frozen for 60 d and analyzed at 28 d of refrigerated storage. Water activity values ranged from 0.97 to 0.99. The main effects of BT frozen storage and refrigerated storage had no effect on shear force values for the *Semitendenosus* m. *Semimembranosus* m. and *Biceps femoris* m. The 4% CR BT exhibited the lowest shear force value (4.04 N/g) but was not statistically different than CNT or FZ I on ham knuckle muscles. As frozen storage time increased shear values tended to increase. Percent protein was the greatest for both 2% and 4% CR with 4% CR exhibiting the greatest percent protein among all BT. Percent protein decreased during frozen storage.

Trained sensory panelists found hammy and salty were the strongest flavor and basic taste attributes throughout the entire study, but no differences in sensory attributes due to BT were detected.

The results of this study confirm that quality attributes and protein functionality were maintained but not significantly improved by injecting a brine solution with cryoprotectants prior to freezing. Other studies have attempted to employ cryoprotectant compounds in the freezing preservation of intact muscle (Fennenma et al., 1989). The intimate association of the cryoprotectant molecules and the protein is difficult to achieve in intact muscle whereby comminuted products have seen success (Mac Donald, 1991). Although the 2% and 4% CR did not significantly differ from hams injected with the CNT brine treatment, the CNT did contain 2% of sucrose.

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APPENDIX A

pH MEASUREMENT PROCEDURE

EQUIPMENT:
Food processor or homogenizer
pH meter with pH electrode
Waring® Blender
Stir plate
Magnetic stir bars
Thermometer

REAGENTS:
Distilled water
Buffer, pH 4.0 and pH 7.0

PROCEDURE:

1. Obtain 50-100 g of product or tissue sample and grind 3 to 4 times or homogenize in a food processor. For pre-rigor sausage it is important to take the pH reading immediately after slaughter and/or use iodoacetate to halt the pH decline prior to rigor mortis resolution.
2. Place approximately 30 g of the finely chopped, minced or ground sample into a blender.
3. Add 90 g distilled water (at least three times the weight of the sample) to the blender and blend on high speed for 15 to 20 seconds to make smooth slurry.
4. Measure the pH of this slurry with a pH meter that has been calibrated with two standard buffer solutions. One buffer at pH = 7.0 and the other having a pH value near that of the final sausage pH (range 4.0 to 4.5).
5. The electrode should be placed in the stirred slurry for about 30 to 60 seconds to allow the electrode to equilibrate. Record the pH of the slurry after the electrode has stabilized. The temperature of the solution being measured should be close to the temperature calibration of the pH meter, otherwise the pH determination will not be accurate.
6. Do **NOT** leave the pH probe in the meat slurry. Remove the pH probe from the slurry and wash it thoroughly with distilled water. Be sure to gently wipe all fat and connective tissue from the probe.
7. Always store the pH probe in **CLEAN** distilled water or pH 7 buffer. **NEVER** let the bulb dry out.

APPENDIX B

HUNTER LAB MINI SCAN XE PLUS STANDARD OPERATING PROCEDURES

** Always handle the black and white standardization plates with care. Do not scratch or chip them.

Plug Mini Scan into electrical outlet.

Wipe the black plate with a Kimwipe to insure it is clean and place the black plate on the circle of the calibration tile holder.

Place the Mini Scan on the calibration tile holder so the two rubber feet are in the two holes of the holder and the aperture is centered on the black plate. The aperture should fit flatly on the black plate to insure that there is no interference when taking readings.

Push the lightning bolt key on the Mini Scan to turn the unit on.

Make sure that the XYZ values on the screen correspond to the XYZ values listed on the back of the white plate.

You are now ready to standardize the unit. Press the lightning bolt key and the Mini Scan will read the black plate.

When the reading is complete, the screen will indicate that the machine is ready to read the white plate.

Remove the black plate from the calibration tile holder and replace it with the white plate. Wipe the white plate with a Kimwipe. Make sure that the aperture of the Mini Scan sits flatly on the white plate.

Press the lightning bolt key to read the white plate.

Press the lightning bolt key three times and the MiniScan will be ready to read the first sample.

The Tristimulus values L*a*b* will be recorded.

Position the aperture of the Mini Scan on the part of the meat sample to be tested. Be sure that the aperture fits flatly on the meat but do not apply pressure. The spot to be

tested should be representative of the Ham **muscle** tissue. There should not be a lot of connective tissue, seam fat or subcutaneous fat where the color reading is taken.

To take a reading, press the lightning bolt key.

Record the L*a*b* values and press the lightning bolt key again to take a second reading of exactly the same spot on the meat sample.

Record the L*a*b* values and take a third reading by pressing the lightning bolt key.

Record the L*a*b* values and press the lightning bolt key to display the average values. Record these values.

Press the lightning bolt key again to display the standard deviations. Record these values.

The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample, make sure that the PVC overwrap covering the aperture is clean and free of fat or anything that might interfere with a clean reading.

When all readings are complete, unplug it from the electrical source.

Be sure that the Mini Scan is clean and that the aperture is clean before putting the machine away.

APPENDIX C

LIPID OXIDATION ANALYSIS PROCEDURE

FOR CURED MEATS

Apparatus:

500 or 800 ml Kjeldahl flasks
Spectrophotometer with 1 cm cells
Hot plate or Bunsen burner
Waring Blender
Boiling chips
250 ml beakers
Balance / Scale
400 ml beakers
Screw cap test tubes
Test tube rack
Graduated cylinder
Timer
Pipette

Reagents:

0.02 M 2-Thiobarbituric Acid (1.442 g 2-Thiobarbituric acid in 500 ml distilled water). Heat just enough to dissolve, DO NOT BOIL.

0.5% Propyl gallate (PG) and 0.5% ethylenediamine tetraacetic acid (EDTA) solution (5g PG + 5 g EDTA in 1 liter distilled water, heat just enough to dissolve, DO NOT BOIL).

Sulfanilamide Reagent (0.5% sulfanilamide in 20% HCL, v/v); For 500 ml: 20% HCL combine 100 ml conc. HCL + 400 ml dd-water

4 N HCL (1 volume concentrated HCL and 2 volumes of distilled water) or (384 g conc. HCL in 1 liter dd-water)

Silicone® Spray (reduces foaming)

PROCEDURE:

Extraction Solution Combinations for Various Nitrite Levels in the Sample (For Step #1)
BLENDING SOLUTION

	Amount of Residual Nitrite (ppm)				
	0	**0-50**	**50-100**	**100-150**	**150-200**
Meat	**30**	**30.0**	**30**	**30.0**	**30**
Sulfa Reagent	**0**	**1.5**	**3**	**4.5**	**6**
dd-water	**45**	**43.5**	**42**	**40.5**	**39**
PG + EDTA	**15**	**15.0**	**15**	**15.0**	**15**

Transfer Solution Combinations for Various Nitrite Levels in the Sample (For Step #2)
DISTILLATION SOLUTION

	Amount of Residual				
Nitrite (ppm)	**0**	**0-50**	**50-100**	**100-150**	**150-200**
dd-water	**77.5**	**78**	**78.5**	**79**	**79.5**
1:2 N HCL	**2.5**	**2**	**1.5**	**1**	**0.5**

1. Blend 30 g of meat with 43.5 ml of 50^{o}C distilled water plus 1.5 ml of sulfanilamide reagent and 15 ml of 0.5% solution of PG and EDTA for 2 min. (Sulfanilamide forms a diazonium salt with nitrite and prevents interference).
2. Quantitatively transfer 30 g of the slurry into a 500 ml Kjeldahl flask using 78 ml of 50^{o}C distilled water. Add 2 ml of HCL solution (1:2).
3. Spray Silicone® into the neck of the flask and add 5 – 6 boiling chips.
4. Turn on Kjeldahl distillation hot plate and the condenser water.
5. After the heating element is hot, connect the flask and collect 50 ml of distillate (12 – 15 min).
6. Remove the distillate and replace with a beaker containing 400 ml distilled water. Turn off the heat and allow the water to be drawn back through the distillation apparatus. Then turn off the cooling water.
7. Add 5 ml of the distillate to a screw cap test tube along with 5 ml of the TBA reagent. Mix and heat in a boiling water bath for 35 min to develop the color. For the blank, use 5 ml distilled water + 5 ml TBA reagent and heat with the sample.
8. Cool in tap water for 10 min, place sample in a cuvette. Read the sample absorbance in the spectrophotometer at 530 nm. The blank should be read first and set at 0 absorbance.

NOTE: For accurate results, a standard curve should be run for quantities of malonaldehyde over the expected range of values.

CALCULATION OF TBA NUMBER:

TBA number = O.D. x K

Where K = 7.8, which was determined for the distillation set up in the lab.

Specifically,

TBA number = Abs 530λ x 7.8 (conversion factor) mg malonaldehyde/kg sample

Standard deviations of the duplicates should be approximately ±0.2 TBA Value.

Slight changes occur in the K value from laboratory to laboratory. Therefore, the K value or standard curve for known dilutions of 1, 1, 3, 5 tetraethyoxypropane should be calculated in each laboratory. K=7.0 is an average value that can be used but may not be the most accurate (Tarladgis et al., 1960).

Expected TBA Values:

0.7 to 1.0	Fresh manufactured product (maximum)
1.0 or higher	Considered to be rancid by some processors
0.1 to 0.2	Reported rancidity for cooked pork
0.5 to 1.0	Considered the threshold level in pork by come researchers
0.3	Maximum allowed for rework material by some packers

1.0 mg malonaldehyde/1000g meat has been reported as the threshold for rancidity by organoleptic detection.

APPENDIX D

INSTRUCTIONS FOR OPERATING THE VARIAN SPECTROPHOTOMETER

TO DETERMINE TBAR VALUES

Turn the spectrophotometer on at least 15 minutes prior to running samples. This allows the machine time to warm up.

The **ON** switch is located on the front of the spec in the lower left corner.

The computer should never be turned off.

With the computer mouse, double click on the icon '**CaryWinUV'**.

On the next screen, double click on the icon '**Advanced Reads'**.

A "Did you know" box will appear, click "**Close**".

Go to the left side of the screen and double click on the "**Setup**" button.

On the next screen, change the wavelength to "**530**" nm. Then click on the "**Samples**" tab and enter the number of samples that you will be running. You can also name the samples by typing each sample name. (If you don't want to do this, just keep track of what sample corresponds to sample 1, sample 2, etc. on you lab paperwork.) Click "**OK**" to close the setup screen.

**You should always wear disposable gloves to keep your hands and the cuvettes clean.

To run the blank, double click on the "Zero" button (which is located directly below the "Setup" key on the left side of the screen).

A box will appear prompting you to load the blank into the spec.

Open the green door on the top of the spec. Wipe the smooth sides of the cuvette with a Kimwipe to remove anything that could interfere with a clear reading. Insert the cuvette containing the sample blank into well #1 of the cell block. The smooth sides of the cuvette should be on the left and right. The grooved sides of the cuvette should face front and back.

Completely close the green door. You are now ready to read the blank.

Click "**OK**" on the computer screen to read the blank.

When the sample reading is complete, the "Zero" value will appear on the screen. Open the door and remove the cuvette.

To remove the cuvette, gently pull up on the silver knob that is located in front of the sample cell block. Using gloved hands or tweezers, pull the cuvette out of the cell block. **DO NOT** discard any samples until you are finished with the run, just in case any samples need to be rerun.

Take the next sample cuvette, wipe the sides clean with a Kimwipe and insert the cuvette into cell block well #1. Close the green door.

On the computer screen, click on the "**START"** traffic light key located toward the top of the screen.

A "Sample Collection" box will appear on the screen. In this box will be a list of your samples. If the list is correct, click "**OK**". (If the list is not correct, go back to the "**Setup**" screen, "Samples" tab and enter the correct information.)

Then a "Save As" box will appear. Type in a file name for your sample run i.e. "Fat Blend Day 7 TBAR 081506". Click "**Save**".

A "Present Sample" box will appear on the screen. It is now ready to read the first sample, which you placed in the cell block.

Click "**OK**".

When the first sample has been read, another box will appear on the screen prompting you to insert the second sample.

Remove sample 1 cuvette from the cell block. Wipe the sample 2 cuvette with a Kimwipe and insert the cuvette into well #1 of the cell block. Close the green door and click "**OK**".

Continue this process until all samples have been read. When the last sample reading is complete, click on the "**Print**" button which is located toward the lower left corner of the screen.

Then click on the "**Clear Report**" button which is just above the "Print" button. This will clear the report from the screen but not from the computer files.

If you have more samples to run, you can start over again by clicking on "Setup" and check the wavelength and set up for the appropriate number of samples.

If you are finished running samples, click on the X in the upper right corner of the screen to escape from the program.

Click the X again and leave the computer turned ON.

Turn off the spec when you're finished.

APPENDIX E

NITRITE ANALYSIS IN CURED MEATS PROCEDURE

(AOAC Official Method 973.31, 2000, 39.1.21, PAGE 8)

EQUIPMENT:

100 ml beakers
1000 ml Volumetric flasks
50 ml Volumetric flasks
Spectrophotometer (UV/VIS 540 nm)
5 ml Pipettes
500 ml Erlenmeyer flasks
Heated Water Bath
Analytical balance
Homogenizer or food processor
Glass rods
500 ml Volumetric flasks
Hot Plate
Spec cuvettes
10 ml Pipettes
Whatman® No. 2 Filter paper

REAGENTS:

NED Reagent: Dissolve 0.2 g N-(1-naphthyl)ethylene diamine • 2HCl in 150 ml 15% (v/v) acetic acid. Store in a glass-stoppered brown glass bottle. If necessary, filter before use.

Sulfanilamide Reagent*: Dissolve 0.5 g sulfanilamide in 150 ml 15% (v/v) acetic acid. *Store in dark or brown glass bottle. If necessary, filter before use.

Standard Curve Preparation:
Nitrite Standard Solution

Stock solution (1,000 ppm $NaNO_2$): Dissolve 1 g (± 0.0001) $NaNO_2$ in distilled water and dilute to 1 L.

Intermediate Solution (100 ppm $NaNO_2$): Dilute 100 ml of Stock Solution to 1,000 ml with distilled water.

Working Solution (1 ppm $NaNO_2$): Dilute 10 ml of Intermediate Solution to 1,000 ml with distilled water.

Filter Paper:
Randomly select 3 to 4 sheets per box. Filter 40 ml water through each sheet.
Add 4 ml sulfanilamide reagent, mix and wait 15 min.
If any sheets are positive, discard entire box.

PROCEDURE:

1. Weigh 5 g (±0.01) of finely comminuted and thoroughly mixed sample into a 100 ml beaker.
2. Add approximately 40 ml distilled water and heat to 80^{o}C. Use a glass rod to break up all lumps and mix thoroughly.
3. Transfer the heated solution to a 500 ml volumetric flask. Quantitatively wash the beaker and rod with successive portions of the hot distilled water, adding all washings to the flask (approximately 300 ml).
4. Transfer the flask to a steam bath (~100^{o}C) and shake occasionally for 2 hour. After cooling to room temperature, bring the volume to 500 ml with distilled water and remix. Filter through two Whatman No. 2 filter papers into flask and mix solution thoroughly (discard the residue). Then transfer 25 ml of the filtrate into a 50 ml volumetric flask then add 2.5 ml sulfanilamide reagent, mix thoroughly.
5. After setting for 5 min, add 2.5 ml NED reagent, mix. Dilute to volume with distilled water, mix and set for another 15 min to let the color develop.
6. Transfer a portion of the solution to the cuvette and read absorbance at 540 nm against a blank of 45 ml distilled water + 2.5 ml sulfanilamide reagent + 2.5 ml NED reagent.

Standard Curve Preparation:

Add 10, 20, 30 and 40 ml of nitrite working solution to individual 50 ml volumetric flasks. The nitrite concentration in each flask is 0.2, 0.4, 0.6 and 0.8 ppm, respectively. Add 2.5 ml of sulfanilamide reagent, mix and proceed as in steps 5 and 6. The standard curve is straight line to 1 µg/ml $NaNO_2$ in final solution.

CALCULATION:

Nitrite Residual (ppm or µg/g) = Absorbance x K x F

Where: K = Standard Curve Slope = 1.7438

F = Dilution Factor = 500 x 2 x 1/5 = 200

OR

The concentration may be read directly off of the spectrophotometer. Thus, K, Abs nor F are required in this case.

NITRITE DETERMINATION

Standard Curve: _____

Absorbance

Blank _____

Sample _____

Rep #1 _____

Rep #2 _____

Rep #3 _____

Rep #4 _____

Rep #5 _____

Average _____

Nitrite Residual (ppm or µg/g) = Absorbance x K x F

Absorbance: 540 nm (Spectrophotometer)

K: Standard Curve Slope _____

F: Dilution Factor (500 x 2 x 0.20) _____

Nitrite Residual:

Blank: _____ Absorbance

Rep #1 _____ Nitrite

Rep #2 _____ Nitrite

Rep #3 _____ Nitrite

Rep #4 _____ Nitrite

Rep #5 _____ Nitrite

Average Nitrite Concentration in the Product: _____

APPENDIX F

MOISTURE ANALYSIS – AOAC PROCEDURE

EQUIPMENT:
Whatman® filter paper, 22 x 40 mm
Stapler
Aluminum dishes, 50 mm diameter x 40 mm deep
Desiccator
Convection oven
Food Processor
Tongs
Analytical balance/ Scale

PROCEDURE:

1. Filter paper/extraction thimbles consisting of Whatman 22 x 40 mm filter paper folded in to a sleeve open at one end and stapled at the opposite end, or a covered aluminum dish at least 50 mm in diameter and not greater than 40 mm deep.
2. Mechanical convection oven, preferably one equipped with a booster heater.
3. Accurately weigh sample to the fourth decimal place (+/- 0.0001). Sample should weigh approximately 2 g. Then place sample into a previously dried and desiccated sleeve, paper thimble or a covered aluminum dish that has been dried and desiccated.

   Note: handle sample container with tongs to avoid moisture from your fingers. Never handle sample containers with gloved or ungloved hands for the most accurate results.

4. Dry sample for 16 to 18 hours at 100 to 102^{o}C, or for four hours at 125^{o}C, in the mechanical convection oven. Drying at higher temperature (125^{o}C) may cause the fat to oxidize (vaporize) creating excessive fat loss and inaccurate fat percentages.
5. Cool the samples in a desiccator (with desiccant) to room temperature so that no additional moisture is absorbed by the sample. Reweigh the dried sample.

CALCULATIONS:

$$\textbf{Percent Moisture} = \frac{100\,(B-C)}{A}$$

Where: **A = Sample weight**
B = Weight of dish/thimble + sample before drying
C = Weight of dish/thimble + sample after dryin

APPENDIX G

RAPID NITROGEN/PROTEIN ANALYSIS PROCEDURE

LECO FP-528

EQUIPMENT:
LECO FP-528 System
Analytical balance

REAGENTS:
Oxygen gas
Helium Gas
Air

PROCEDURE:
Instrument Start-Up:

Assumes instrument switch has been turned "ON", but gases have been turned "OFF". In the "OFF" mode, no helium is flowing.

QUICK MENU – First Screen

1. Perform leak detection – See operation manual for this procedure.
2. Standard parameter settings for the LECO FP528 Nitrogen/Protein System:

Gases	**Pressure**
Oxygen	40 psi
Air	40 psi
Helium	40 psi

When gas tanks reach 300 psi – **CHANGE TO NEW TANK**
Combustion Tube Temperature – 850ºC

•**Furnace Filter** – Change when the metal shavings have begun to rust ~1" down the tube. Change daily if the machine is used 8 hr/day.

•**Filter Materials**

Anhydrone (Mg Perchlorate) – Absorbs H2O
LecoSorb (NaOH with silica coating) – Absorbs CO2

•**Thermal Conductivity Cell**

Reference flow of Helium = 30 cc
Sample Flow = 200 cc/min – Red line is the indicator

3. To turn gases "ON"

NOTE: Superscript "S" denotes prompts on the LECO FP528 Screen while superscript "B" denotes Button below screen.

[ANALYZE]^{S} → [l]^{S} → [MENU]^{B} → [5]^{S} → [CARRIER GASES]^{S} →
(ANALYZE) (SYSTEM CONTROLS)
[ON]^{B} → [EXIT]^{B}

4. To calibrate the BLANKS prior to standardization and analysis

[l]^{B} → [SELECT]^{B}* → [NEXT]^{B}** → [START]^{B} → [EXIT]^{B} →

[3]^{S} → [YES]^{B***}→ [MENU]^{B} → [6]^{S} → [EXIT]^{B****}→ TO QUICK MENU
(CALIBRATE) (CALIBRATE BLANK)

*Press key two times to move to ID Code; Input Code by pressing key pad buttons until appropriate letter or number appears.
**Press key to input multiple blanks >5.
***Press to select each blank to be run.
****Press 2 times.

(Allow 5 or more blanks to run until blank values are near zero (0), i.e., 0.012 or -0.012).

5. To Run Standards:

[l]^{S} → [WEIGHT]^{S*} → [SELECT]^{B**} → [NEXT]^{B***} → [WEIGHT]^{S****} → (ANALYZE)

[NEXT]^{B*****} → [ENTER REMAINDER OF STD'S] → [START]^{B}
Runs Standard

*Enter weight of standard
**Press 2 times and input ID Cod, i.e. "Oats"
***Press 1x to enter
****Enter 2nd weight of standard
*****Enters 2nd standard

6. To Delete Blanks:

[PREVIOUS]^{B} to select for DELETION
[NEXT]

[ANALYZE]^{B} → [SELECT]^{B} → (Change any blanks as needed) →
Scrolls through [MENU]^{B} → [l]^{S} DELETE

ID Code, Weight, P Factor

[EXIT]^{B} TO MAIN MENU

7. To Run Samples:

Weigh ~0.5000 g of sample into gel cap.

Under the ANALYZE menu, press SELECT to obtain
"Weight Input"

[l]^{S} → [WEIGHT]^{S*} → [SELECT]^{B**} → [NEXT]^{B} → [WEIGHT]^{S} →
Press #1 (ANALYZE) Press 1x to enter Enter 2nd wt of Sample

[NEXT]^{B} → [ENTER REMAINDER OF SAMPLES]^{S} → [START]^{B}
Enter 2nd Sample (Maximum sample number is 10) Runs samples

*Enter weight of sample.
**Press 1, 2 etc.times and input ID Code, i.e. "Oats" or other sample name.

APPENDIX H

CHILLED – MIRROR DEWPOINT DETERMINATION OF WATER ACTIVITY (a_w)

EQUIPMENT:
Chilled Mirror Dewpoint Machine
Disposable sample cups
Homogenizer or Food Processor

REAGENTS:
Salt solution Standards
Distilled water

PROCEDURE:
Instrument Calibration
Linear Offset is a means of checking the calibration of the instrument and can be checked by using standardized salt solutions and distilled water. Verification standards are specially prepared salt solutions that have a specific molality and water activity that is constant and accurately measurable. Performance Verification Standards come in three water activity levels listed below.

Verification Standard @ 20°C	Water Activity
0.5 m KCl	0.983 ± 0.001
6.0 m NaCl	0.760 ± 0.003
8.5 m LiCl	0.500 ± 0.003
13.3 m LiCl	0.250 ± 0.003

Linear offset should never be verified against distilled water, but should be tested with a standard near the water activity level to be measured.

Verification for Linear Offset

1. Choose a verification standard that is close to the a_w of the sample you are measuring. Make sure that your standard is at ambient temperature before you load it into the sample drawer, and that the AquaLab has warmed up at least 15 minutes.
2. Empty the whole vial of solution into a sample cup and place it carefully into the sample drawer.
3. Carefully slide the drawer closed, being especially careful that the solution doesn't splash or spill and contaminate the chamber.

4. Turn the drawer knob to the READ position to make an a_w reading. Make two readings. The readings should be within ± 0.003 of the given value for the salt solution.
5. If the reading is within 0.003 of the salt solution, prepare a sample cup half full of distilled water and make two readings. The first reading may be low. The second reading should be 1.000 ± 0.003. If the salt reading is correct and the distilled water is not, it is probably due to contamination of the sensor chamber. For cleaning instructions, see Chapter 10 in the instruction manual.
6. If you consistently get readings that are outside of the a_w of the salt solution standard by more than ± 0.003, a linear offset has probably occurred. See Chapter 5 in the instruction manual for adjusting the linear offset.

Sample Preparation:
Make sure that the sample to be measured is **homogeneous**. For meat products, this requires homogenization of the sample in a food processor to create a homogeneous mass. Samples should be stored in a moisture proof container to avoid loss of moisture.

Place the sample in a disposable sample cup, **completely covering** the bottom of the cup if possible. Samples that contain propylene glycol in concentrations >10% will not damage the instrument, but a_x values for consecutive samples will not be accurate. Propylene glycol condenses on the mirror during the reading, but does not evaporate from the mirror as water does.

Do **NOT** fill the sample cup **more than half full**. Overfilled cups will contaminate the sensors in the sensor chamber. Make sure the rim and the outside of the sample cup are clean.

If a sample will be read at some other time, put the sample cup's disposable lid on the cup to restrict water transfer.

Taking a_w Readings:
1. Prepare the sample as described previously and place in the plastic sample cup. ALLOW THE SAMPLE TO EQUILIBRATE TO ROOM TEMPERATURE.
2. Turn the sample drawer knob to the OPEN/LOAD position and pull the drawer open.
3. Place the sample cup with sample in the drawer. CHECK THE TOP LIP OF THE CUP TO MAKE SURE IT IS FREE FROM SAMPLE RESIDUE.
4. Carefully slide the drawer closed, being especially careful if you have a liquid sample that may splash or spill and contaminate the chamber.
5. Turn the sample drawer knob to the READ position to seal the sample cup with the chamber. Readings normally take 5 minutes. Some extremely dry samples, highly viscous samples may require up to 10 minutes or more to reach an accurate measurement of a_w.

6. Take the a_w reading directly from the screen.

CAUTIONS:

- Never leave a sample in the water activity meter after a reading has been taken. The sample may spill or contaminate the instrument's chamber.
- Never try to move the instrument after a sample has been loaded. Same reason as above.
- Take special care not to move the sample drawer too quickly when loading or unloading liquid samples.
- If a sample has a temperature that is 4^{o}C higher than the sample chamber, the instrument will display "Sample too hot". Warm samples cause condensation on the mirror surface.
- The operating temperature range of the instrument is 5^{o} to 43^{o}C.
- If a triangular warning symbol appears in the top right hand corner, this indicates that the mirror has become too dirty to give accurate measurements. Clean mirror.
- If a sample has a lower a_w than about 0.08, a display message will indicate that the a_w <0.078 at 24.7^{o}C.

APPENDIX I

CARVER PRESS WATER HOLDING CAPACITY

1. Remove a sheet of 9 cm Whatman #1 filter paper from a desiccator containing a saturated solution of KCl (RH = 80%).

2. Tare the filter paper on the scale.

3. Add 500 mg of meat sample to paper. Record weight of sample.

4. Place filter paper containing meat sample between two plexiglass plates.

5. Place plates on Carver Lab Press and press the sample at 500 psi for 1 minute.

6. Place a sheet of acetate paper over the pressed sample on the filter paper.

7. Trace, with a pencil, around the outer edge of the pressed meat sample and around the outer edge of the pressed moisture.

8. Measure the six inner circle diameters (cm) and six outer circle diameters (cm). Use average of the six diameters to calculate area of each circle.

9. Using a companion sample, determine the % moisture of the meat sample using the CEM or AOAC Air Drying Oven Method.

10. Calculate the % Free Water & % Bound Water.

$$\% \text{ Free Water} = \frac{[(\text{Total Surface Area} - \text{Meat Film Area}) \, 61.60 \times 100]}{\text{Total Moisture (mg) of meat sample}}$$

$$\% \text{ Bound Water} = 100 = \% \text{ Free Water}$$

APPENDIX J

BRADFORD PROTEIN SOLUBILITY DETERMINATION

1. Homogenize 6 g sample and 30 ml deionized water or PBS (PO_4 buffer, pH 7.0) for 30 sec on, 30 sec off, 30 sec on.
2. Weigh out 30 – 35 g of each sample and place each sample in a 50 ml polycarbonate centrifuge tube.
3. Centrifuge at 20,000 x g for 20-30 minutes at 2°C.
4. After centrifugation, leave centrifuge door open to evaporate the condensate and dry out the centrifuge.
5. Take 1 ml sample from the supernatant (below the fat layer and above the pellet) and store in a micro centrifuge tube as a reserve.
6. Allow the refrigerated Coomassie Plus (Bradford) Reagent to come to room temperature.
7. Mix just prior to use by gently inverting the bottle several times without shaking.
8. Dilute the sample if necessary (raw meat extract may require 1:10 dilution).
9. Add 50 µL of sample to a disposable 2 ml plastic micro centrifuge tube.
10. Add 1.0 ml of the straight Bradford Reagent (Coomassie Plus) to each standard tube, starting with the blank.
11. Read the standards at 595 nm (within 5 to 90 minutes after the reagent addition) on the spectrophotometer. Zero the spectrophotometer using the 0 standard.
12. Add 1.0 ml of the straight Bradford Reagent to each sample tube.
13. Read the samples at 595 nm (within 5 to 90 minutes after the reagent addition) on the spectrophotometer.

OPERATING THE BECKMAN COULTER CENTRIFUGE

1. Keep the rotor in the cooler (approx. 4°C). The rotor should be stored upside down to allow for drainage.
2. Turn on the centrifuge 5-10 minutes prior to use and set at 2°C.
3. Push Set/Actual button to “Set”.
4. Set “Rotor ID” to “JA25.50”.
5. Open door using foot pedal.
6. Match locking pins in the centrifuge with the rotor head.
7. Attach lid and tighten screw to seal.
8. Close the door.
9. Set “Speed” by pushing the button to “RCF”. Adjust to 20,000 x g.
10. Set “Time” to 20-30 minutes.
11. Set “Temperature” to 2-4°C.
12. Push “Start” button.
13. Observe centrifuge until it reaches the set speed.
14. “Accel” should be set a max, do not need to change.

15. Push “Stop” button if there is any problem.

APPENDIX K

Table Ballot used for sensory analysis of ham samples.

Sensory Ballot

Name _____ **Date** _____

Sample	Textures				Flavors									Tastes		Afterflavors			
	Hardness (1st Bite)	Springiness	Surface Wetness	Juiciness (1st Chew)	Hamy	Cured Fat	Mature Animal	Canned Meat	Smoke	Cardboard	Painty	Fishy	Other	Salt	Sweet	Cured Lean	Salt	Sweet	Other
W/U																			

Surface Wetness: Feel Surface of sample with lips and tongue. Amount of wetness or oiliness (moistness of both) on surface.

Dry to wet/oily/moist.

Springiness: Compress partially between molars without breaking and release. Degree of which sample returns to original shape after a certain time period.

No recovery to very springy.

Hardness (1st bite): Bite through sample with incisors. Forces require to bite through.

Very soft to very hard.

Juiciness (1st chew): Bite through sample with molars. Amount of wetness/juiciness released from sample.

None to very juicy.

APPENDIX L

WARNER-BRATZLER SHEAR TEXTURE ANALYSIS PROCEDURE

EQUIPMENT:
Warner-Bratzler shear device
Instron Universal Testing Machine (Model 1011, Canton, MA 02021)

The Warner-Bratzler shear device consists of a stainless steel blade 0.040 in. thick in which an equilateral triangle circumscribed around a 2.54 cm diameter circle has been cut and the edges rounded off to a radius of 0.02 in. This blade is attached to a load cell or force measuring device and pulled or pushed at 200 mm/min between two fixed stainless steel plates. Meat samples ranging from 0.5 to 1.0 inches (1.27 to 2.54 cm) in thickness are typically cored to yield cylinders 0.5 to 0.75 inches (1.27 to 1.91 cm) in diameter and parallel to the muscle fiber orientation. The cores are placed individually in the blade hole equidistant from front-to-rear of the cylinder and then sheared across the fiber (force is perpendicular to the muscle fiber orientation) by pulling or pushing the blade with the core between the two fixed plates. The Warner-Bratzler Shear device can be easily attached to an Instron Universal Testing Machine (Model 1011, Canton, MA 02021) with the appropriate load cell to record the force of the shear measurement.

PROCEDURE:

Sample Preparation:
Samples are cored parallel to the muscle fibers with a 1.27 cm diameter cork borer yielding cylinders of tissue with the fibers running lengthwise. After coring, samples are thawed to room temperature. Core samples are carefully positioned in the notch of the "V" shaped blade of the Warner-Bratzler shear.

WARNER-BRATZLER SHEAR USING THE UNITED TESTING SYSTEM, INC. MACHINE:

1. Turn on computer.
2. Turn on "ON" switch on shear machine.
3. Push the "START" button shear machine.
4. On computer desktop, double click Datum Software.
5. On opening screen, click "Template Manager" on wizard window. From the menu on the next screen, select "BIT SHEAR" and click "OK".
6. Back on the wizard, click "Specimen Preparation".
   On next screen, enter Operator - Client Name is optional.
   Click "Measurement" and enter "Specimen ID".
   Click in the "Specimen Area" and 1 should appear. Leave the 1 and click on "Batch Mode".
   Enter "6". Click "OK".
7. A plotting area should appear on the next screen.

Jog testing head/probe and make sure that the FORCE number changes (Check periodically).
Push "Z" to zero the testing head/probe – STRAIN number should zero.
Position core.
Push "T" twice (testing head/probe moves once you push "T" the second time).
Write down the FORCE number as your shear force on the broiling or cooking record form.
Push "O" for OK to refresh the plotting area.
Position core and repeat process again.

8. Once you've finished all 6 cores/sample, push "Q" for quit. That will bring you back to the wizard screen.
   Select "Data Report".
   Click "Report" on the next screen and from the menu select "Print Std. Test Report".
   Exit twice.
9. The wizard box should be on the screen again.
   Click "Specimen Preparation" and begin again at #6.

INSTRON UNIVERSAL TESTING MACHINE WITH WARNER-BRATZLER SHEAR

1. Place the meat sample through the hole in the W-B shear blade and center lengthwise. The shear blade should be positioned between the two parallel plates (anvils) and the shear blade hole located beneath or above the plates, depending upon the direction of the applied force.
2. Activate the Instron and move the blade at a rate of 200 mm/min forcing the meat sample into the V-frame of the triangle (hole) until it is sheared completely through.
3. Read the Instron recording device for the maximum force (kg or lbs) expressed by the load cell during the shearing procedure.
4. Record and calculate the average shear force value (lbs or kg).

APPENDIX M

SAMPLE PREPARATION PROCEDURE FOR SCANNING ELECTRON MICROSCOPE

EQUIPMENT:
Desiccator
20 ml Scintillation vials with caps
CD for picture storage
Hummer Sputtering System
Double sided carbon tape

REAGENTS:

Buffer:	Sodium phosphate, monobasic	1.16 g
	Sodium hydroxide	0.27 g
	Dilute to 100 ml with distilled water	

Trump's Fixative:

Sodium phosphate, monobasic	1.16 g
Sodium hydroxide	0.27 g
Deionized water	88 ml
40% Formaldehyde (Commercial formalin)	10 ml
50% Glutaraldehyde (Biological grade)	2 ml

Reference: McDowell, E.M. and Trump, B.F. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Lab. Med. 100:405-414.

1% OsO_4:
6 ml buffer + 2 ml 4% Os O_4

PROCEDURE:
Fixing

1. Cut meat sample into ½ cm cubes and place in labeled scintillation vial containing Trump's fixative.
2. The fixative should completely cover the samples.
3. Samples can be held in Trump's fixative until preservation for up to a year's time.

DAY 1 of Preserving (**Conduction**)

1. Wash samples with buffer by pouring off the Trump's fixative and immediately adding buffer to the vial. This will insure that the samples will not dry out. The used Trump's fixative should be placed in a waste beaker.
2. Microwave Set-up:
   a. The two vacuum lines behind the microwave should be opened by turning levers.
   b. Top left knob on mw oven should be set on "Vacuum Cycle".
   c. Lower left knobs set at minimum level (vacuum time & vent time).
   d. Press "Time Entry" (4^{th} button from bottom)
   e. Press "1", Press "0", Press "0", Press "Start"
   f. The wattage should be set at 250.
   g. A red light will turn on when the microwave is on and a green light will light when the microwave is off and ready to be used.
3. Place the vials in the microwave set at 20°C (37°C is temp limit). Vials should sit inside the portable vacuum chamber. Place lid on chamber, close the stop cock and slip other end of vacuum hose onto vacuum nozzle in mw oven.
4. Remove the vials from the microwave and pipette off the buffer. The used buffer should be placed in a waste beaker.
5. Repeat washes for second and third time by pulling off almost the entire buffer before adding more.
6. After the third wash, pipette off almost the entire buffer and add enough 1 % OsO_4 to cover all of the samples.
7. Screw the caps onto the vials and wrap in parafilm as a precaution.
8. Place the vials in the refrigerator overnight.
9. The OsO_4 preserves the samples and makes them conductive. The samples will turn black from the OsO_4. Everything black must be handled under the hood and never come into contact with bare skin.

DAY 2 of Preservation (**Dehydrating**)

1. Wash samples one time with deionized water by pipetting off the OsO_4 and adding enough water to cover the samples. The used OsO_4 should be placed in a waster beaker.
2. Set microwave to 20°C, 250 wattage. Place vials in microwave and run 1 min.
3. Pipette off the rinse water (place in waste beaker) and dehydrate the samples in 5% steps using the methanol/water combinations listed on the attached Dehydration Table. The waste beaker contents may be poured down the sink drain with copious amounts of water.
4. Never let the samples sit dry without being covered with fluid.
5. After pipetting off the methanol, pour the next dehydration wash on the sample before moving to the next sample.

6. After pipetting off the last methanol, add enough HMDS to the vial to completely cover the samples. Place cap tightly on vial, wrap caps with parafilm and allow sitting overnight under the hood.
7. The HMDS should be changed once, halfway through the night.

DAY 3 of Preservation (**Mounting/Sputter Coating**)

Mounting

1. Remove the vial caps and allow the HMDS to volatilize off under the hood.
2. Using a permanent marker, identify the sample by writing on the side of the aluminum stub.
3. Using a microscope, view the sample cubes and determine proper orientation for mounting.
4. Place a small piece of double sided carbon tape on a stub mount and, using tweezers, place the sample cube on the tape. If sample does not adhere to the carbon tape, a small amount of carbon glue can be placed on the carbon tape, and then adhere the sample. Additional time for drying is needed prior to sputter coating.

Sputter Coating (Gold/Palladium Coating)

1. Place stubs on the stage of the Hummer Sputtering System
2. Adjust the height of the stage by using the cardboard guide. The top of the samples should align with the top of the cardboard guide.
3. Assemble the remaining apparatus (clear cylinder tube and vacuum lid)
4. Knob Settings
   a. Voltage = zero
   b. Right Pressure knob = turn completely to “decrease”
   c. Left Pressure knob = closed
   d. Process Control = Auto
5. Turn on Argon gas supply
6. Turn on vacuum. Allow vacuum for 5 minutes
7. Press start on Process Control
8. Allow samples to be coating for approximately 15 minutes
9. Once complete, turn off process control and release vacuum
10. Remove samples from stage
11. Place stubs in desicator for storage

VITA

Name: Denise Gail Phillips

Address: Columbia Packing Company
2807 E Eleventh
Dallas, TX 75203

Email Address: nissiep@hotmail.com

Education: B.S., Biomedical Science, Texas A&M University, 2003
M.S., Animal Science, Texas A&M University, 2009