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Hydrocolloids in processed meat

by

Gitanjali Acharya Prabhu

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Departments:	Food Science and Human Nutrition;
	Animal Science
Co-majors:	Food Science and Technology;
	Meat Science
Major Professors:	Joseph G. Sebranek and Dennis G. Olson

Iowa State University Ames, Iowa

1996

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DEDICATION

To my children Krishna and Mythili

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GENERAL INTRODUCTION

Meat processors have responded to consumer demands for leaner, healthier food by changing product composition and carefully controlling fat levels. The functional properties of meat systems are primarily dependent on the interaction of the protein fraction with other components. These interactions have a direct impact on how well the meat system binds water, stabilizes fat, produces desirable textural properties and achieves cohesion. In products with high added water or reduced sodium chloride levels, however, the functionality of the traditional myosin heat set protein matrix may be limited due to low ionic strength, palatability may be compromised. Safety in low ionic strength systems may also become a concern if microbial inhibition is reduced.

Macromolecular hydrocolloids are commonly used by the food industry as texture modifying agents in many different types of products. They have a wide range of functional properties and are used for several different functions, including stabilization, thickening and structure formation (gelling agents). These functional properties are related in part to the ability to imbibe a large amount of water and interact with that water in solution. In order to select the best hydrocolloid for a meat application and to optimize its functionality in a meat system, it is necessary to thoroughly understand the specific properties of these ingredients when used in a meat system.

Hydrocolloid gelling agents like starch and carrageenan have been used by the meat industry as binders to improve water retention in meat, especially

when high volumes of water are added. When a large volume of water is added, the salt-soluble proteins extracted to the surface of the meat are diluted. As a consequence, the binding forces among meat pieces decrease and it also becomes more difficult to retain the added water.

Carrageenans and starches are added to meat products as binding agents. The functionality of carrageenan in meat products is related to its thermally reversible gelation properties. Carrageenans are known to improve water retention, consistency, sliceability and texture in meat products with high levels of added water. Starch has traditionally been used in meat products to improve quality and occasionally to extend the more expensive meat fraction of various products. The effect of starch addition is based on the ability of the starch to gelatinize when heated in a water-containing medium, thereby binding relatively large amounts of water.

Freezing and frozen storage can produce profound effects on the structural and chemical properties of muscle foods including changes in muscle fibers, lipids and proteins. All of these components have potential for significantly influencing the quality attributes of meat and meat products. Frozen storage of finely comminuted meat products requires particular attention due to fat oxidation and freezing-induced protein denaturation.

It is well known that frozen storage causes alterations in protein functionality. Total extractable proteins, sarcoplasmic proteins and myofibrillar proteins are adversely affected during frozen storage. Evidence indicates that as the quality of the protein and fat deteriorates during frozen

storage, meat products manufactured from the frozen meat gradually decrease in quality characteristics as well.

To overcome problems associated with the freezing or frozen storage of meat, attempts have been made to study the mechanism and causes of change in functional properties of meat proteins. Meat proteins are known to exhibit significantly better retention of functional properties if the meat is frozen with cryoprotectants. The most commonly used cryoprotectants in the food industry have been low-molecular weight sugars and polyols such as sucrose and sorbitol which are used for surimi manufacture. Studies have also shown the effectiveness of high-molecular weight carbohydrates, such as starch hydrolysis products, as cryoprotectants of functional properties during long term frozen storage of meat.

The research presented in this dissertation had three objectives. First, to evaluate the effects of hydrocolloids such as starch and carrageenans on the quality characteristics of hams containing high levels of added water. Second, to investigate the cryoprotectant effects of hydrocolloids such as maltodextrins and corn syrup solids for freeze-thaw stability in mechanically deboned pork. Finally, to study the quality and sensory characteristics of pork sausage made from mechanically deboned pork containing these cryoprotectants.

Dissertation organization

This dissertation consists of a general introduction, a general review of the literature, three papers to be submitted for publication and a general summary. Each individual paper consists of an abstract, introduction, materials and methods, results and discussion, conclusions and references.

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LITERATURE REVIEW

Hydrocolloids

Hydrocolloids or hydrophilic colloids are substances that have the ability to thicken or gel aqueous systems (Graham, 1977; Glicksman, 1979). These materials can be obtained from extracts of plants or seaweed, flours of seeds or grains, gummy slimes from fermentation processes and many other natural products (Glicksman, 1982-83). While most hydrocolloids thicken and impart viscosity to aqueous solutions, a few of them have another major property of being able to form gels. Gelation is the phenomenon involving the association or cross-linking of polymer chains to form a three-dimensional continuous network which traps or immobilizes the water. The result of this network is a firm, rigid structure that is resistant to flow under pressure (Glicksman, 1982-83). The degree of thickening varies, with a few hydrocolloids giving low viscosities at fairly high concentrations but most giving high viscosities at very low concentrations, usually less than 1%.

The useful properties of hydrocolloids are due largely to physical effects, primarily those dealing with their interaction with water. Although hydrocolloids may interact with proteins, lipids and other environmental molecules commonly found in food, they must primarily and continuously interact with water molecules. By such interactions, hydrocolloids perform useful functions which include viscosity, solution stability, emulsifying action, suspendability and gelation (Danishefsky et al., 1970). All food-grade hydrocolloids are basically derived from the following: exudates, extracts, flours, fermentation or biosynthesis, chemical modification and chemical synthesis (Glicksman, 1982-83). Sources include starches (raw, pregelatinized, modified), cellulose and cellulose derivatives, seaweed extracts (alginates, carrageenans, agar and furcellaran), plant exudates or gums (arabic, karaya and tragacanth), seed gums (locust bean, guar), plant extracts (pectins) and microbial gums (xanthan) (Sanderson, 1981; Glicksman, 1979)

Hydrocolloids are used by the meat industry as water-binding and texture-modifying agents in many different types of meat products but especially in low-fat processed meats (Shand et al., 1990). Addition of hydrocolloids to low-fat meat products improves water-retention, consistency, sliceability and texture. Hydrocolloid gelling agents, like carrageenan and starch, have been successfully used in hams and emulsion-style meat products as water-binding agents. The hydrocolloids seem to be a cost effective means of replacing a significant portion of the fat in meat products (Geise, 1992).

Another important function of hydrocolloids in meat may be cryoprotection. Cryoprotection is the process of protecting meat from freezing damage (Reid, 1993). Cryoprotectants are added to meat prior to freezing to ensure long term storage stability of proteins during frozen storage. This, in turn, assures good functionality of the proteins in the manufacture of meat products, expressed primarily as gel forming potential with its manifestations of texture formation and water-binding properties (MacDonald and Lanier, 1991). Protective treatments using high molecular weight hydrocolloids have

been suggested as cryoprotectants in meat in order to maintain stability of muscle proteins during frozen storage (Lanier and Akahane, 1986; Park et al., 1987 a, b).

Starch

Starch is the most commonly used food hydrocolloid and occurs widely in nature. Starches are carbohydrate polymers of glucose and usually contain amylose and amylopectin components. Amylose is a linear, straight-chain α -1,4 glucopyranose polymer; amylopectin, on the other hand, is a randombranched configuration of α -1,4 glucopyranose which has periodic branching of side chains with α -1,6 linkages (Wurzburg, 1986). Granule size, shape and size distribution as well as amylose/amylopectin ratio of a starch varies with botanical source (potato, corn, wheat, tapioca, rice) (Oakenfull, 1987).

Gelling properties of starches are dependent upon the amylose component which, because of its structure, can form hydrogen bonds with neighboring molecules to build up a three-dimensional network. The highly branched structure of amylopectin does not allow close physical association between molecules and this prevents gelation (Shand et al., 1990). During starch gelatinization, a number of changes take place simultaneously or successively, which include granule swelling, disruption of the crystalline regions, loss of birefringence, increase in viscosity and possible fragmentation of the granule (Wu et al., 1985). Starch gelatinization can be influenced by water-to-starch ratios, lipid, sucrose, salt content and processing conditions (Lund, 1984).

Several types of starches are available for use in meat products. They originate from various plant sources: grains (wheat, corn), tubers (potatoes) and roots (tapioca) (Skrede, 1989). A number of starches available today are physically and/or chemically modified (Luallen, 1985; Langan, 1986). Pregelatinization of starches renders swelling in cold water and involves simultaneous cooking and drying procedures. Starches may also undergo a variety of chemical modifications to alter their functional properties, for example, to improve freeze-thaw stability, reduce syneresis and/or to enable the starch to withstand high shear or heat conditions (Langan, 1986).

Starches are often added to emulsion-style meat products and are popular not only for their functional properties but also for their low cost relative to alternatives (Shand, 1990). Starches help stabilize meat batters by absorbing or binding excess water, thus improving cooking yields. However, starches do not participate in the actual emulsifying process or improve water binding of the meat itself (Schut, 1976). The use of these binders thus enables the producer to add more water than the meat emulsion itself could hold.

The advantages of using food starches in low-fat meat products are their low cost, familiar ingredient technology and positive acceptance by consumers (Berry, 1991). They seem to offer the most cost effective means of replacing a significant portion of fat in meat products and duplicating the textural and sensory characteristics of animal fat (Keeton, 1991).

Odio (1989) evaluated ten starches or maltodextrins from rice, tapioca corn, peas, potatoes and oats. The author reported that modified waxy maize starch, tapioca dextrin and rice flour have potential when added at 2.5-5% levels to frankfurters containing 9% or 15% fat. Low-fat franks containing modified waxy maize starch, tapioca dextrin and rice flour had similar flavor and texture profiles except for a slightly detectable starch flavor at the 9% level. Cooking yields were deceased by 1% to 2% for modified waxy maize starch and rice flour and 5% for the tapioca dextrin.

Potato starch swells at a faster rate than the other starches, and in highly extended systems, may give most stable homogenates (Comer, 1979). However, under retort conditions, potato starch granules rupture more rapidly and consequently produce softer products than other native starches. Skrede (1989), evaluated cooked Norwegian sausages containing 21.1% fat and 4% levels of potato flour, modified potato starch and wheat, corn or tapioca starch. The author found potato starch to be the best suited starch and tapioca to be the least after yield and sensory determinations were made.

In another study, Berry and Wergin (1990) evaluated the replacement of lean with an 8% modified pregelatinized potato starch gel (3% starch, 5% water) in 4% and 20% fat beef patties. The gel-treated patties had lower sensory flavor and juiciness scores, higher tenderness scores and improved cooking yields of 4% to 6%. Starch addition (2%) has been reported to improve the surface appearance, sliceability and texture of sectioned, cooked ham containing 80% added water (Trudso, 1985).

On January 31, 1992, the USDA Food Safety and Inspection Service proposed the use of modified food starch in hams at a level not to exceed 2% of the product formulation to prevent purging of the pumped brine solution from the products (USDA, 1992a). Modified food starch has been approved for use in cured pork products labeled as "ham-water added" or "ham and water product" where X% of the weight is added ingredients.

Carrageenans

Carrageenans are hydrocolloids extracted from red seaweed of the class *Rhodophyceae* and are sulfated linear polysaccharides of D-galactose and 3,6anhydro-D-galactose (Oakenfull, 1987). Three main carrageenan fractions have been defined: kappa, iota and lambda. They differ chemically in their content of 3,6-anhydro-D-galactose and the number and position of ester sulfate group (Glicksman, 1982-83).

Kappa-carrageenan is formed of alternating 1,3 linked D-galactose 4sulfate and 1,4 linked 3,6-anhydro-D-galactose units. It is soluble in hot water (65°C) and forms strong and brittle thermoreversible gels in the presence of calcium ions (Glicksman, 1982-83), and elastic gels in the presence of potassium ions. Iota-carrageenan is formed of alternating 1,3-linked Dgalactose 4-sulfate and 1,4 linked 3,6-anhydro-D-galactose. It is soluble in hot water (50°C) and forms elastic gels in the presence of calcium cations. Lambda is a non-gelling carrageenan and is formed of units of 1,3 linked Dgalactose 2-sulfate and 1,4 linked D-galactose 2,6 disulfate. It is cold water soluble (Glicksman, 1982-83). In the presence of potassium ions, lambdacarrageenan does not precipitate.

Carrageenans can perform several different functions in meat products. They may be used either for gelation of broth surrounding meat or of liquid contained in meat (Shand et al., 1990). Carrageenans can be used as a brine ingredient and introduced into meat products either by massaging or by multineedle injection. For brine preparation they are normally incorporated into the brine after dissolution of the salt and phosphate (Shand et al., 1990). The federal meat inspection regulations permit the use of carrageenans as extenders and stabilizers in meat products (USDA, 1992b). The amount of carrageenan used may not exceed 1.5% of the product formulation in ham, and is not permitted in combination with other binders (such as starch) approved for these meat products (USDA, 1992a).

Carrageenan has been evaluated as a water-binding agent in cooked ham (Anonymous, 1984) and other processed meat. Kappa-carrageenan, a preferred binding agent in ham, resulted in products with good water binding during storage, good sliceability, decreased cooking losses and improved texture (Anonymous, 1984). Bater et al. (1992a) studied kappa-carrageenan effects on the gelation properties of oven-roasted turkey breast juice. They found that combining the protein contained in the juice with carrageenan resulted in a significant synergistic increase in gel strength, interpreted by authors as a consequence of protein/carrageenan interaction. The same authors (Bater et al., 1992b) reported that addition of 0.5% kappa-carrageenan

to oven-roasted turkey breasts manufactured with 70% added brine resulted in better yield, sliceability, water retention and higher rigidity.

The mechanism by which carrageenans might interact with meat proteins is not yet known. Carrageenan particles bind water between 46°C and 60°C during heating and gel at 44°C upon cooling. Meat proteins start to coagulate at 52°C and are completely gelled at 67°C. The overlap of these two temperatures may explain the efficiency of carrageenans in meat (Bater et al., 1992a). Carrageenan molecules could interact directly with positively charged groups on protein surfaces or indirectly with negatively charged proteins through cation bridges. Upon protein denaturation, the number of sites on proteins increases as well as the flexibility of the random coil, maximizing potential protein-polysaccharide interaction (Carr, 1993).

The current consumer trends towards low-fat meat products has prompted the food industry to develop low-calorie foods with acceptable sensory characteristics (Brewer et al., 1992). This can often be done by addition of water. In cooked sausages, such as frankfurters and bologna, decreasing fat content to 10% by water addition causes lower cook yield, increased purge and decreased firmness. Also, the added water may affect the microbial shelf-life of the product, as well as flavor (Keeton, 1991).

Polysaccharide gums are often used to hold water and enhance texture properties of low-fat meat products. Barbut and Mittal (1992) used kappacarrageenan, iota-carrageenan and xanthan gum (0.5%) in reduced-fat breakfast sausage. In their study, kappa-carrageenan was considered the best gum for a low-fat product resulting in good texture and juiciness. Foegeding

and Ramsey (1986) added iota-carrageenan, kappa-carrageenan and other gums to low-fat, water-added meat batters. They concluded that kappa- and iota-carrageenans helped retain water, and kappa increased hardness of the product. In a later study, the same authors (Foegeding and Ramsey, 1987), evaluated the textural and water-holding properties of gelled meat batters containing iota-carrageenan, kappa-carrageenan or xanthan gum (0.5% or 1%). Iota-carrageenan was the most effective in increasing force-to-fracture, true shear strain, and water-holding ability. On the other hand, kappacarrageenan was more effective than iota in increasing hardness and was equivalent to iota in affecting true shear stress.

Shand et al. (1993) studied the effect of two levels of kappa-carrageenan (0.5% and 1%) and three levels of NaCl (1-3%) on the yield of restructured beef rolls with 33% added water. The addition of kappa-carrageenan improved cook yield and textural properties at all levels of NaCl. Furthermore, reduction of salt to 1% and incorporation of carrageenan resulted in products with better characteristics than controls. These researchers, therefore, suggested the use of kappa-carrageenan to improve the overall characteristics of low-salt beef products.

Maltodextrins and corn syrup solids

The Food and Drug Administration (FDA) defines maltodextrins $(C_6H_{10}O_5)_n \bullet H_2O$, CAS Reg. No. 9050-36-6, as non-sweet, nutritive saccharide polymers that consist of D-glucose units linked primarily by α -1-4 bonds, having a DE less than 20 (21 CFR 184.1444). They are prepared as a white

powder or concentrated solution by partial hydrolysis of corn starch with acid/enzyme hydrolysis (Anonymous, 1993). Starch conversion products are classified by their dextrose equivalent (DE). This is a measure of the reducing sugar content calculated as anhydrous dextrose and expressed as a percentage of total dry substance.

Corn syrup solids are obtained by starch depolymerization in an acid/enzyme conversion process that is halted as soon as the desired composition is reached. Corn syrup solids $(C_6H_{10}O_5)_n \cdot H_2O$, CAS Reg. No. 68131-37-3, are defined by the FDA as dried glucose syrup (21 CFR 168.121) in which the reducing sugar content is 20 DE or higher (Anonymous, 1993). They are classified into 4 types based on their DE: Type I (20 DE up to 38 DE), Type II (38 DE up to 55 DE), Type III (55 DE up to 73 DE), and Type IV (73 DE and above). In general, Type I syrups and maltodextrins have a relatively small concentrations of low molecular weight sugars.

Maltodextrins and corn syrup solids have long polysaccharide structures and act similar to starch when used in meat products. However, it has been suggested that they result in a product with a cleaner flavor and mouthfeel compared to starch (Shand et al., 1990). Maltodextrins are good water-binding and fat-binding agents and may be used up to a level of 3.5% in the finished meat products (Giese, 1992). Addition of these binders to meat formulations can improve cooking yields, slicing characteristics, and flavor (Schmidt, 1986). They may also reduce the cost of the finished meat product by replacing some of the more expensive meat fraction. However, these compounds that have dextrose, can contribute to non-enzymatic browning in meat products, and thus maltodextrins with low DE have often been used as fat substitutes (Inglett and Grisamore, 1991). An oat-based maltodextrin (oat trim) has been developed which forms a fat-like gel with less than one calorie per gram.

Maltodextrins and corn syrup solids may have a potential to serve as cryoprotectants in meat products. In order to maximize functionality of frozen meat for use a ingredient in processed meat products, some means of preventing or inhibiting freeze-induced denaturation is needed (Park et al., 1987 a, b). High molecular weight carbohydrates when included prior to freezing have potential to impart long-term stability to the proteins during frozen storage (MacDonald and Lanier, 1991).

Technology of freezing meat and meat products

Fundamental physicochemical aspects of freezing

Freezing implies two linked processes: lowering the temperature and changing the phase from liquid to solid. Both these processes are accompanied by a reduction of heat energy of the material. The first process, lowering of temperature will tend to reduce rates of chemical change, and hence in the absence of special factors might be expected to enhance the storage life of a product (Reid, 1993). There are two important aspects of the transformation of water to ice - thermodynamic factors that define the positions of equilibrium of compounds and kinetic factors that describe the rates at which these equilibria may be approached (Reid, 1993). These factors may also influence chemical changes in the products in which they occur.

Thermodynamics of water to ice transformation

When heat is removed from a system, if there is no phase change, the temperature of change may be described by a relationship of the form:

$$\Delta T = Q/C$$

where ΔT is temperature change, Q is amount of heat removed and C is the heat capacity or specific heat (Atkins, 1982). When a phase change occurs, a further change in heat content can take place without accompanying change in temperature. The heat associated with this phenomena is known as latent heat.

It is useful to employ phase diagrams to describe the changing composition of the constituent phases as a function of temperature (Reid, 1983). Even in a simple binary system as illustrated in Fig. 1, several types of behavior are possible. The first (Fig. 1A) illustrates the crystallization of both ice and solute, and shows a eutectic system. The second (Fig. 1B) illustrates a system where solute crystallization does not occur. This produces a glasscontaining system which can be termed a state diagram as it does not illustrate an equilibrium system.

Kinetic factors in the water to ice transformation

Nucleation For a liquid-solid transformation to take place, it is necessary for there to be a seed upon which the solid phase can grow (Reid, 1993). These seeds are termed as nuclei. Without this seed, growth is not





Fig. 1 Temperature concentration phase relationships: (A) a binary system forming a eutectic mixture; and (B) a system in which solute crystallization is kinetically constrained (Reid, 1983). possible as the molecules in the liquid phase do not easily align into the configurations required for the solids. At any temperature below the equilibrium melting point, any volume of the solid phase will have a lower volume of free energy than the same molecules in the liquid phase (Chalmers, 1964). However, the clusters of molecules will have an interface with the remaining liquid and the formation of this interface is thermodynamically unfavorable.

Crystal growth becomes possible once nucleation takes place. The rate of crystal growth is controlled in part by the rate of heat removal and also the direction of heat removal (Reid, 1983). The interaction of nucleation and crystal growth has a result on the size of the ice crystal formed, at least during initial freezing. Rapid freezing tends to produce small ice crystals because the propagation rate of the initial ice crystals is insufficient to keep pace with the rate of heat withdrawal. During slow freezing, the propagation of ice can keep better pace with the withdrawal of heat resulting in fewer nuclei, hence fewer but larger ice crystals are formed (Reid, 1983).

During storage even at constant temperatures, ripening or maturation occurs in which smaller ice crystals decrease in size while larger ice crystals grow in size. With time, the number of ice crystals decreases and their average size increases - in part reversing the initial effects produced by rapid freezing (Reid, 1983).

Glasses Once nucleation and crystal growth takes place, an approach can be made to achieve thermodynamic equilibrium. However, this approach

may be subject to another constraint. The concept of "glass dynamics" seeks to explain some of the observations relating to the differential temperature stability of frozen foods in terms of the physical properties of the unfrozen matrix between the ice crystals within the frozen food (Levine and Slade, 1988a). In Fig. 1B, as ice is formed from the liquid phase, the concentration of the liquid phase increases causing an increase in viscosity of this phase. As the temperature is reduced, more ice tends to form and the concentration of the unfrozen medium increases (Reid, 1990). Provided that the solute does not crystallize, continued separation of ice can lead to a state where the liquid phase has very restricted mobility and it is no longer possible for ice to crystallize. Both increasing concentration and decreasing temperature result in increasing viscosity so this phase from which no more ice can crystallize will have the characteristics of a glass. The glass reached by this route of freeze concentration can be termed as "maximally freeze-concentrated glass" (Franks 1982; Levine and Slade, 1988a) and the temperature at which it is attained (T_g'), has great significance for the stability of frozen foods because below this temperature, change might be expected to be slow and diffusion rate will be very slow. However, in this subfreezing region, the properties of the matrix may better be described as rubbery and the kinetics would be expected to follow the relationship described by William-Landel-Ferry (WLF) equation rather than Arrhenius equation (Reid, 1990). This has an important result that the rate of change is expected to increase as an exponential function of the difference between the system's temperature and its T_{g} ', rather than depend on an exponential function of the absolute temperature of the system. Manipulation of the T_g' might be expected to have dramatic effects on reaction

kinetics. Below the melting point, but above the T_g ', the reaction rates might vary with the difference between the storage temperature and T_g ' rather than with the reciprocal temperature (Levine and Slade, 1988a, b, 1991). Thus knowledge of T_g ' for different tissues might be important to an understanding of relative stability of frozen tissues in frozen storage (Reid, 1990).

Effect of freezing and freezing rate on muscle tissue

Animal cells do not possess a structurally strong cell wall. Characteristically, the animal cell membrane has a greater hydraulic permeability than many plant cell-walls. The animal membrane is however much less effective in preventing propagation of ice and so internal freezing of animal cells is more common than plant cells (Reid, 1993).

Freezer damage refers to irreversible changes in tissues brought about by the freezing process and manifested after the thawing of the tissue (Reid, 1993). Freezer damage is a consequence of four contributory processes, namely, chill damage, solute-concentration damage, dehydration damage and damage from ice crystals (Mazur, 1966). Of these, the most important contributor to freezing damage of animal tissues is solute-concentration damage. Due to the formation of ice, the concentration of solutes in the unfrozen medium increases (Fig. 1). In particular, ionic concentrations increase and ionic strength goes up resulting in protein denaturation (Reid, 1993). Charged molecules will have interactions modified by the increasing ionic strength of the medium. Aggregation and precipitation of such molecules are likely to occur. As a result of increased solute concentrations in the unfrozen medium, there may also be osmotic transfer of water from the cells interior to the exterior environment. The cell interior will thus dehydrate and shrink in volume (Mazur, 1966). Many of the structures within the cell may have to change configuration to accommodate this decrease.

In raw meat, owing to the solutes present in the water phase, freezing starts at approximately -2°C (28°F). At -5°C (23°F) approximately 80% of the freezable water is frozen and at -30°C (-22°F), the figure rises to 90% as shown in Fig. 2 (Desrosier, 1970).

Under controlled conditions, it has been demonstrated that in the freezing of thin sections of beef, supercooling occurs first followed by an increase in temperature of the cooled section to the actual freezing point when the change in phase occurs. Supercooling is a phenomenon in which the temperature of the solute is lower than the temperature needed for ice crystal formation. This change in phase continues, provided a temperature differential is maintained, until the free water becomes ice. Fig. 2 shows supercooling, the latent heat pause and the progressive decrease in freezing point as the concentration of dissolved solids increases. The degree of the ice crystal formation may be estimated from the slope of the cooling curve once freezing begins (Desrosier, 1970).

The rate at which meat is frozen can have a great influence on the quality of the meat after thawing - particularly the amount of drip loss. The rate of ice crystallization and the size of the crystals formed depends on the mass of the meat, freezing method, the freezing conditions and the packaging (Marsden and Hendrickson, 1993; Offer and Knight, 1988).



Freezing time (minutes)

Fig. 2: Freezing curve of a thin section of beef showing the period of ice crystallization and percentage of frozen water (Desrosier, 1970).

There are several advantages of rapid freezing rates: production of uniformly dispersed small ice crystals upon freezing, less fluid loss during freezing and upon thawing and less chemical deterioration (Love, 1968; Luyet 1968; Fennema, 1968; Rasmussen, 1976; Menegalli and Calvelo, 1979).

If animal tissue is frozen rapidly, the cellular fluids retain their location and freeze as tiny crystals uniformly distributed throughout the tissue (Marsden and Hendrickson, 1993). With faster transition from 0-5°C (i.e. shorter the latent heat pause), less translocation of water occurs during freezing (Lawrie, 1985; Powrie, 1973; Fennema, 1977; Nusbaum et al., 1983; Bevilacqua and Zaritzky, 1980). A study by Anon and Calvelo (1980) confirmed that drip loss is reduced by shortening the freezing period, although Bechtel (1986), reported that protein in the drip was unaffected by the freezing rate. Jalang et al. (1987) found that regardless of the species, frozen muscle at -19°C provided more expressible (press) fluid than fresh muscle, although pork had more press fluid than beef or lamb. Wagner and Anon (1986), explained the effect of freezing rate on protein denaturation by noting that myofibrillar proteins denature in two stages, with an initial rapid reaction followed by a slower, second stage, with the myosin molecule continuing to denature with time, leading to an overall decrease in viscosity and solubility.

Menegalli and Calvelo (1979) and Mascheroni and Calvelo (1980) demonstrated differences in ice crystal nucleation in beef due to different rates of supercooling (as might be determined by product mass). These authors discussed the freezing rates and related this to increased thaw exudate produced in meat subjected to slower rates. Penny (1974) also studied the freezing effects on drip losses and found slightly less drip at low temperatures (faster rate). He emphasized, however, that many factors will influence thaw exudate (pH, size of the muscle, surface area of meat, thawing conditions etc.) and that these must be standardized before comparisons between studies may be made. The same would be true for other quality changes, such as tenderness, flavor, juiciness or texture.

It is well documented that slow freezing results in more thaw drip and less expressible juice than quick freezing (Kahn and Lentz, 1977; Skenderovic

and Rankov, 1977; Nusbaum, 1979a). Slow freezing causes fluid in the extracellular spaces to freeze first, thus increasing the concentration of solutes and drawing water osmotically from the still-unfrozen cell through the semipermeable cellular membrane (Marsden and Hendrickson, 1993). There is, therefore, extensive translocation of the tissue fluid such that the fibers appear shrunken and, in some cases, damaged through rupture of myofibrillar walls.

Slow freezing rates produce greater damage to the mitochondria in the cells than fast freezing, and consequently there is a greater release of enzymes (Hamm and Gottesmann, 1984). These workers found that the quantity of enzymes released from muscle frozen post-rigor increased substantially when meat was frozen in a -10°C or -20°C environment but that little was released when the temperature of the environment was between -40°C and -80°C. Hamm (1979) postulated that membrane damage (enzyme release) was caused by dehydration at low temperatures rather than by structural disruption from ice crystals.

Perhaps the greatest interest in the red meat area has been in ground beef patties because of the large volume of ground beef consumed in the U.S. Sebranek et al. (1978) found improved palatability, decreased freezing shrink and decreased cooking shrink in patties frozen either with liquid nitrogen or carbon dioxide as compared with slow-frozen patties. The quality differences observed seem related to structural rather than to chemical alterations in the tissue (Sebranek et al., 1979; Nusbaum, 1979a) and led to a follow-up study investigating several freezing rates for their effect on patty structure and palatability (Nusbaum, 1979b). Palatability declined with increased freezing

time, showing the largest drop between 80 and 100-min. freezing rates. Thus, the effects of freezing rates on ground-beef patties seem to represent gradual transition of product alterations rather than a sharp "critical" freezing rate with sudden product changes (Sebranek, 1982).

Effect of frozen storage on meat quality

The quality of a frozen meat product involves several aspects: microbial, nutritional and organoleptic quality, the latter including color, tenderness, juiciness and flavor (Daudin, 1992). While the microbial and nutritional qualities as well of quantity of exudate upon thawing can be easily measured by objective methods, the organoleptic qualities (with the exception of color) need to be measured by human sensory panels.

During freezing, the cellular integrity of a portion of the microbial population is impaired. Slow freezing flavors the destructive action of microorganisms since the increase in ionic strength of the liquid phase (which is considerable between -2°C and -7°C) is the likely cause of the denaturation reactions affecting the proteins of the cellular membranes and enzymes (Daudin, 1992).

The nutritional quality of meat is relatively stable to freezing (Jul, 1984). The extent of protein denaturation does not have any significant negative effects on the nutritional quality of meat. Vitamins (especially the B vitamins) and minerals such as iron seem to be unaffected by the freezing process.

The eating quality of frozen meat is influenced both by the amount of drip loss and the temperature of storage -especially if the temperature fluctuates (Marsden and Hendrickson, 1993). The latter effect can lead to ice crystal growth in the extracellular spaces and damage to proteins within muscle fibers; storage temperature also has a marked effect on the rate of chemical reaction such as oxidative rancidity. Oxidative rancidity plays an important role in determining the shelf-life of frozen meat especially mechanically deboned meat. Due to the comminuted nature of mechanically deboned meat, this product is prone to rapid quality deterioration if not frozen properly (Mast et al., 1979).

Drip loss is not only disadvantageous economically but can give rise to an unpleasant appearance. Furthermore, the free moisture is a medium for potential bacterial spoilage, and also represents losses of nutrients such as vitamins, proteins and minerals which are dissolved in the exudate (Marsden and Hendrickson, 1993).

The influence of freezing rate on the tenderness of meat was studied by Berry (1983). The author concluded that meat tenderness was independent of the rate of freezing. However, freezing of prerigor meat while the muscle still contains ATP can give rise to very severe contraction upon thawing called thaw rigor. This can result in toughening as well as substantial loss of fluid (Locker, 1985). Cold shortening can also occur in the course of rapid freezing of muscle before the onset of rigor mortis. Hence, it is preferable to freeze meat containing negligible amount of ATP.

The key control points that determine the quality of frozen meat and meat products are the intrinsic quality of the raw materials, the freezing conditions used, the protection and storage of the product throughout the cold chain and how the meat is packaged (Marsden and Hendrickson, 1993; Offer and Knight, 1988).

Freezing of meat and meat products continues to be recognized in general as an excellent method of preservation which can result in very little changes in food quality or nutritive value, and potentially offers the consumer food very similar in quality to that when fresh. However, if done improperly or if raw meat which is particularly susceptible to damage is used, considerable freezing damage may occur.

Cryoprotection

Cryoprotection is the process of protecting material from freezing damage (Reid, 1993). Muscle tissue structure is affected to a greater extent by freezing and frozen storage than are its chemical properties (Uijttenboogaart et al., 1993). This is mainly due to the crystallization of water in the muscle tissue; and to the size and location of the ice crystals produced (Sebranek, 1982). Investigation of the protective action of chemical agents against damage caused by freezing and thawing have been carried out on a variety of biological systems (Smith, 1961). Some cryoprotectants, particularly sugars and polyalcohols, are useful for both food proteins and living cells, suggesting that the mechanism of freezing injury and cryoprotection might be similar for both (Fennema, 1973, 1982).
Red meat and poultry muscles are commonly stored frozen without cryoprotective additives. This is because red meats and poultry are stored in the whole-muscle form, in which reactive components of the muscle are more compartmentalized from one another (MacDonald and Lanier, 1991). Despite their stability, mammalian and poultry meats may suffer substantial losses in functionality during frozen storage. Inclusion of a cryoprotective component prior to freezing would ensure long term stability of the proteins in frozen storage. This, in turn, would assure good functionality of the material for manufacturing characteristics such as gel-forming potential with its manifestations of texture formation and water-binding properties (MacDonald and Lanier, 1991).

Muscle proteins express maximum functionality when the salt-soluble proteins are fully extracted (solubilized) and cryoprotection is only possible when intimate association of the cryoprotectant and the protein molecules occurs (MacDonald and Lanier, 1991). Thus, both the expression and cryoprotection of muscle protein functionality will optimally occur in a minced or comminuted muscle system. One such example where freezing and frozen storage are critical, is mechanically deboned meat.

In finely comminuted meat products, the occurrence of fat oxidation and freezing-induced protein denaturation may markedly reduce some functional properties (Uijttenboogaart et al., 1993). Niewiarowicz et al. (1990, 1991) found that enhanced oxidative changes occurring in mechanically deboned poultry meat during frozen storage caused a marked deterioration in the functional properties of proteins.

Among the various muscle proteins, myofibrillar proteins such as myosin have shown to be more susceptible to freeze damage, while sarcoplasmic proteins such as globulin, and stromal proteins such as collagen and elastin, are not affected significantly by freezing (Park, 1985). During frozen storage, it might be expected that the conformation of myofibrillar proteins would change, that proteins would interact to form insoluble complexes, and that the enzymatic activity of myosin and sarcoplasmic proteins would be reduced.

Connell (1960, 1963) suggested that toughening of frozen fish meat was caused by the denaturation of actomyosin, of which denaturation is attributed to myosin and not actin. Akahane (1982) confirmed that the denaturation of carp myosin was not only due to aggregation but also conformational changes during frozen storage.

Alterations in myofibrillar proteins and their functionality has been observed in frozen muscle and isolated protein systems as indicated by changed protein solubility (Connell, 1962; Partmann, 1980; Park et al., 1987a, b) and decreased gel-forming ability (Shenouda, 1980; Kim et al; 1986; Park et al., 1987a).

Fennema (1982) noted that all proteins would be expected to have optimum stability at a temperature just above the freezing point of water. At higher temperatures, thermal destabilization would ensue as a result of destabilization of hydrogen bonds and increased molecular motion. At lower temperatures, despite enhanced intramolecular hydrogen bonding, hydrophobic interaction would weaken to the point that a net destabilization

may be expected to occur. The optimum temperature of stability might actually be below the freezing point, but the onset of ice crystal formation could induce changes in the protein environment that effect denaturation.

Cryoprotective additives

Noguchi (1974) surveyed a wide variety of chemical compounds for their ability to maintain the solubility of carp actomyosin in dilute solutions over brief periods of frozen storage. A variety of carbohydrate compounds, including most of the mono- and disaccharides evaluated, and several lowmolecular-weight polyols, many amino acids and carboxylic acids were found to be cryoprotective. Nucleotides (ATP, ADP, and IMP) have been shown to exert a protective effect on fish actomyosin stored at -20°C (Jhiang et al., 1987a, b). Watanabe et al. (1988) demonstrated the cryoprotective ability of certain surfactants, particularly certain polyoxyethylene sorbitan esters and sucrose esters, in preventing loss of gel-forming ability in surimi.

Sucrose and/or sorbitol, typically alone or mixed 1:1 and added at 8% w/w to leached fish muscle, serves as the primary cryoprotectant commercially used in manufacture of surimi from Alaskan pollack. Polyphosphate (0.2-0.3%) was also added as a synergist (Park et al., 1988). Lanier and Akahane (1986) compared the effectiveness of polydextrose with that of sucrose-sorbitol and a 10 DE maltodextrin in maintaining the saltsolubility and gel-forming properties of Alaskan pollack surimi. While the three additives maintained similar high levels of solubility of the myofibrillar proteins at -28°C over several months compared to a control, the surimi

containing the 10 DE maltodextrin failed to form cohesive gels. These results were interpreted to mean that the 10 DE maltodextrin interfered with the gelation of the surimi myofibrillar protein in the same way, similar to what occurs with pregelatinized starch and certain gums (Lim et al 1990; Foegeding and Ramsey, 1986, 1987). Similarly, Park et al. (1990, 1987a, b) showed that polydextrose was equivalent to sucrose-sorbitol mixture in cryoprotecting beef actomyosin during frozen storage. While the addition of either the sucrosesorbitol mixture or polydextrose resulted in higher salt-solubility and gelforming ability in both pre- and post rigor beef during frozen storage, the presence of salt in the product contributed to denaturation of the protein. In a subsequent study (Park et al., 1990), when the salt was omitted from the cryoprotected sample, the gel forming abilities of both the pre- and post-rigor muscles were maintained at near unfrozen levels by polydextrose addition.

Lactitol and lactulose reportedly have low sweetness, and have been demonstrated to effectively cryoprotect surimi protein (Sych et al., 1990a, b). Maltitol, isomalt, and hydrogenated glucose syrups could also be considered for special applications (Sych et al., 1990a). Edible gums have been proposed to function as effective cryoprotectants but tests have failed to demonstrate their effectiveness (da Ponte et al., 1985a, b, c). The reduced functionality of muscle proteins in the presence of gums may result from the gum competing for water with protein, or from interaction of the gum with proteins, which results in poor protein gelation. Addition of gums in the fully hydrated form without adding excess water, and the attainment of concentrations sufficient

for cryoprotection, are additional problems in the practical applications of gums as cryoprotectants (MacDonald and Lanier, 1991).

Mechanism of protein cryoprotection

The most commonly used cryoprotectants in the food industry have been low-molecular weight sugars and polyols such as sucrose and sorbitol used in surimi manufacture (MacDonald and Lanier, 1991). While the mechanism of cryoprotection by such molecules is not fully understood, it is known that they are able to stabilize proteins through their interactions with the surrounding water.

Denaturation of proteins is thermodynamically less favorable in sugar solution than in water (Arakawa and Timasheff, 1982). These authors indicate that the stabilizing solute molecules (low molecular weight sugars and polyols) were excluded from the surface of the protein molecule thus water was "preferentially hydrating" the protein. This would result in a positive (unfavorable) free energy change because the sugar is excluded from the protein surface. The magnitude of this unfavorable free energy shift is proportional to the surface area of the protein. Since the protein cavity is assumed to be greater when the protein is unfolded, this means that the native state of the protein is thermodynamically favored in a sugar-low molecular weight polyol solution (MacDonald and Lanier, 1991).

High molecular weight carbohydrates seem to work by another mechanism. Carpenter and Crowe (1988) theorized that certain high

molecular weight polymers such as polyvinylpyrrolidone, polyethyleneglycol and dextran are good cryoprotectants because they are stearically excluded from the protein surface by their size. However, an entirely different mechanism has been postulated by other workers to explain the cryoprotective effects of many high molecular weight polyols and glucose polymers (starch hydrolysis products). This "cryostabilization" theory is based on the ability of high molecular weight solutes to raise the glass transition temperature (T_g') of a solution (Levine and Slade, 1988a, b). At higher concentrations of solute, the T_g , occurs at temperatures above freezing. Thus, the mixture cools to form a "glass" directly from the liquid state. At solute concentrations below T_g ', when the temperature falls below the freezing curve, the solution will exist either as a viscous supersaturated solution in the liquid state or more commonly as a mixture of ice crystals and supersaturated solution. This system is termed as "rubber", exhibiting high viscosity. This rubber state is a departure from the Arrhenius kinetics of chemical and enzymatic interactions, following instead William-Landel-Ferry kinetics (Levine and Slade, 1988b, c).

Hence, cryostabilization of proteins involves addition of a solute to raise the T_g ' to a temperature above that of the storage temperature, thereby ensuring that the system is in the glass state. This effectively shuts down the deteriorative processes and no ice crystals are formed because the water is immobilized in the glass structure.

Levine and Slade (1988b, c) indicate a direct relationship between molecular weight (or inversely, dextrose equivalent) for starch hydrolysis products and T_g' . Lim et al. (1990) demonstrated the principle of cryostabilization for the freezing of leached fish muscle (surimi). In model

studies using salt-soluble proteins to represent the surimi, they found that maltodextrin (T_g' =-10°C) protected the solubility of the protein in a much more temperature-dependent fashion than did sucrose.

Several studies have been done using high molecular weight carbohydrates as cryoprotectants. Park et al. (1987a, b, c) reported the effectiveness of polydextrose (a non-sweet, low calorie bulking agent) in the stabilization of pre-rigor beef muscle proteins and fish myofibrils during frozen storage, and its use in muscle food has been patented (Lanier and Akahane, 1986). MacDonald and Lanier (1991) studied the effects of hydrolyzed starch as cryoprotectants of meat and surimi. Popiel et al. (1992) studied the thermal properties of myofibrillar proteins isolates from mechanically deboned turkey meat. They reported a significant cryoprotective effect of starch during repeated freeze/thaw treatments. More recent studies on protein protection have focused on four groups of compounds: carbohydrates, polyalcohols, protein hydrolysates and hydrocolloids (Sych et al., 1990b; MacDonald and Lanier, 1991).

Meat proteins

The amount of protein in lean muscle tissue is approximately 20% but it varies with sex, age, species, genetic makeup and state of nutrition of the animal from which it is obtained (Morrisey et al., 1987).

Muscle proteins can be classified as sarcoplasmic, myofibrillar and stromal protein based on their solubility and functionality. Sarcoplasmic proteins are soluble in water and low ionic strength (0.1μ) salt solution, are located in the sarcoplasm (muscle cell cytoplasm) and account for approximately 30-35% of the total protein(Morrisey et al., 1987). They consist of globular proteins and are very similar to the proteins that are present in the cell cytoplasm except for the presence of myoglobin and glycolytic proteins (Asghar et al., 1985). They have isoelectric points between pH 6.0-7.0 (Schmidt and Trout, 1982) and molecular weights in the range of 30,000 to 100,000 (Bendall, 1964). The sarcoplasmic proteins have very low water-binding capacity and gel forming ability, but contribute a great deal to the meat color because myoglobin is a part of this group of proteins.

Myofibrillar proteins constitute between 50-55% of the total muscle protein (Acton et al., 1983). They are located in the myofibrils which are the contractile elements of the muscle. The major proteins in this salt-soluble fraction are myosin, actin (actomyosin in post-rigor meat), tropomyosin, troponin and α -actinin (Morrisey et al., 1987). Myofibrillar proteins are usually extracted from muscle with high ionic strength solutions, but once extracted, some of them are soluble at low ionic strength. Myosin, with a molecular weight of 470,000-500,000 is the most abundant and accounts for approximately 35% of the muscle protein. The isoelectric point of myosin is approximately 5.4 (Schmidt and Trout, 1982). The salt soluble protein, mainly myosin or actomyosin possesses high water binding capacity and viscosity. It has extensive emulsification and gel forming ability resulting from proteinlipid and protein-protein interactions (Acton et al., 1983).

Stromal proteins, also referred to as connective tissue proteins, comprise 10-15% of the total protein content (Morrisey et al., 1987). This group of proteins mainly consist of three fibrillar proteins: collagen, reticulin and elastin (Asghar et al., 1985). The biological function of connective tissue is to cover the body as well as connecting the muscle, organs and other tissue structures to the skeleton and to each other. Collagen, the main protein is a triple helix, fiber-like protein that contains a higher hydroxyproline content than any other meat protein. Collagen fibers shorten to about one-third their original length when heated to 70°C and at 80°C or higher, collagen is converted to gelatin.

On the basis of their physiological function in the muscle, the myofibrillar proteins are divided into two subgroups: contractile and regulatory proteins; and intermediate filaments and proteins (Asghar et al., 1985). Myosin and actin are major components of the thick and thin filaments respectively. They are directly involved in the contraction-relaxation cycle of the live muscle. Myosin is composed of two large polypeptide chains, "heavy chains" each having a molecular weight of about 200,000 daltons and three of four small chains, "light chains". Studies conducted on isolated myosin molecules have shown three important intrinsic properties of myosin. It is an enzyme with ATPase activity, it has the ability to bind actin and it can form aggregates with itself to form filaments (Bandman, 1987). Actin is the major constituent of the thin filament and accounts for 22% of the myofibrillar protein. Depending on the environmental conditions, actin can be found in two forms. In low ionic strength solutions, actin exists as a single-chain globular

protein (G-actin). Under high salt conditions, G-actin polymerizes to form a fibrous protein (F-actin) (Morrisey et al., 1987).

Regulatory proteins are the myofibrillar proteins involved in the contraction-relaxation cycle and are divided into two subgroups; the major (tropomyosin and the troponins) and the minor (M-protein, C-protein, F-protein, H-protein, X-protein, I-protein, paramyosin, actinins, α -actinin, β -actinin, γ -actinin, Eu-actinin and Z-protein) regulatory proteins.

Protein extraction

Myofibrils and myofibrillar filaments can be released from the sarcolemma by grinding and chopping of the meat. This facilitates the extraction of maximum amount of myofibrillar protein which is the main functional protein in meat. The procedure for extracting myofibrillar proteins involves blending of the meat with a salt-phosphate brine followed by centrifugation (Camou, 1989).

Gillett et al. (1977) studied the effect of various parameters (stirring, time, temperature and salt concentration) on the extraction of meat proteins in a model system. They observed an increase in soluble protein with mixing time, high NaCl concentrations and optimum temperature of 7.5°C. Other researchers have shown a linear relationship between salt concentrations and extractable protein up to 10% NaCl. However, in practical applications for processed meats, concentrations higher than 2.5% are not recommended due to flavor considerations. Turner et al. (1979) reported greater protein extraction using high salt concentrations in the presence of phosphates (1 M salt and 0.25% tripolyphosphate) than with low salt concentrations.

The addition of sodium chloride (NaCl) during myofibrillar protein extraction produces a shift in the pI of the myofibrillar protein to a lower pH value, increasing net negative charge on the protein. Repulsion between the negatively charged groups causes the protein to unfold, thus increasing water holding capacity (Acton et al., 1983). Polyphosphates are also effective in increasing the water holding capacity in meat due to their high pH. Offer and Trinick (1983) reported maximum swelling of myofibrils at 0.4 M NaCl and 10 mM sodium phosphate. Equivalent swelling was also observed in solutions containing 0.8 mM NaCl (Asghar et al., 1985). Common extraction procedures for myofibrillar proteins in meat systems are performed with ionic strengths of 0.6 to 1.3 and a pH range 5.8 to 6.2. In buffered solutions these parameters vary between studies but seldom fall below pH 6.0 and ionic strength of 0.5-0.6 (Acton et al., 1983).

Protein gelation

Protein gelation in model systems has been extensively used to study the mechanism responsible for texture and water holding characteristics of meat products. The process of gelation involves the formation of a three dimensional continuous protein network with a certain degree of order that occurs during heat processing of meat batters and which physically and chemically stabilizes fat and water (Acton et al., 1983; Ziegler and Acton, 1984). Heat induced gelation is an irreversible process that determines the development of textural characteristics of meat products, binding of meat pieces and emulsion stabilization (Pomeranz, 1991). Extensive studies have shown that myosin was the main protein responsible for binding meat pieces together, hence, many researchers have used myosin as the model system (Yasui et al., 1979; Ishioroshi et al., 1979; Ishioroshi et al., 1980; Dudziak et al., 1988). More complex systems such as meat batters and intermediate systems that include salt-soluble proteins and myofibril suspensions have also been used to study the chemical/physical events that occur during the manufacturing of meat products (Trout and Schmidt, 1987; Camou et al., 1989; Foegeding et al., 1991).

Heat-induced gelation was described by Ferry (1948) as a two-step process that involves the initial denaturation of native proteins into uncoiled polypeptides, followed by aggregation of denatured proteins into a cross-linked gel network during heating (Fig. 3).

The aggregation step must occur slowly, to allow the denatured protein molecules to orient and interact, allowing the gel matrix to be formed (Acton and Dick, 1984). Quick aggregation of protein molecules results in random

heatheat and/or coolingNative protein ----->Denatured protein ----->AggregatedPhase 1Phase 2protein

Fig. 3: Myosin gelation mechanism (Ferry, 1948)

protein-protein interactions that promote coagulation, characterized by syneresis, low elasticity and high opacity (Morrisey et al., 1987).

Transition temperatures $(T_m's)$ have been used to identify temperature points where protein conformational change occurs with absorption of thermal energy. Ishioroshi et al. (1979) found two $T_m's$ for myosin at 43°C and 55°C and concluded that in the gelling reaction, myosin undergoes at least two conformational changes. The first phase occurs at temperatures between 30°C and 50°C, and rapid aggregation and gelation occurs. At this stage, there are structural changes of the helical rod segments of myosin and cross-linking (Acton and Dick, 1984; Ferry, 1948; Ziegler and Acton, 1984).

During the heating process as the temperature increases, hydrogen bonds weaken, protein-water interactions tend to decrease and hydrophobic protein-protein interactions increase. Optimal conditions for heat gelation of myosin are pH of 6.0, temperature of 60°C to 65°C and an ionic strength greater than 0.3M, although when the meat batter is formed ionic strength greater than 0.6M is needed (Whiting, 1988b). Acton et al. (1983) reported that the optimal pH for gelation was in the range of 5.8 to 6.1 and described the process to be dependent on pH and ionic strength.

It is generally accepted that polypeptide chains of myosin cross-link to form five or six crystalline regions per molecule during gelation. These interactions may involve multiple hydrogen bonds, disulfide or peptide bonds and may also be electrostatic and hydrophobic in nature (Asghar et al., 1985)

Several factors influence the gelation process and the gel strength of myosin in meat systems. Siegel and Schmidt (1979) reported that salt and phosphate increased the ability of myosin to bind meat pieces through gel formation following solubilization of myofibrillar proteins. In addition, phosphates are able to dissociate the actomyosin complex. Both effects increase the amount of free myosin that can participate in the formation of the gel network. Temperature and protein concentration were found to increase binding capacity in the range of 45-80°C and 0-8% respectively. There were no significant differences in binding ability of myosin within the pH range of 6 to 8.

Functionality of meat proteins

The functionality of meat proteins can be defined as their ability to provide desirable performance attributes and textural characteristics to different meat products. As described by Smith (1988), functionality is an expression of the physical and chemical properties of meat proteins when they are modified by external conditions. In comminuted muscle, functionality is commonly taken to mean how well the muscle produces desirable sensory texture and stabilizes fat and water in thermally processed products (Hamann, 1988). The gelling properties of muscle components which determine the development of structured meat products (Acton et al., 1983; Asghar et al., 1985) are critical to the production of a large variety of manufactured meat products. Development of a product texture exhibiting proper hardness (strength) and cohesiveness (deformability) is important.

The myofibrillar fraction, formed of high molecular weight saltextractable fibrous protein accounts for 50-55% of the total proteins of muscle tissue. From this fraction, myosin is considered to contribute the most to functionality of processed meat products (Acton et al., 1983; Ziegler and Acton, 1984). The amount and nature of myosin is closely related to the binding strength of meat products. Salt-soluble proteins are crucial for the formation of interfacial films which are essential for batter stabilization and fat emulsification (Asghar et al., 1985). Muscle proteins have different emulsifying capacities that vary in the order: myosin > actomyosin > sarcoplasmic proteins > actin (Hegarty et al., 1963).

The functional properties of meat proteins include swelling, solubility, viscosity, water-binding ability, gelation and emulsification (Acton and Dick, 1984; Smith, 1988). In raw meat emulsions, the most important functional properties are water binding, fat binding and emulsification although Gordon and Barbut (1990) described some gelation occurring at this stage. In cooked products, water binding, fat binding and gelation are the main functional properties. All these functional properties are the result of three type of interactions: protein-water interactions determining water-binding capacity, protein-fat interactions determining fat stabilization and emulsification and protein-protein interactions determining protein gelation (Acton and Dick, 1984; Jones, 1984; Regenstein, 1984; Smith, 1988).

The functionality of salt-soluble proteins determines the characteristics of processed meats, and the functional properties of the protein matrix that are most important for the quality of the meat products are binding, emulsification and gelation (Schmidt et al., 1981; Smith, 1988).

Factors influencing functionality

Several factors influence the gelation process and the gel strength of myosin in meat systems. These factors include meat ingredients, protein concentration and denaturation, frozen storage and the effect of ingredients.

The different sources of meat used can influence protein functionality. Skeletal beef and pork muscle, an excellent protein source, is the usual meat ingredient for processed meat products. Poultry meat is also frequently used in comminuted products. Other protein sources such as fish and rabbit have been explored by Lanier (1985) and Whiting and Jenkins (1981) but are not common in commercial meat products.

Mechanically separated or deboned meats (MDM) are a major meat source in the poultry industry (Froning, 1981). In general, the processing properties of MDM compare favorably to hand-boned meats (Field, 1981) although products containing too much MDM have been described as having "less texture". The bone marrow content which is often relatively high in MDM, may improve processing properties by raising the pH and increasing the water-holding capacity, but increased iron, copper and magnesium from bone marrow may reverse this gain (Whiting, 1988a).

Fresh meat just after slaughter has good functionality because of the relatively high pH and lack of sarcomere shortening. Prerigor muscle has superior water-holding capacity and batter-forming ability because myosin is readily extractable at high pH values. As muscle approaches the rigor state, water-holding decreases with decreasing pH caused by glycolysis, until the

proteins' isoelectric point is reached, near pH 5.4 in the rigor state. The optimum for gelation is pH 5.5-6.0 (Acton et al., 1983; Whiting, 1984).

Protein concentration affects the gel strength of myosin. Heat-induced gel strength of myosin increased proportionally to protein concentration, independently of ionic strength and presence of actin in the system (Asghar et al., 1985). Similar results were observed by Ishioroshi et al. (1979) who evaluated the effect of protein concentration (0.1-1%) on gel strength of myosin. Cofrades et al. (1993) studied the effect of protein concentration on the apparent viscosity of natural actomyosin from hake, pork and chicken muscles. In diluted solutions, the effect of protein-solvent interaction predominated and there was a linear relationship between viscosity and protein concentration. However, when protein concentrations were higher, protein-protein interaction was increased and a non-linear relationship was found.

Frozen meat is often used in further processed products, although it is not as good as fresh meat. Ice crystals formed during freezing can damage the muscle structure and result in protein denaturation. The localization and size of ice crystals can be controlled by freezing rate. Grujic et al. (1992) investigated the effect of different freezing rates on the structure and ultrastructure of beef M longissimus dorsi. It was found that by slow freezing, ice crystals formed intracellularly were relatively large; at fast freezing rates, smaller ice crystals were formed intracellularly. These authors found that muscle frozen at a freezing rate of 3.95 cm/hr showed the least change, therefore the best quality of frozen meat could be obtained. The length of frozen storage and degree of protein denaturation occurring during storage also

influences the texture property of meat products (Smith, 1988; Miller et al., 1980).

Sarcoplasmic proteins are less soluble after freezing meat tissue (Kronman and Winterbottom, 1960). Total extractable protein, sarcoplasmic protein and actomyosin extractability decreased with frozen storage (Awad et al., 1968). Miller et al. (1980) reported a significant decrease in extractable protein, water binding and emulsifying capacity in beef and pork during 37 weeks of frozen storage.

Non-meat ingredients that are added to meat products to improve functionality include salt, phosphate, binders and others (Whiting, 1988a). Salt allows dispersion and solubilization of myofibrillar proteins. Maximum water-holding capacity is acheived with 0.8-1.0 M NaCl (4.6-5.8% salt) (Offer and Trinick, 1983) but 0.4-0.6 M is generally sufficient for good functionality (Trout and Schmidt, 1983). During processing, salt is added during comminution and the effect of NaCl and tissue disintegration leads to an increase in water-binding capacity of the myofibrillar protein (Acton et al., 1983; Whiting, 1988b). The effect of salt on water- holding capacity of a meat batter is mainly due to the chloride anion (Hamm, 1960) but sodium is the principle element that makes NaCl taste like salt and gives characteristic flavor and palatability to meat products (Olson and Terrell, 1981). Besides improving functionality, sodium chloride decreases purge loss during storage, increases binding properties of proteins, gives flavor and has a bacteriostatic effect prolonging shelf-life of meat products.

Phosphates are widely used to increase water-binding capacity, increase strength of meat binding, retard lipid oxidation, improve emulsification and color development and provide certain antimicrobial effects (Steinhauer, 1983; Trout and Schmidt, 1983). Overall, phosphates increase juiciness, yield, batter stability and texture of processed meats. The most common forms for use in meat products are sodium acid pyrophosphate (SAPP), tetrasodium pyrophosphate (TSPP) and sodium tripolyphosphate (STPP). Current regulations allow a total addition of 0.5%.

Binders are added to meat products to improve water retention (Whiting, 1988a). Proteins, modified proteins, and carbohydrates can be chosen depending on the specific product and process. Carbohydrates used in meat products include sugars, glycerol, cereals, starches and gums. Carrageenans have been tested as a water-binding agent in cooked ham (Anonymous, 1984) and other processed meat. Carrageenan is also the most promising gum tested in low-fat frankfurters (Foegeding and Ramsey, 1986, 1987).

To improve functionality of frozen meats for use as ingredients in processed meat products, means of preventing or inhibiting freeze-induced denaturation and/or aggregation are needed (Park et al., 1987a, b). Carbohydrates like sucrose and sorbitol can potentially serve as cryoprotectants in meat products (Park et al., 1987a, b) and surimi (Lee, 1986).

Measurements of meat protein functionality

Back extrusion Texture properties of gels are usually evaluated by instrumental measurements. There are many fundamental and empirical

rheological tests that can be used to measure texture properties of gels depending on the different physical properties to be measured, differences existing in solution/heating conditions or in amounts and types of proteins (Lavelle and Foegeding, 1993).

The term back extrusion has also been referred to as compressionextrusion testing or annular pumping (Bourne, 1982). The test consists of applying a downward force to a gel within a container. The gel is compressed until the structure of the gel is disrupted and it extrudes, or flows back past the rod or plunger. Usually, the maximum force required to accomplish extrusion is measured and used as an index of textural quality. The back extrusion test has been used by several researchers to evaluate the rheological characteristics of meat protein gels (Hickson et al., 1982; Xiong and Brekke, 1990, 1991). Camou (1989) and DeFreitas (1994) measured gel strength of protein extracts by the compression-extrusion method using an Instron equipped with a plunger of 35 mm diameter. Trius (1994) also used the back extrusion technique to measure rheological behavior of raw meat batters.

Seman et al. (1980) used the back extrusion technique for meat emulsions and related the extrusion peak force to the meat emulsion stability. Payne and Rizvi (1988) described a similar technique using a capillary extrusion method to evaluate rheological behavior of comminuted meat batters. Harper et al. (1978) evaluated the strength of bovine plasma gels by annular pumping.

Emulsifying capacity An emulsion as defined by Becher (1965) is a heterogeneous system, consisting of at least one immiscible liquid dispersed in

another, in the form of droplets, whose diameters in general, less than 0.1 micron. Emulsions in food products are of two types: water-in-oil; such as margarine and butter, or oil-in-water such as salad dressing, ice-cream and cheese. In either case, small droplets of the discontinuous phase are dispersed in the continuous phase. The two phases in an emulsion, by definition, repulse each other and attract like phases, resulting eventually, in aggregation of each phase into distinct layers (Petrowski, 1979; Friberg and El-Nokaly, 1983).

Model systems have been used to estimate meat protein functionality in emulsions because they can closely mimic the steps and ingredients of commercial meat emulsions (Pour-El, 1981). The first model system was developed by Swift et al. (1961). In this method, oil was added continuously to a protein/water dispersion. The total volume of oil added relative to protein at the point of phase coalescence, is emulsifying capacity (EC). Another objective method to measure EC is by detecting a drop in electrical conductivity at breakpoint caused by fat release. This method was developed by Webb et al. (1970). Another variation in end-point determination is the color change which occurs when an emulsion formed with Oil-Red-O dyed oil separates into different phases, releasing bright red oil to the surface (Marshall et al., 1975).

Several non-protein factors affect the EC test. The pH and ionic environment of the protein solution affects the ability of the protein to solubilize and participate in the interface formation. Kamat et al. (1978) indicated that near the iso-electric point, a solubilized protein will have its greatest EC value because the protein adsorption and elasticity at the oil/water interface is the

greatest. Solubility at the isoelectric point, however, is relatively low. Crenwelge et al. (1974) found, that pH 7.0 results in greater total EC in meat proteins than the isoelectric point.

Final temperature at emulsion breakpoint appears to have a great effect on EC. Swift et al. (1961) and Carpenter and Saffle (1964) found a negative correlation between temperature rise and EC.

Increased mixing speed has been clearly demonstrated to decrease EC. Carpenter and Saffle (1964) found a negative correlation between blender speed and EC. Effect of fat and oil addition rate also has an effect on EC. Swift et al. (1961) found a high correlation between increased EC with increased rate of oil addition. This was probably due to the protein layer being formed instantaneously and with greater oil flow, a greater amount of oil could be emulsified prior to the onset of denaturation.

The literature reviewed indicates that hydrocolloids such as starches, carrageenans, maltodextrins and corn syrup solids exert different effects in meat systems and may differ in their functional contributions to the meat system. Functional properties dominate the decision-making process for the use of hydrocolloids in meat products and the choice of which hydrocolloid to use. Hence the major part of this research examines the effects of these hydrocolloids as texture modifying agents, water binders and cryoprotectants in different meat systems. A better understanding of the properties of hydrocolloids in conjunction with meat is critical in order to maximize product functionality.

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QUALITY CHARACTERISTICS OF HAM FORMULATED WITH MODIFIED CORN STARCH AND KAPPA-CARRAGEENAN

A paper submitted to the Journal of Food Science G. A. Prabhu¹ and J. G. Sebranek¹

ABSTRACT

Effects of modified corn starch and kappa-carrageenan in a ham product containing 55% added brine were evaluated. Eight treatments were formulated: kappa-carrageenan (0%, or 1.5%) x starch (0%, 2%, 3.5% or 5%). Hams were evaluated for cooking yields, purge, color, texture and sensory characteristics. Incorporation of carrageenan at a level of 1.5% increased yield, decreased purge and resulted in a sensory perception of reduced juiciness. Increasing levels of starch increased perception of juiciness. There was no synergistic effect on moisture retention due to a combination of starch and carrageenan. Microscopic analysis revealed that starch and carrageenan were randomly distributed in localized areas with no evidence of an interaction between them.

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INTRODUCTION

Hams made with 55% added water are prepared by tumbling the meat with the brine for extraction of salt-soluble proteins and then the meat pieces are bound together by subsequent thermal processing. Binding of ham muscle pieces results from gelation of salt-extracted myofibrillar proteins (McFarlane et al., 1977; Siegel and Schmidt, 1979). The binding among the whole ham muscle pieces and the water retained are together responsible for the final product texture.

The effects of the addition of salt and phosphate on water retention in processed meats are well known. However, the amount of water retained by using only these ingredients is not satisfactory when high levels of brine are added. The brine dilutes the protein in the surface as exudate of the meat; binding between meat pieces decreases and cooking purge increases (Acton, 1983). Hydrocolloid gelling agents, like carrageenan and starch, are added to hams as binding agents. The functionality of carrageenan in meat products is related to its thermal-reversible gelation properties. Carrageenan dissolves throughout the meat during thermal processing and gels when cooled (Bater et al., 1992a).

Several researchers have reported using carrageenan in meat products (Egbert et al., 1991; Fox et al., 1983; Trudso 1985). There are three major commercially available types of polymers designated as kappa, iota and lambda carrageenans (Bater et al., 1992b). Of these, kappa-carrageenan is an important gelling agent utilized in food products. Kappa-carrageenan

produces a strong and brittle gel with water (Pomeranz, 1985). It was reported that kappa-carrageenan synergistically interacts with the potassium ion (Pomeranz, 1985), locust bean gum (Whistler and Daniel, 1985), and milk protein casein (Lin, 1977) for gel formation.

The functional properties of carrageenan in meat products are related to its gel-structure formation with meat protein. During processing of meat, carrageenan is first dispersed in a brine solution, then introduced into the meat by injecting and massaging, and dissolved and distributed throughout the meat by thermal processing (Bater et al., 1993). During cooling of the cooked products, the carrageenan solution together with the meat exudate forms a firm and cohesive gel structure (Trudso, 1985).

Cooking losses have been reported to be unaffected by carrageenan type, but the use of brine injection (use of multineedle injection + massaging rather than massaging alone) increased the yield by 2.2% in hams containing carrageenan (Orovio et al., 1987). Incorporation of 0.5% kappa-carrageenan increased yield, improved visual appearance, sliceability, and rigidity and decreased expressible juice compared with controls in oven-roasted turkey breasts with 70% added brine (Bater et al., 1992a).

Incorporation of 0.5% kappa-carrageenan in turkey ham-like products significantly increased cooked yield, visual appearance, sliceability and bind, and decreased freeze and thaw purge compared with the control (Bater et al., 1993). The bind values were further increased with a blend of 0.5% kappacarrageenan and 2% non fat dry milk or starch. Starch has been used in meat products to improve quality and occasionally to lower cost of the formulation. The effect is based on the ability of starch to gelatinize when heated in a water-containing medium, thereby binding relatively large amounts of water (Hodge and Osman, 1976).

Several types of starches are available for use in meat products, and in recent years the choices have been extended by development of various modified starches (Skrede, 1989). Chemical modification of native starches introduces new substitutes in the starch molecule and results in changed technological properties (Howling, 1980). Several of the modified starches have been approved for human consumption (FDA, 1993).

Skrede (1989) compared various types of starches that may be used in meat products for storage stability, optimal cooking temperature and sensory properties. Potato starch was rated as the best-suited starch, followed by wheat starch, whereas tapioca was rated as the least suited. Corn starch required temperatures above 75°C and showed relatively low freeze-thaw stability.

Berry and Wergin (1993) studied the effects of modified pregelatinized potato starch (MPPS) in ground-beef patties containing 5% and 20% fat. Inclusion of MPPS increased tenderness and cooking yields but reduced juiciness and beef flavor.

Starch and carrageenans have also been used as potential fat-replacers in ground-beef products. Iota-carrageenan used at a 0.5% level in a 8% fat ground-beef formulation imparted sensory characteristics similar to those obtained by using a 20% fat formulation (Egbert et al., 1991). A three-way combination of polydextrose, potato starch and dietary fiber reduced firmness

and cohesiveness of beef patties containing 5% and 10% fat such that they were similar to those with 20% fat (Troutt et al., 1992).

Starch addition (2%) has been reported to improve the surface appearance, sliceability and texture of hams containing 0.5% carrageenan. The surface of the products with carrageenan alone appeared to be jelly-like and wet (Trudso, 1985). Hams containing carrageenans showed a significantly better break strength as well as improved rigidity and cohesiveness.

The objective of our study was to investigate the effects of kappacarrageenan alone, starch alone and various combinations of kappacarrageenan with starch on the quality characteristics of hams containing 55% added brine. A second objective was to observe the distribution of carrageenan and starch in the ham slices and to determine their microstructural characteristics.

MATERIALS AND METHODS

Ham Manufacture

Fresh hams were obtained from a commercial processing plant in Iowa on three different occasions. Kappa-carrageenan was provided by Sanofi Bio-Industries, Inc. (Waukesha, WI). Modified corn starch was provided by Grain Processing Corporation (Muscatine, IA). Eight treatments were formulated (Table 1). Treatments containing 1.5% carrageenan, 2% starch, 3.5% starch and 5% starch were designed to investigate the effects of carrageenan and the various levels of starch alone on product qualities. Treatments consisting of 1.5% carrageenan + 2% starch, 1.5% carrageenan + 3.5% starch and 1.5% carrageenan + 5% starch were designed to test the effects of a combination of carrageenan and increasing levels of added starch on the product characteristics.

For each treatment, 6.8 kg of hams were macerated by using a macerator (Stork Protecon, Oss, Holland) to increase the surface area. Brines for each treatment were prepared by dissolving sodium tripolyphosphate (STPP), the least soluble ingredient, followed by sodium chloride, sodium erythorbate and sodium nitrite, in 10°C tap water, followed by dispersion of the hydrocolloids (kappa-carrageenan and/or starch) in the solution.

The brine was added to the ham in a vacuum tumbler (Globus Laboratories Inc., South Hackensack, NJ 07606) and a 1.4 atmosphere vacuum was drawn inside the vacuum tumbler. The hams were tumbled at 4°C continuously for 4 hr. After refrigeration overnight, the hams were stuffed by hand into 18.7-cm-diameter fibrous casings (Teepak, N. Kansas City, MO). For each treatment, 2 ham-chubs of approximately 4 kg each were obtained. Hams were thermally processed in a Maurer and Sohne system oven (Maurer and Sohne Rauch-Und Warmetechnik GmbH and Co. KG, Irsel Reichenam, West Germany) to an internal temperature of 68°C by using a stepwise thermal-processing schedule. The relative humidity was kept at 74%. The cooked product was stored overnight in a cooler at 4°C. The hams were

then sliced into 1.25-cm-thick slices by using a Hobart slicer (Model 1712 Hobart Manufacturing Co., Troy, OH), placed in Curlon 892 (Curwood Inc., New London, WI) bags, vacuum-sealed at 1 kPa by using a Multivac MG-2 packaging machine (Sepp Haegenmuller KG, Allgau, West Germany), and stored in a 4°C cooler until evaluated.

Evaluations

Cooked vield

For each individual treatment, product yield was calculated by dividing the cooked product weight by the uncooked product weight (cooked product weight/uncooked product weight x 100). Yield, therefore, represents product evaporation losses that occurred primarily during thermal processing. Weight loss due to exudate remaining in the tumbler was small because tumbler surfaces had been scraped with a spatula to reclaim as much of the exudate as possible.

Purge

Individually packaged ham-slices were weighed (sample + bag + purge), and the initial weight (g) was recorded. The sample was then removed from the bag, and the bag was dried with a paper towel and weighed. The sample and the dry bag were weighed again (final weight (g)). Purge was calculated by using the formula: weight of sample + bag + purge - weight of bag / weight of sample + bag - weight of bag x 100. Purge was measured 2 weeks after sample preparation. Four samples from each treatment were tested.

Instrumental texture evaluation

Texture was evaluated by using an Instron Universal Testing Machine (Model 4502, Instron Corp., Canton MA) equipped with a star probe of 9-mmdiameter, and a cyclic computer-software program (cyclic 2, Instron Corp., Canton, MA) was used to measure texture characteristics. Samples were cut into squares of 10 cm^2 with a thickness of 1.25 cm. The measurements were taken perpendicular to the cut surface in five separate locations. The probe was set at 30 mm from the bottom of the plate and moved downward at a speed of 100 mm/min., stopping when it sensed the sample. It was used to puncture the sample completely. Peak load expressed as Newtons (force required to puncture the sample), peak energy (total work expended in puncturing until peak load was reached) and total energy (total work expended in completely puncturing the sample) were registered.

Instrumental color evaluation

Instrumental color determinations were made on the surface of the packaged slices by using a Hunter Lab Labscan 6000 Spectrocolorimeter (Model JB-1201 M (A), Hunter Asso. Laboratory Inc., Reston, VA) standardized by using the white LS-12029 standard plate (X=81.60, Y=86.68, Z=91.18). Packaging material was compensated for during instrument standardization by covering the white LS-12029 standard plate with saran wrap before standardization. Measurements were taken directly on the surface of the packaged slices on 3 different locations on 2 slices. Samples were measured for Hunter Lab color "L", "a" and "b" values. Mean value of a sample was obtained from six readings.

Sensory evaluation

Sensory analysis was conducted by using a 15-cm line scale. The tastepanel members were primarily graduate students and departmental staff who had participated in previous sensory evaluations of ham products. Samples (cut into cubes of 1.5 cm^2) from the eight treatments were randomly placed on a sample plate and labeled with three-digit random numbers. The samples were presented simultaneously to about 15 panelists in individual booths under fluorescent light and at room temperature. The panelists consumed unsalted crackers and warm (20°C) water between samples. Samples from all eight treatments were evaluated during each session. The samples were scored on a 15-cm line by placing a slash perpendicular to the line at the point that best described the attribute. The attributes evaluated were toughness, juiciness, cohesiveness, flavor intensity, flavor desirability, color and overall acceptability. The score for each attribute was measured (cm) by using a ruler from the left-hand side of the 15-cm line scale, and the scores obtained were averaged to give the final score for each attribute.

Proximate analysis

Two slices from each treatment were ground through a 4.8-mm plate and analyzed for moisture, fat and protein content. Moisture was determined

by weight loss after 8-12 hr of drying at 100°C in a vacuum oven (Precision Scientific Model 524 A, Precision Scientific Co., Chicago, IL). Fat was determined by weight loss after 16 hr of extraction in a Soxhlet apparatus with petroleum ether, and protein was analyzed by using a nitrogen analyzer (Model FP 428, LECO Corp., St. Joseph, MI), a combustion method used to determine nitrogen released at high temperature and measured by thermal conductivity (AOAC, 1990). A nitrogen-to-protein conversion factor of 6.25 was used. Analyses were conducted in duplicate.

Carrageenan distribution

Cross-sectioned slices of hams treated with carrageenans were stained with a methylene blue solution (0.1% methylene blue in 50% isopropyl alcohol) to show the distribution of kappa-carrageenan in the product (Bater et al., 1992a). Black-and-white and color photographs of the slices were taken after decolorizing the methylene blue color with 50% isopropyl alcohol. Carrageenan stained a very dark blue color in contrast to light-blue-stained muscle tissue.

Starch distribution

Cross-sectioned slices of hams containing starch were stained with an iodine solution (0.5% iodine in 5% potassium iodide solution) to show the distribution of starch in the product (Anonymous, 1988). Black-and-white and color photographs of the slices were taken after washing the iodine off with water. Starch particles stained dark brown/purple in contrast to yellowstained muscle tissue.

Microscopic analyses

Cylindrical sections of 1.25-cm length and 0.3-cm diameter were cut from the ham slices with a cork borer. They were then fixed in 10% formalinacetic acid (FAA) solution and embedded in paraffin by using standard procedures for paraffin embedding (Sass, 1958). 10 to 15µm-thick sections were cut on a Spencer Model 820 rotary microtome (American Optical Corporation, Buffalo N.Y.) and mounted onto glass slides. They were then deparaffinized with three changes of xylene for 10 min each, followed by a graded series of ethanol (200%, 100%, 95%, 70% and 50%), and washed with distilled water. Two staining procedures were used:

Carbohydrate staining

Carbohydrates were stained by using perodic acid-Schiff's regent. This staining procedure consisted of 0.5% perodic acid for 20 min., Schiff's 4°C dark for 30 min. and 2% sodium bisulfite for 2 min. Distilled water was used to wash off excess stain (H. T. Horner, personal communication). Water soluble carbohydrates stained purplish red.

Differential staining for carrageenan, starch and muscle tissue

Sections were deparaffinized to 50% ethanol (same as before, but without distilled water wash), and 0.1% methylene blue in 50% isopropyl alcohol was used for 5-10 min. to stain the carrageenan. Excess stain was washed off with isopropyl alcohol. Starch granules were stained with 0.5% iodine in 5% potassium iodide solution for 5-10 min. The excess stain was washed off with distilled water. Counter-staining of the muscle tissue was done with 0.1% hematoxylin for 2-3 min. A coverslip with permount was used, and the slides were examined with a Leitz Orthoplan phase-contrast microscope (Leitz GMBH Ernst Wetzler, Germany). Black-and-white and color photomicrographs were taken by using a Leitz camera at 88x and 133.5x magnification. Carrageenans stained dark blue, starch stained purple/dark brown and the muscle tissue stained a light blue.

Statistical analysis

The experiment was replicated three times. A completely randomized block design with treatments in a factorial arrangement (2 carrageenan levels x 4 starch levels) was used. Statistical analysis was performed by using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., 1991). The Least Significance Difference (P<0.05) test was used to test difference between means.

RESULTS AND DISCUSSION

The cooked hams for all treatments averaged at 73.91% moisture, 17.42% protein and 2.49% fat (Appendix A, Table 1A). The cooked yield of all treatments based on the stuffed weight after brine addition varied from 85.3% to 88.1% (Table 2). The measured weight loss occurred primarily during thermal processing and chilling. Product weight loss due to left over exudate in the tumbler was negligible. Addition of 1.5% kappa-carrageenan caused significantly higher yields (P<0.05) compared with those treatments without carrageenan (that is control or treatments with starch alone). There was a slight increase in yield as the level of added starch increased; however, it was not significantly different (P>0.05).

Bater et al., (1993) reported similar effects in turkey ham-like products. In their study, there was a significant increase in yield in treatments containing 0.5% carrageenan compared with the control. However, a combination of carrageenan with 2% added starch or 2% added non fat dry milk resulted in no further increase in yield compared with those treatments with carrageenan alone. Trudso (1985) reported a significant loss of brine during the massaging step of ham manufacture containing 40% brine in treatments without carrageenan.

One of the objective methods of measuring free water is purge measurement. The higher the purge, the greater the free water content and vice versa. Purge values indicated that as the level of added starch increased,

the amount of purge increased (Table 2). The highest purge values were for treatments containing 3.5% and 5% added starch (Table 2).

Bater et al. (1993) reported a significantly lower freeze-and-thaw purge in turkey ham-like products containing carrageenan compared with the control. The purge was further decreased when starch was used in combination with carrageenan or non fat dry milk.

There was no significant difference (P>0.05) in instrumental texture measurement for any of the treatments evaluated (Appendix A, Table 2A). These observations were in agreement with those of Orovio et al (1987) that hams treated with a different combination levels of carrageenan did not show any instrumental texture differences.

Addition of starch or carrageenan did not seem to significantly (P>0.05) affect the instrumental measurements of color in any of the treatments studied (Appendix A, Table 3A). These results were confirmed by the sensory panel, which was unable to detect significant differences in color for any of the treatments.

Panelists perceived the treatments with added starch to have significantly more juiciness compared with treatments containing carrageenan (Table 3). The treatment containing the most starch (5%) was perceived by panelists to be most juicy followed by 3.5% added starch. Treatments with 1.5% carrageenan received the lowest score for juiciness. Starch and carrageenan used in combination had a significant impact on juiciness scores (P<0.05). This indicates that starch may increase juiciness of products with carrageenan.

Berry and Wergin (1993) reported lower juiciness scores in beef patties containing modified pregelatinized potato starch. Bater et al (1992a) indicated significantly lower scores for juiciness, chewiness and firmness in ovenroasted turkey breasts with added starch alone compared with those with added carrageenan alone.

Cooking yield was higher and purge was lower in treatments containing carrageenan compared with the control or those containing starch, indicating that those treatments should have received high juiciness ratings. However, it seems that although carrageenans suitably bind water, it is unavailable for early juice release. Starch, on the other hand, seems to enhance the perception of juiciness during mastication because the water held by starch is much more easily released.

Panelists were unable to detect differences between treatments in any of the other sensory attributes evaluated. There were no significant differences (P>0.05) between any of the treatments for toughness, cohesiveness, flavor intensity, flavor desirability, color or overall acceptability. The overall acceptability values for all treatments ranged from 7.5 to 8.0 (on a scale of 15), and all treatments were considered acceptable by the panelists (Appendix A, Table 4A).

On January 31, 1992, the USDA Food Safety and Inspection Service (FSIS) proposed to allow the use of carrageenan at a level not to exceed 1.5% and modified food starch at a level not to exceed 2% of the product formulation in ham and water products (USDA, 1992). However, these binders have not been permitted in combination.

Figure 1A shows an example of starch distribution in ham slices stained with 0.5% iodine in 5% potassium iodide. Starch particles stained dark brown/purple and did not seem to have any specific distribution. Starch formed a complex with iodine and solubilized from the product surface.

Figure 1B shows an example of kappa-carrageenan distribution in ham slices stained with 0.1% methylene blue in 50% isopropyl alcohol. Kappacarrageenan stained a darker purple color in contrast to the light-blue-stained muscle tissue. The carrageenan was uniformly distributed along the macerated cuts in the muscle tissue as stripes.

Light microscopy was used to observe the distribution and microscopic structure of starch and carrageenan in the ham slices. Figure 2A illustrates key microstructural features of the control. The muscle tissue is stained light blue with 0.1% hematoxylin. The striations in the muscle structure are evident in several areas where the tissue is fragmented. This could be due to the maceration of the muscle during processing or to the sectioning procedure.

Figure 2B illustrates the distribution and structure of kappacarrageenan obtained from hams treated only with kappa-carrageenan. The carrageenan, stained dark blue, did not appear to have any defined structure and was localized in spots. The carrageenan was also dispersed randomly in the voids that could be either air or aqueous regions and in the spaces between the muscle where the binding of the meat pieces took place. This could probably be due to the fact that kappa-carrageenan gels at 60-70°C, a temperature at which myosin has already gelled, preventing the uniform distribution of the carrageenan throughout the meat matrix.

Figure 2C illustrates the structure and distribution of starch granules obtained from hams treated with only starch. The starch granules stained dark brown/purple with iodine and had a dense, well-defined structure, polygonal or oval shaped and 10-25µm in diameter. The granules were randomly distributed in the meat matrix in the voids between the cells. Most of the granules seemed to retain their shape.

Figure 2D illustrates a photomicrograph obtained from hams treated with both starch and kappa-carrageenan. Starch (stained dark brown) can be identified by its well defined polygonal or oval structure where as carrageenan (stained dark blue) did not have any specific structure. It is evident that both starch and carrageenan are found in localized spots; however, they are localized in separate regions. Interaction between starch and carrageenan is not evident.

CONCLUSIONS

No synergistic effect on moisture retention due to a combination of starch and carrageenan was observed. In fact, starch and carrageenan seemed to have opposite effects: increasing the level of starch increased perception of juiciness, and purge was increased. Addition of carrageenan increased cooking yields, but decreased purge and resulted in a sensory perception of reduced juiciness. Combinations of starch and carrageenan showed that starch may increase juiciness of products with carrageenan.

Microstructural examination indicated that starch and carrageenan are randomly distributed in localized areas and evidently do not interact.

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Treatments ^a	Ham (Kg)	Water (Kg)	Starch (g)	Carrageenan (g)
Control	6.8	3.1		
1.5% Carrag.	6.8	3.1		143
2% Starch	6.8	3.1	191	
3.5% Starch	6.8	3.1	333	
5% Starch	6.8	3.1	476	
1.5% Carrag.+2% Starch	6.8	3.1	191	143
1.5% Carrag.+3.5% Starch	6.8	3.1	333	143
1.5% Carrag.+5% Starch	6.8	3.1	476	143

Table 1: Ham formulations containing kappa-carrageenan and modified corn starch

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^a All treatments were formulated with 215g salt, 47.8g sodium tripolyphosphate, 5.2g sodium erythorbate and 1.5g sodium nitrite.

Treatment	Yield (%)	Purge (%)
Starch (%)		
0	85.3 ^a	2.53 ^b
2	86.3 ^a	2.58 ^b
3.5	86.4 ^a	3.38 ^a
5	88.1 ^a	3.18 ^a
SEM ¹	0.50	0.11
Carrageenan (%)		
0	85.7 ^b	3.46 ^a
1.5	87.3 ^a	2.38 ^b
SEM ¹	0.71	0.23
Probabilities		
Replication	0.0010	0.0007
Starch	0.0816	0.0042
Carrageenan	0.0462	0.0001
Starch*Carrageenan	0.6854	0.0819

Table 2:Yield and purge values for hams treated with kappa-
carrageenan and modified corn starch

^{a,b}Means with different letters are significantly different (P<0.05).

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¹Standard error of the mean.

Treatment	Juiciness ² (cm)	
Starch (%)		<u></u>
0	6.58 ^b	
2	7.92 ^a	
3.5	8.05 ^a	
5	8.16 ^a	
sem ¹	2.38	
Carrageenan (%)		
0	8.14 ^a	
1.5	7.21 ^b	
SEM ¹	3.37	
Probabilities		
Replication	0.5288	
Starch	0.0166	
Carrageenan	0.0146	
Starch*Carrageenan	0.0396	

Table 3:	Juiciness scores for hams treated with kappa-carrageenan and	l
	modified corn starch	

 a,b Means with different letters are significantly different (P<0.05).

¹Standard error of the mean.

²Sensory measurements using a 15-cm line scale. Juiciness (0=not juicy, 15=very juicy).

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Figure 1. Distribution of starch (A) and kappa-carrageenan (B) and in ham samples.



Figure 2. Photomicrographs of ham sections examined by light microscopy (magnification 86X): Control (A), carrageenan distribution (B), starch distribution (C), starch and carrageenan distribution (D). Bar = $3 \mu m$.





Figure 2 continued

CRYOPROTECTANT EFFECTS OF CORN MALTODEXTRINS AND CORN SYRUP SOLIDS IN MECHANICALLY DEBONED PORK DURING FROZEN STORAGE

A paper to be submitted to the Journal of Food Science G. A. Prabhu¹ and J. G. Sebranek¹

ABSTRACT

Changes in functional properties (protein solubility, gel-forming ability and emulsifying capacity) of mechanically deboned pork were measured over 240 days frozen storage, as affected by addition of cryoprotectants (5 DE, 10 DE maltodextrins and 20 DE corn syrup solids). Proteins were destabilized during freezing and frozen storage as reflected by decreases in protein solubility, gel strength and emulsifying capacity. Freeze-induced protein denaturation was reduced effectively by 10 DE maltodextrin and 20 DE corn syrup solids used at the 8% level, and, less effectively when used at the 4% level. The 5 DE maltodextrin used at the 4% level adversely affected salt-soluble protein extractability after 240 days of frozen storage.

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INTRODUCTION

Frozen storage has proven to be an important long term storage method for muscle foods as it prevents microbial spoilage and minimizes the rate of biochemical reactions in muscles (Park and Lanier, 1987). However, it is inevitably associated with some deterioration of meat protein functionality (Matsumoto, 1980; Park et. al, 1987a). Extended frozen storage can produce profound effects on the structural and chemical properties of muscle proteins, which in turn can significantly influence the quality attributes of muscle food products. This is mainly due to the crystallization of water in the muscle tissue, and to the size and location of the ice crystals produced (Sebranek, 1982). Frozen storage of finely comminuted meat products requires particular attention due to the possible occurrence of fat oxidation and freezing-induced protein denaturation which may markedly reduce functional properties.

Muscle proteins express their functionality only when the salt-soluble proteins are fully extracted (solubilized) and cryoprotection is only possible when intimate association of the cryoprotectant and the protein molecules occurs. Thus, both the expression and cryoprotection of muscle protein functionality will optimally occur in a minced or comminuted muscle system (MacDonald and Lanier, 1991).

Miller et al. (1980) and Verma et al. (1985) reported a marked decline in textural quality of frankfurters prepared from prerigor meat which was frozen. According to Miller et al., the total extractable protein decreased by approximately 50% for pork and 36% for beef after 25 weeks at -17.8°C. Large

changes in functionality (as measured by gel-forming ability, water-holding capacity and protein solubility) takes place during temperatures as low as -28°C (Park et al., 1987a, b). Furthermore, the degree of deterioration would be expected to be accentuated if the mechanically deboned form of these materials were evaluated during frozen storage (Macdonald et al., 1990; Shimuzu and Fujitu, 1985).

Uijltenboogart et al. (1993) reported that sorbitol in combination with 4% starch was a very effective cryoprotectant for chicken myofibrillar protein isolates obtained from mechanically deboned chicken. Niewiarowicz et al. (1990, 1991) found that enhanced oxidative changes occurring in mechanically deboned poultry meat during frozen storage caused a marked deterioration in the functional properties of proteins.

Of particular interest are studies on the use of high molecular weight carbohydrates as cryoprotectants. These high molecular weight carbohydrates "cryostabilize" proteins by raising the glass transition temperature T_g ' of a solution to a temperature above that of the storage temperature, thereby ensuring that the system is in the glass state. This effectively shuts down the deteriorative processes because no ice crystals are formed while the water is immobilized in the glass structure. Levine and Slade (1988a, b) indicated a direct relationship between molecular weight (or inversely dextrose equivalent) for starch hydrolysis products and T_g '.

Lanier and Akahane (1986) discovered and patented the use of polydextrose, a non-sweet, low calorie bulking agent (Pfizer Specialty

Chemical Group, New York, NY) for the cryoprotection of muscle foods. They compared its effectiveness with sucrose-sorbitol and a 10 DE maltodextrin in maintaining the salt-solubility and gel forming properties of Alaska pollack surimi. While the three additives maintained similar high levels of solubility in the myofibrillar proteins at -28°C over several months compared to control, the surimi containing 10 DE maltodextrin failed to form strong cohesive gels. Park et al. (1990, 1987a, b) showed that polydextrose was equivalent to sucrosesorbitol mixture in cryoprotecting beef actomyosin during frozen storage. Popiel et al. (1992) studied the thermal properties of myofibrillar protein isolates from mechanically deboned turkey meat. They reported a significant cryoprotective effect of starch during repeated freeze/thaw treatments. The effect however was less than that found with sorbitol/sucrose.

The purpose of this study was to investigate and compare the effects of high molecular weight carbohydrates such as maltodextrins and corn syrup solids on the freeze-thaw stability and quality characteristics of mechanically deboned pork during frozen storage.

MATERIALS AND METHODS

Fresh mechanically deboned pork (MDP) was obtained from a commercial source, directly off the processing line. The maltodextrins and corn syrup solids were provided by Grain Processing Corporation (Muscatine, IA). Maltodextrins are non-sweet, nutritive saccharide polymers that consist
of D-glucose units linked primarily by α -1-4 bonds, having a dextrose equivalent (DE) less than 20. Corn syrup solids are obtained by starch depolymerization by an acid/enzyme conversion process where the resulting reducing sugar content is 20 DE or higher.

The study was designed to include 7 treatments: control, 4% and 8% each of 5 DE, 10 DE maltodextrins and 20 DE corn syrup solids. The MDP (27.3 Kg.) was mixed immediately after collection with the respective maltodextrin or corn syrup solids for each treatment using a bowl chopper (Kramer & Grebe model VSM65, GmbH & Co. KG, Wallau/Lahn, Germany) at low speed under vacuum for 1 minute. The MDP was then divided into seven 2.27 Kg. batches and placed in 2 gallon freezer bags (Ziploc, Dowbrands Inc., Indianapolis, IN). Portions consisting of approximately 115 g of meat from each treatment were simultaneously placed into freezer bags (Ziploc, Dowbrands Inc., Indianapolis, IN) with dimensions of 16.5 x 15 cm. Half the bags were frozen in a air-blast freezer at -40°C (Vollrath Refrigeration, Inc., River Falls, WI) while the other half were frozen in the still-air freezer at -10°C. After 3 days, all the samples were moved into a freezer at -28°C (Vollrath Refrigeration Inc., River Falls, WI).

Sampling

Samples were evaluated on the day of preparation of the mixtures (non-frozen), and after 2, 60, 120, 180 and 240 days of frozen storage for both the air-blast and still-air frozen samples.

Evaluations

Proximate analysis

MDP from each treatment and for each freezing method was analyzed for moisture, fat, protein and ash content using AOAC methods (AOAC, 1990, 1993). Moisture was determined by weight loss after 8-12 hr of drying at 100°C in a vacuum oven (Precision Scientific Model 524 A, Precision Scientific Co., Chicago, IL). Fat was determined by weight loss after 16 hr of extraction in a Soxhlet apparatus with petroleum ether, and protein was analyzed by using a nitrogen analyzer (Model FP 428, LECO Corp., St. Joseph, MI), a combustion method used to determine nitrogen released at high temperature and measured by thermal conductivity. A nitrogen-to-protein conversion factor of 6.25 was used. Ash was measured by heating the sample to 525°C by using a muffle furnace. Analyses were conducted in triplicates.

<u>Color</u>

Instrumental color determinations were made by using a Hunter Lab Labscan 6000 Spectrocolorimeter (Model JB-1201 M (A), Hunter Asso. Laboratory Inc., Reston, VA) to measure the surface of the 115 g packaged portions after thawing the samples for 24 hr at 2°C. The instrument was standardized by using the white LS-12029 standard plate (X=81.60, Y=86.68, Z=91.18). Compensation for packaging material was achieved during instrument standardization by covering the white LS-12029 standard plate with saran wrap before standardization. Measurements were taken directly on the surface of the packaged portions. Samples were measured for Hunter Lab color "L", "a" and "b" values. Mean value of each sample was obtained from 4 readings.

<u>Thaw drip</u>

Individually frozen meat samples (approximately 115 g) from each treatment and for each freezing method were weighed and the initial weight (g) was recorded. The frozen sample was then removed from the bag and put in a funnel which was placed on a graduated cylinder to facilitate draining of the released fluids. The top of the funnel was covered with aluminum foil to prevent evaporation. The samples were thawed at room temperature. After 8 hr, the samples were reweighed and the final weight (g) was recorded. Thaw drip was calculated as: final weight/initial weight x100. Two samples were used for each thaw drip measurement.

Oxidative rancidity measurements

MDP from each treatment and for each freezing method was tested for oxidative rancidity using the TBA (2-thiobarbituric acid) test, as described by Tarladgis et al. (1960). TBA values for each sample were determined as an average of three measurements.

Protein extraction and preparation

Salt-soluble protein was extracted using the method of Camou, (1989). 150g of MDP from each treatment and for each freezing method was thawed in a cooler at 2°C for 48 hr, then homogenized (Waring Blender, Model 7010, New Harford, CT) by blending 1 part of meat with 2.2 parts of a 1°C solution containing 0.56 M NaCl, 17.8 mM Na5P3O10, pH 8.3 and 1 mM NaN3 for 30s. This solution corresponded to 2.25% salt and 0.45% sodium tripolyphosphate, similar to the levels used in commercially processed meat products. The meat/brine mixture was chilled at 2°C for 1 hr and then centrifuged (Beckman Model J-21C Centrifuge, Palo Alto, CA) at 12,000 g, 2°C for 1 hr and the protein extract was separated from the residue and strained through gauze. The protein extract was kept overnight at 1°C for subsequent evaluation.

Soluble protein

The volume of protein extract (ml) was measured for each treatment. Protein concentration in each extract was measured using a nitrogen analyzer (Model FP-428, LECO Corporation, St. Joseph, MI) and recorded as mg of soluble protein per ml of solution. The total protein concentration (g) for each treatment was determined by the formula: volume of protein extracted (ml) x protein concentration (mg/ml). The % of extracted salt-soluble protein for each treatment was calculated as: total protein concentration in extract/protein concentration in MDP x 100.

<u>Gel strength</u>

The protein concentration of samples from each treatment was first adjusted by diluting the initial protein extract to the desired concentration range (approximately 35 mg/ml) with a salt-phosphate solution of the same ionic strength as the original.

Aliquots of 30 g of protein extract were placed in 50 ml beakers. Aluminum foil was placed on top of the beakers to avoid evaporation during heating. The samples were allowed to equilibrate at room temperature for 30 min. before heat treatment which was done by submersion in a controlledtemperature water bath (Model 2562, Forma Scientific Inc., Marietta, OH) at 70°C. Both the water bath and sample temperature was recorded. When the internal temperature of the sample reached 70°C, the heating was stopped, the beakers were placed in ice and stored at 1°C overnight.

Gel strength was measured by the compression-extrusion method (Camou and Sebranek, 1991). The gels were compressed in the beaker by the Instron Universal Testing Machine (Model 4502 Instron Corporation, Canton, MA) equipped with a 1kN load cell. A cyclic computer software program (Cyclic 2, Instron Corp., Canton, MA) was used to measure texture characteristics. An anvil of 35 mm. diameter was used to compress and extrude the protein gels at a constant speed of 100 mm./min. to a final 20% of original height. The height of the peak gave the force needed to compress the protein gel (to about 80% of its height) and gel strength was recorded in N/cm². The gel strength was determined on 3 samples per treatment.

Emulsifying capacity

Emulsifying capacity was measured by using the method of Swift et. al (1961) with several modifications. Wesson corn oil was used for determination of emulsification capacity. Colored corn oil was prepared by adding 0.3 g of the biological stain Oil-red-O to 1 liter of oil and mixing for 60 min. by use of a magnetic stirrer at low speed. The colored oil did not require further mixing prior to use since Oil-red-O is highly soluble in most lipids and will not sediment. 10 ml of protein extract (adjusted to 35 mg/ml) obtained from each treatment was initially mixed with 10 ml of Wesson corn oil (mixed with Oilred-O) and blended for one min. using a stirrer (Fisher Scientific StedFast Stirrer, Model SL 2400, Fisher Scientific Co., Pittsburgh PA) at a constant speed setting of 5. The oil was added at a steady rate of 1 ml/min. The emulsifying capacity endpoint was determined using the Oil-Red-O dye release method of Marshall et al. (1975). A change in color and a drop in viscosity occurred simultaneously at the end point. The ml of oil needed to reach the end point where the emulsion separated into two phases was recorded. Emulsifying capacity for each sample was determined as an average of 3 measurements.

Statistical analysis

The experiment was replicated twice. A randomized-block design (Cochran and Cox, 1992) with treatments in a factorial arrangement (3 dextrose equivalents x 2 concentrations) was used. A positive control (no treatment applied) was also used. The Statistical Analysis System (SAS

Institute Inc. 1991) was used to determine means, standard errors and analysis of variance. The Least Significance Difference (P<0.05) test was used to test difference between means.

RESULTS AND DISCUSSION

The proximate analysis results are shown in Tables 1-4. There were significant differences (P<0.05) in the percentages of moisture and protein between the control, the treatments containing the cryoprotectants at the 4% level and at the 8% level. This was due to the fact that as the level of cryoprotectant was increased, the moisture and protein content based on the same sample weight decreased. For example, from Table 2, the treatment containing the 8% 20 DE corn syrup solid has a moisture content of 60.46% which would correspond to 65.39% on a meat weight basis. The 62.53% moisture in the treatment containing 4% 20 DE would correspond to 65.14% on a meat weight basis. There were no significant differences (P>0.05) for L, a and b values for any of the treatments or for either of the freezing methods used in this study (Appendix B, Tables B1-B3).

Drip loss

The effects of frozen storage on the thaw exudate volume is shown in Table 5. The amount of drip loss was significantly affected (P<0.05) by the freezing method used. Rapid freezing produced a significantly lower drip loss (P<0.05) compared to the slow freezing method. This is probably because rapid freezing produces smaller ice crystals which are uniformly distributed throughout the tissue resulting in less fluid loss during thawing. Slow freezing causes fluid in the extracellular spaces to freeze first, thus increasing the concentration of solutes and drawing water osmotically from the stillunfrozen cell through the semipermeable cellular membrane (Marsden and Hendrickson, 1993). There is, therefore, extensive translocation of the tissue fluid resulting in damage of the myofibrillar walls.

There was no significant effect (P>0.05) of the maltodextrin or the corn syrup solids used on the drip loss measurements throughout the period of frozen storage. Drip loss values did not show an increasing trend with time for either the rapid or slow frozen samples.

There are several other factors that could contribute to thaw exudate losses. These include pH of the meat, storage temperature, time the meat is stored in the temperature range of 1-5°C and time of freezing postmortem (Miller et al., 1980).

Oxidative rancidity

It has been well recognized that lipids in frozen meat are likely to undergo oxidative deterioration. The common measurement of these changes is the TBA test. Mechanically deboned meat is especially prone to lipid oxidation because of its comminuted nature, relatively high concentrations of polyunsaturated fatty acids and high heme pigment concentration which acts as a prooxidant (Kunsman et al., 1978). TBA values, a measure of oxidation products and rancidity, of frozen stored mechanically deboned meat are shown in Table 6. Samples which were frozen rapidly had significantly lower (P<0.05) TBA values compared to the slow frozen samples for as long as 180 days of frozen storage. However, after 240 days of frozen storage there were no significant differences (P>0.05) between the rapid and slow frozen samples. This is probably due to the fact that degradation of the hydroperoxides to carbonyl compounds took place which resulted in a decrease in TBA numbers in the slow frozen samples, whereas the hydroperoxides production continued in the rapidly frozen samples during the 240 days of frozen storage. A similar rise in TBA values followed by a decrease was noted by Awad et al. (1968, 1969). There was no effect (P>0.05) of maltodextrins or corn syrup solids on the TBA values up to 180 days of frozen storage. However, after 240 days of frozen storage, the TBA values for treatments containing 4% 5 DE, 4% 10 DE maltodextrins and 4% and 8% 20 DE corn syrup solids were significantly (P<0.05) lower than the control. Hence, the maltodextrins and corn syrup solids appear to provide some protection for the mechanically deboned meat from oxidative rancidity during long-term frozen storage.

Theories regarding frozen storage-induced deterioration of meat postulate interactions between proteins and lipid degradation products. Free fatty acids, the products of hydrolytic rancidity, have been shown to bind to proteins in model systems (Bull and Breese, 1967a) and will denature proteins (Bull and Breese, 1967b). In addition, Buttkus (1969) has shown that malonaldehyde, a product of polyunsaturated fatty acid oxidation, reacts with meat proteins to produce denaturation. Observations made on the possible effects of peroxides in meat emulsion systems suggests that these compounds

may exhibit deleterious effects on emulsion stability, implying protein alteration.

Protein solubility

Changes in the concentration of extractable salt-soluble proteins during frozen storage are shown in Table 7. Significant differences (P<0.05) were observed between different treatments after 2 days of frozen storage. The treatment containing 8% 20 DE corn syrup solid had the lowest concentration of extracted salt-soluble proteins followed by the control and the 4% 5 DE maltodextrin.

After 60 days of frozen storage, the amount of extractable salt-soluble protein in the control decreased by approximately 31% whereas, the protein extractability decreased by 7-12% in the treatments containing the cryoprotectants. Further reduction of 15% was observed in the control after 120 days of frozen storage whereas the treatments containing the cryoprotectants were unaffected. After 180 days of frozen storage, the salt-soluble proteins from the control for both rapidly and slow frozen samples showed very limited solubility. After 240 days of frozen storage, the treatments containing 4% 5 DE maltodextrins for both the rapid and slow frozen samples developed similar limited solubility of these proteins. The 4% and 8% levels of both the 10 DE maltodextrin and the 20 DE corn syrup solids were effective in preventing losses of protein solubility throughout the entire period of frozen storage; however, the mean values were not significantly different (P>0.05). The

method of freezing (rapid vs. slow) did not seem to contribute to the extractability of salt-soluble proteins (P>0.05).

The relatively poor salt-soluble protein extractability of the 5 DE maltodextrin may be attributable to an interference by the high molecular weight maltodextrin with the myofibrillar protein in the meat. Being a low DE derivative of starch, the maltodextrin is chemically quite similar to pregelatinized starch. The reduced functionality of muscle proteins in the presence of starch may result from their competition for water with protein, or from interaction with proteins which result in poor protein extractability and poor protein gelation (MacDonald and Lanier, 1991). Pregelatinized starch was shown by Wu et al. (1985) to interfere with surimi proteins with respect to their gel-forming ability.

Decreased protein extractability during frozen storage has been reported by Park et al. (1987a). These authors observed a 30% loss of protein extractability in the control and a 20% loss in treatments containing cryoprotectants after 2 months of frozen storage of prerigor beef. Miller et al. (1980) reported that total extractable proteins decreased by approximately 50% for pork and 36% for beef after 25 weeks at -17.8°C. Awad et al. (1969) observed a change from 90.99% to 50.84% of total extractable protein in beef muscle over an 8-week storage at -40°C.

As stated by Matsumoto (1980), a decrease in the amount of salt-soluble protein (particularly actomyosin) is regarded as a primary criterion of freeze denaturation and/or aggregation. Insolubilization of proteins during frozen storage is known to occur as a result of the formation of inter-molecular

hydrogen or hydrophobic bonds as well as disulfide bonds or ionic interactions (Akahane, 1982).

The solubility of actomyosin was found to decrease by approximately 23% in a 0.1M sucrose treatment and by approximately 28% in a 0.1M sorbitol treatment after 7 weeks frozen storage, while a 54% decrease was noted in the control treatment (Noguchi et al., 1976). Buttkus (1970) similarly observed a 70% decrease in solubility of rabbit myosin held at -10°C for 2 weeks and only a 30% decrease of the solubility in the presence of 5.4M glycerol.

Protein gelation

Protein gelation, as measured by gel forming ability was monitored as an indicator of muscle protein functionality. Changes in gel strength for the several treatments following frozen storage are shown in Table 8. A significant difference (P<0.05) in gel strength was observed between the control and treatments containing 8% 10 DE maltodextrin and 8% 20 DE corn syrup solids after 60 days of frozen storage. A decrease of approximately 60% in gel strength was noted for gels prepared from the control following 60 days storage. Gels prepared from the cryoprotectant group maintained significantly higher levels of gel strength, even though values decreased over time.

After 120 days of frozen storage, the treatment containing 8% 10 DE maltodextrin showed highest gel strength (P<0.05) compared to the control. However, it was not significantly (P>0.05) different from the rest of the treatments containing the cryoprotectants. Thus, cryoprotectant addition was effective in reducing loss of gel properties which occurs due to protein denaturation and/or aggregation. The method of freezing (rapid vs. slow) did not have a significant (P>0.05) effect on the gel strength up to 120 days of frozen storage. However, after 240 days, the rapid frozen samples had significantly higher (P<0.05) gel strength compared to the slow frozen samples.

Similar results were observed by Park et al. (1987a). A decrease of 45% in stress-at-failure and 30% in strain-at-failure was noted upon mechanical failure of control gels prepared from pre-rigor beef following 2 months frozen storage. However, the cryoprotectant treated group maintained higher levels of stress and strain at failure during 6 months frozen storage. Uijttenboogaart et al. (1993) observed a significantly higher gel strength in treatments containing sucrose/sorbitol and polydextrose compared to the control after 2and 3 week frozen storage of chicken myofibrillar protein isolates.

Emulsifying capacity

Emulsifying capacity is generally defined as the maximum amount of lipid emulsified by a protein dispersion (Mangino, 1994). This test is often used as an indictor of the quality of proteins for use in meat emulsions. Table 9 shows the changes in emulsifying capacity for the various treatments during frozen storage. Both the freezing method used (rapid vs. slow) and the various cryoprotectants used had a significant effect on the emulsifying capacity measurements. Rapidly frozen samples exhibited a significantly higher (P<0.01) emulsifying capacity compared to the slow frozen samples during all samples times (except the two day sampling time). The higher level (8%) of maltodextrins and corn syrup solids used caused a significant increase (P<0.01) in emulsifying capacity compared to these used at the lower level (4%). The lowest emulsifying capacity was observed for the control, while the highest emulsifying capacity was achieved by the treatment with 8% 10 DE maltodextrin and 8% 20 DE corn syrup solids throughout the period of frozen storage.

Swift et al. (1961) showed that higher levels of extracted salt-soluble proteins increased emulsifying capacity. Miller et al. (1980) observed a dramatic diminution of emulsifying capacity between 0 and 7 weeks of frozen storage for pork and 0 and 1 week of frozen storage for beef.

Evidence obtained here indicates that the quality of both protein and fat deteriorated during frozen storage. While there was no emulsion instability as a result of 240 days of frozen storage of MDP in most of the treatments containing the cryoprotectants, the trend suggests an eventual failure following further storage. The first signs of change were evident after 60 days of freezing and by 240 days, all treatments showed significant degradative changes.

Sikroski et al (1976) in a review of protein changes in frozen fish, included partial dehydration and the formation of formaldehyde as additional factors influencing the denaturation of muscle proteins. The observed changes in the present study support the hypothesis that formation of degradation products and interaction with muscle tissue during frozen storage result in deceased protein solubility, emulsifying capacity and gel strength for mechanically deboned pork. For these reasons, one option for long-term frozen storage of mechanically deboned meat is supplementation with cryoprotectants to inhibit freeze-induced protein denaturation and protect functional properties of the proteins. The most effective cryoprotectants in this study were the 10 DE maltodextrin and 20 DE corn syrup solids used at the 8% level.

CONCLUSIONS

The results of this study suggest that addition of 10 DE maltodextrin or 20 DE corn syrup solids may contribute to stabilizing the myofibrillar proteins during frozen storage of mechanically deboned meat. The evidence for this included greater retention of protein solubility, gel strength and emulsifying capacity in treatments containing these cryoprotectants. The 5 DE maltodextrin used at the 4% level resulted in no improvement, however, at the 8% level, some degree of cryoprotection was observed.

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Treatments	Days of frozen storage						
	0	2	60	120	180	240	
Control	18.39 ^a	18.95 ^a	18.49 ^a	17.67 ^a	18.00 ^a	17.91 ^a	
$4\%5\mathrm{DE}^1$	18.23 ^a	17.84 ^a	18.08 ^a	17.33 ^a	17.54 ^a	18.25 ^a	
8% 5 DE	17.68 ^a	17.85 ^a	17.44^{a}	17.16 ^a	17.38 ^a	17.55^{a}	
4% 10 DE	18.09 ^a	18.30 ^a	17.87 ^a	16.60 ^a	17.76 ^a	18.00 ^a	
8% 10 DE	17.85 ^a	17.46^{a}	17.73^{a}	$16.70^{\mathbf{a}}$	17.10^{a}	17.74^{a}	
4% 20 DE	18.09 ^a	18.12^{a}	18.12^{a}	17.11 ^a	17.53^{a}	17.81 ^a	
8% 20 DE	18.01 ^a	18.05 ^ª	18.19 ^a	16.99 ^a	17.43 ^a	18.18 ^a	
SEM ²	0.16	0.13	0.28	0.31	0.18	0.29	
Freezing method	l						
Rapid freezing		17.92^{a}	17.85 ^a	17.03 ^a	17.49 ^a	17.93^{a}	
Slow freezing		18.24 ^a	18.13 ^a	17.13 ^a	17.41 ^a	17.78 ^a	
SEM ²		0.17	0.15	0.17	0.09	0.16	

Table 1. Fat content (%) in mechanically deboned pork treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean.

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^aMeans with the same letters within the same column are not significantly different (P>0.05).

Treatments	Days of frozen storage						
	0	2	60	120	180	240	
Control	64.81 ^a	64.35 ^ª	64.77 ^a	65.45 ^a	65.19 ^a	65.27 ^a	
$4\% 5 \mathrm{DE}^1$	62.44^{b}	63.14^{b}	62.82 ^b	62.97 ^b	63.37 ^b	62.63^{b}	
8% 5 DE	60.67 ^c	60.34 ^c	60.63 ^c	61.65 ^c	60.79 ^c	60.62^{c}	
4% 10 DE	62.73 ^b	62.63 ^b	63.00 ^b	63.78 ^b	6 2.84 ^b	62.99^{b}	
8% 10 DE	60.33 ^c	60.79 ^c	60.44 ^c	61.27 ^c	60.92 ^c	60.52^{c}	
4% 20 DE	62.53^{b}	62.61^{b}	62.53^{b}	63.42^{b}	63.00^{b}	62.87^{b}	
8% 20 DE	60.46 ^c	60.43 ^c	60.31 ^c	61.33 ^c	60.82 ^c	60.34 ^c	
SEM ²	0.19	0.27	0.21	0.37	0.19	0.31	
Freezing method							
Rapid freezing	*****	62.20 ^a	62.23 ^a	62.94 ^a	61.95 ^a	61.38^{a}	
Slow freezing		61.88 ^a	61.91 ^ª	62.71 ^a	61.97 ^a	61 .56 ^a	
SEM ²		0.14	0.11	0.20	0.10	0.16	

Table 2. Moisture content (%) in mechanically deboned pork treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage							
	0	2	60	120	180	240		
Control	16.08 ^a	15.79 ^a	15.74 ^a	15.76 ^a	15.88 ^a	16.41 ^a		
$4\% 5 \text{ DE}^1$	15.66 ^{a,b}	$15.25^{\mathbf{b}}$	15.21^{b}	15.23^{b}	15.38^{b}	16.04 ^{a,b}		
8% 5 DE	14.87 ^d	14.70°	$14.72^{c,d}$	14.56^{d}	14.80^c	$15.28^{\rm c,d}$		
4% 10 DE	$15.37^{b,c}$	15.27^{b}	15.16 ^b	15.14^{b}	15.32^{b}	$15.60^{\mathrm{b,c}}$		
8% 10 DE	15.10 ^{c,d}	14.75 ^c	14.76 ^c	14.82^{c}	14.88 ^c	$15.28^{\mathrm{c,d}}$		
4% 20 DE	15.58 ^{b,c}	15.31^{b}	15.41^{b}	15.36^{b}	$15.42^{\rm b}$	$15.76^{\mathrm{b,c}}$		
8% 20 DE	14.62 ^d	14.42 ^c	14.43 ^d	14.39 ^d	14.47 ^c	15.01 ^d		
SEM^2	0.14	0.11	0.10	0.07	0.13	0.18		
Freezing method								
Rapid freezing		15.14^{a}	15.13^{a}	15.07^{a}	15.00^{a}	15.57^{a}		
Slow freezing		15.00 ^a	14.99 ^a	15.01 ^a	15.09 ^a	15.20 ^a		
SEM ²		0.06	0.05	0.04	0.07	0.19		

Table 3. Protein content (%) in mechanically deboned pork treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b, c, d}Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage						
	0	2	60	120	180	240	
Control	1.07 ^a	0.98 ^a	1.04 ^a	1.01 ^a	1.02 ^a	1.01 ^a	
4% 5 DE ¹	1.04^{a}	0.94 ^a	$1.02^{a,b}$	0.96 ^b	0.98 ^a	1.01 ^a	
8% 5 DE	0.99 ^a	0.95^{a}	$0.98^{\mathrm{b,c}}$	$0.92^{\mathrm{b,c}}$	0.95 ^a	0.94 ^a	
4% 10 DE	1.00^{a}	0.92^{a}	$1.02^{\mathrm{a,b}}$	0.96 ^b	0.99 ^a	0.94 ^a	
8% 10 DE	0.98 ^a	0.94 ^a	$1.00^{a,b,c}$	$0.94^{\mathrm{b,c}}$	0.95^{a}	0.94 ^a	
4% 20 DE	1.02^{a}	0.95^{a}	1.03 ^a	$0.95^{\mathrm{b,c}}$	1.05^{a}	0.97^a	
8% 20 DE	1.02 ^a	0.85 ^a	0.96 ^c	0.91 ^c	0.92 ^a	0.95^{a}	
SEM ²	0.02	0.03	0.02	0.01	0.04	0.03	
Freezing method							
Rapid freezing		0.95^{a}	1.03 ^a	0.94^a	1.01 ^a	0.98^{a}	
Slow freezing		0.92^{a}	0.98 ^b	0.96 ^a	0.95 ^b	0.95 ^a	
SEM ²		0.02	0.01	0.01	0.02	0.01	

Table 4. Ash content (%) in mechanically deboned pork treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b, c,} Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage							
	0	2	60	120	180	240		
Control	1.49 ^a	10.07 ^a	10.97 ^a	10.34 ^a	12.48 ^a	13.71 ^a		
$4\% 5 \mathrm{DE}^1$	0.4 6 ^a	7.72 ^a	9.66 ^a	9.16 ^a	9.83 ^a	11.53^a		
8% 5 DE	0.50^{a}	8.80 ^a	8.62 ^a	8.30 ^a	9.86^a	8.74 ^a		
4% 10 DE	0.68 ^a	8.96 ^a	10.55^{a}	10.20^{a}	12.04 ^a	12.05^{a}		
8% 10 DE	1.34 ^a	10.97 ^a	11.55^{a}	12.56^{a}	10.27 ^a	9 .29 ^a		
4% 20 DE	1.22^{a}	8.66 ^a	9.16^a	8.35 ^a	9.74 ^a	9.98^a		
8% 20 DE	0.67 ^a	11.66 ^a	11.03^a	10.08 ^a	11.77 ^a	10.55 ^a		
SEM ²	0.38	0.89	0.87	0.70	0.99	1.06		
Freezing method		<u>.</u>				_		
Rapid freezing		7.99 ^b	8.88 ^b	8.76 ^b	9.56 ^b	8.55 ^b		
Slow freezing	684 # 8	11.11 ^a	11.56 ^a	10.95 ^a	11.60 ^a	11.70 ^a		
SEM^2		0.47	0.46	0.37	0.53	0.56		

Table 5. Effect of maltodextrins and corn syrup solids on the drip loss (%) measurements in mechanically deboned pork.

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¹Dextrose equivalent. ²Standard error of the mean. ^{a, b}Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage						
	0	2	60	120	180	240	
Control	1.71 ^a	1.92 ^a	4.21 ^a	4.98 ^a	6.04 ^a	6.41 ^a	
$4\% 5 \text{DE}^1$	1.23 ^a	1.45^{a}	3.76 ^a	4.95^a	3.48^a	4.50^{b}	
8% 5 DE	1.80 ^a	2.01^{a}	5.13^{a}	5.35 ^a	4.28 ^a	$4.82^{\mathrm{a,b}}$	
4% 10 DE	1.52^{a}	1.64^{a}	3.98^a	4.54 ^a	3.71 ^a	4.54^{b}	
8% 10 DE	1.61 ^a	1.64 ^a	5.64^{a}	4.98 ^a	4.12^{a}	4.76 ^{a,b}	
4% 20 DE	1.12^{a}	1.38 ^ª	3.46 ^a	5.30^a	4.61 ^a	3.80^{b}	
8% 20 DE	0.85 ^a	1.16 ^a	2.49 ^a	3.22 ^a	3.44^a	3.34^{b}	
SEM ²	0.36	0.48	0.99	0.81	0.61	0.54	
Freezing Method			<u>.</u>				
Rapid freezing	e	0.95 ^b	3.4 ^b	4.29 ^b	3.44 ^b	4.38 ^a	
Slow freezing		2.24^a	4.78^a	5.23 ^a	4.44 ^a	4.12 ^a	
SEM ²		0.25	0.52	0.43	0.32	0.29	

Table 6. Effect of maltodextrins and corn syrup solids on the thiobarbituric acid (TBA) values in mechanically deboned pork.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b}Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage							
	0	2	60	120	180	240		
Control	34.80 ^a	38.47 ^{b,c}	26.41 ^b	22.53 ^b		*****		
$4\% 5 \text{ DE}^1$	33.33 ^a	38.22 ^{b,c}	35.37 ^a	38.02 ^a	37.09 ^a			
8% 5 DE	38.27 ^a	42.09 ^a	37.31 ^ª	40.62^{a}	40.03^{a}	34.11 ^a		
4% 10 DE	32.95 ^a	40.85 ^{a,b}	37.07 ^a	38.94 ^a	37.99 ^a	33.65 ^a		
8% 10 DE	34.23 ^a	$39.12^{a,b}$	34.95 ^{a,b}	38.86 ^a	36.43 ^a	$31.52^{\mathbf{a}}$		
4% 20 DE	38.45 ^a	41.92 ^a	38.55 ^a	41.76 ^a	35.98 ^a	$31.48^{\mathbf{a}}$		
8% 20 DE	36.13 ^a	35.36 ^c	33.50 ^{a,b}	41.44 ^a	38.28 ^a	26.23 ^a		
SEM ²	2.03	1.10	2.84	1.86	1.68	4.28		
Freezing method		9	9		9			
Rapid freezing		39.26 [°]	35.57	38.35	37.52°	34.72 ^ª		
Slow freezing		39.60 ^ª	33.91 [°]	36.5 6 ª	37.74 ^ª	28.07 ^a		
SEM ²		0.59	1.52	0.10	0.97	2.71		

Table 7. Effect of maltodextrins and corn syrup solids on the protein solubility (%) in mechanically deboned pork.

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¹Dextrose equivalent. ²Standard error of the mean. ----- = none extracted. ^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

	Days of frozen storage						
Treatments	0	2	60	120	180	240	
Control	8.94 ^a	5.05 ^{a.}	1.98 ^c	1.91 ^b			
$4\% 5 \mathrm{DE}^2$	5.66 ^a	4.87 ^a	$2.28^{b,c}$	$2.55^{\mathrm{a,b}}$	2.36 ^ª	**===	
8% 5 DE	6.59 ^a	4.41 ^a	2.61 ^{a,b,c}	$2.52^{\mathrm{a,b}}$	2.24 ^a	2.13 ^a	
4% 10 DE	8.13 ^a	4.00 ^a	$2.57^{\mathrm{a,b,c}}$	2.69 ^{a,b}	2.16 ^a	2.18^{a}	
8% 10 DE	7.46 ^a	4.79 ^a	3.50 ^a	3.25 ^a	2.31^{a}	2.46 ^a	
4% 20 DE	8.54 ^a	4.37 ^a	2.44 ^{a,b,c}	2.55 ^{a,b}	2.01 ^a	2.06 ^a	
8% 20 DE	8.35 ^a	4.49 ^a	3.34 ^{a,b}	2.66 ^{a,b}	2.37 ^a	2.17 ^a	
SEM ³	1.37	0.70	0.39	0.31	0.81	0.19	
Freezing method		_			-		
Rapid freezing		4.85 ^a	2.95 ^ª	2.67 ^a	2.36 ^a	2.54 ^ª	
Slow freezing		4.29 ^a	2.39 ^a	2.50^{a}	2.12^{a}	1.86 ^b	
SEM ³	#===#	0.37	0.21	0.16	0.10	0.12	

Table 8. Effect of maltodextrins and corn syrup solids on the gel strength¹(N) of protein extracts from mechanically deboned pork.

¹Protein concentration of the extract was adjusted to 35 mg/ml. ----- = not measured. ²Dextrose equivalent. ³Standard error of the mean.

^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

Treatments	Days of frozen storage							
	0	2	60	120	180	240		
Control	131.50 ^a	138.75 ^a	109.25 ^f	97.00 ^f				
$4\% 5 \mathrm{DE}^2$	143.00^{a}	142.50^{a}	123.00 ^e	110.25^{e}	$94.50^{\mathbf{d}}$			
8% 5 DE	132.75^{a}	139.63 ^a	137.25°	126.00^c	100.50 ^{c,d}	$85.00^{ m c}$		
4% 10 DE	144.50 ^a	139.00 ^a	128.75^{d}	116.00 ^d	103.00 ^{b,c}	88.25 ^{b,c}		
8% 10 DE	136.00 ^a	146.50 ^a	163.25 ^a	147.25 ^a	$110.00^{a,b}$	94.50 ^a		
4% 20 DE	146.25 ^ª	145.38 ^a	$127.75^{\mathrm{d,e}}$	$117.50^{\rm d}$	102.00^c	89.00 ^b		
8% 20 DE	145.25 ^a	144.13 ^a	$155.75^{\rm b}$	138.75 ^b	112.00^a	96.25 ^a		
SEM ³	6.74	2.76	1.75	1.85	2.37	1.19		
Freezing method				-	-	_		
Rapid freezing		141.54 ^ª	140.36 ^a	125.86 ^ª	106.33 ^ª	93.80 ^ª		
Slow freezing		143.00 ^a	129.64 [°]	117.79 ⁰	101.00 ^b	87.40 ^b		
SEM ³		1.47	0.93	0.99	1.37	0.75		

Table 9. Effect of maltodextrins and corn syrup solids on the emulsifying capacity¹ (ml of oil) of protein extracts from mechanically deboned pork.

¹Emulsifying capacity was measured for 10 ml of the adjusted (35 mg/ml) protein extract. ²Dextrose equivalent. ³Standard error of the mean. ---- = not measured.

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^{a, b, c, d, e}Means with different letters within the same column are significantly different (P<0.05).

EFFECT OF FROZEN STORAGE AND CRYOPROTECTANTS ON THE QUALITY AND SENSORY CHARACTERISTICS OF PORK SAUSAGE MADE FROM MECHANICALLY DEBONED PORK

A paper to be submitted to the Journal of Food Science G. A. Prabhu¹ and J. G. Sebranek¹

ABSTRACT

Changes in the quality and sensory characteristics of pork sausage made from frozen mechanically deboned pork with added cryoprotectants (5 DE, 10 DE maltodextrins and 20 DE corn syrup solids) was investigated. Incorporation of cryoprotectants in mechanically deboned pork significantly improved the storage stability (oxidative rancidity) and affected the quality (texture) of the pork sausage. Sensory evaluation showed the most notable quality differences to be color, tenderness and flavor desirability.

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INTRODUCTION

Use of mechanically deboned meat (MDM) has been studied extensively in recent years because it has potential to contribute greatly to the production of low cost meat products (Uebersax et al, 1978). Due to the comminuted nature of MDM, this product is prone to quality deterioration if not handled and/or stored properly (Mast et al., 1979). Spoilage caused by microbial growth is a problem in non-frozen MDM, whereas oxidative rancidity is the major cause of deterioration in frozen MDM. Lipid oxidation may be especially acute in MDM from beef, pork and lamb due to the relatively high concentrations of polyunsaturated fatty acids of the phospholipids (Kunsman and Field, 1976). The heme pigment concentration, in MDM from red meats is also high (Field, 1976). Thus, with adequate substrate available (polyunsaturated fatty acids of the phospholipids), increased levels of known pro-oxidants present (heme pigments) as well as the finely divided physical nature of the product, MDM from red meats are a product highly susceptible to lipid oxidation (Kunsman et al., 1978).

To improve the functionality of frozen meats for use as ingredients in processed meat products, means of preventing or inhibiting freeze-induced denaturation and/or aggregation of proteins are needed (Park et al., 1987b). Cryoprotectants, added to meat prior to freezing, have been shown to improve long-term storage stability of proteins during frozen storage. This, in turn, assures good functionality of the material in the subsequent manufacture of meat products. Protein functionality, in this case, is expressed primarily as

gel-forming potential with its manifestations of texture formation and waterbinding properties (Macdonald and Lanier, 1991). This was observed by Park et al. (1987a) during frozen storage of prerigor salted beef.

Contrary to earlier shelf-life studies on beef where it was assumed that no significant changes took place at -30°C or -40°C (Jul, 1984), it is now realized that significant changes in functionality take place during storage at temperatures as low as -28°C (Park et al., 1987a, b). Furthermore, the degree of deterioration in protein functionality would be expected to be accentuated in frozen mechanically deboned meat due to its comminuted nature (MacDonald and Lanier, 1991).

The use of mechanically deboned red meat has been studied in cooked salami (Joseph et al., 1978), dry fermented salami (Berry et al., 1979), ground beef (Cross et al., 1977, 1978; Seideman et al., 1977), bologna (Field et al., 1975; Chant et al., 1977; Misock et al., 1979) and frankfurters (Bushway et al., 1982; McMillin et al., 1980; Meiburg et al., 1976; Marshall et al., 1977).

McMillin et al. (1980) reported that frankfurters containing 30% or less mechanically separated pork (MSP) were similar in smoke house yields and percentage of water and fat lost during processing compared to frankfurters containing 40% or 50% MSP.

Cross and Kotula (1978) used 0%, 10% and 20% mechanically deboned beef (MDB) in ground beef formulations followed by storage at -12°C, -18°C and -23°C. Formulation (level of MDB) and storage time had no significant effect on any of the palatability traits evaluated. Joseph et al. (1978) used 0, 10, 20 and 30% levels of MDB in cooked salami stored for 0, 14, 28, 42 and 56 days at 6°C. Less desirable flavor, juiciness, tenderness and texture scores were found in salami containing 20-30% levels of MDB. The flavor profile panel results indicated higher aroma and flavor intensities and more rancid flavors with increasing level of MDB and extended storage time.

A study by Cross et al. (1977) on the palatability of ground beef patties containing different levels of MDB indicated that patties containing 5, 10, 15 and 20% MDB were considered more acceptable than the control. As the percentage of added MDB increased, panel ratings for tenderness and juiciness increased.

A recent amendment by USDA's Food Safety and Inspection Service to the Federal Regulations allows meat produced by advanced meat and bone separation machinery that does not crush or grind bones to be labeled as "beef" or "pork" instead of mechanically separated "beef" (or pork) as required in the past (USDA, 1995). This rule went into effect in January, 1995. This has resulted in a potential increase in the usage of this low cost meat source especially in processed meat products. MDM has been approved for use in fresh pork sausage provided the finished product contains less than 50% fat (USDA, 1995).

Because previous work has shown cryoprotectants to protect functional properties of proteins in MDM during long-term frozen storage, it seems likely that cryoprotectants could improve quality of products in which the MDM is used (Prabhu and Sebranek, 1996).

The purpose of this study was to evaluate the chemical and sensory characteristics of pork sausage in which 50% of the pork was replaced with mechanically deboned pork which had been frozen containing two different levels of 5 DE, 10 DE maltodextrins and 20 DE corn syrup solids.

MATERIALS AND METHODS

Product Manufacture

Fresh mechanically deboned pork (MDP) was obtained from a commercial source, directly off the processing line. The maltodextrins and corn syrup solids were provided by Grain Processing Corporation (Muscatine, IA). The MDP was immediately mixed with the maltodextrins or corn syrup solids using a bowl chopper (Kramer & Grebe model VSM65, GmbH & Co. KG, Wallau/Lahn, Germany) at low speed with a vacuum for 1 minute. The maltodextrins and corn syrup solids evaluated included two different levels (4% and 8%) of 5 DE, 10 DE maltodextrins and 20 DE corn syrup solids. Maltodextrins are non-sweet, nutritive saccharide polymers that consist of D-glucose units linked primarily by α -1-4 bonds, having a dextrose equivalent (DE) less than 20. Corn syrup solids are obtained by starch depolymerization by an acid/enzyme conversion process where the reducing sugar content is 20 DE or higher. After the addition of the maltodextrins or corn syrup solids, the MDP was divided into five 2.27 Kg. batches and placed in 2 gallon freezer bags (Ziploc, Dowbrands Inc., Indianapolis, IN). Half of the bags were frozen in a

air-blast freezer at -40°C (Vollrath Refrigeration Inc., River Falls, WI) while the other half was frozen in the still-air freezer at -10°C. After 3 days, all the samples were moved into a freezer at -28°C (Vollrath Refrigeration Inc., River Falls, WI). After 1 week of storage at -28°C, the frozen MDP from each treatment and for each freezing method was flaked using a flaker (Butcher Boy, Model CMF, Lasar Manufacturing Co. Inc., Los Angeles, CA) and divided into 0.5 Kg. batches and re-frozen in freezer bags (Ziploc, Dowbrands Inc., Indianapolis, IN) with dimensions of 16.5 x 15 cm. until further use.

Fresh pork sausage was formulated to contain 50% flaked MDP from each treatment and 50% fresh pork containing approximately 20%-25% fat. A control, with no maltodextrin or corn syrup solids was also formulated. Pork sausage seasoning mix (A. C. Legg Spice Company, Auburn, AL) was added to the meat mixtures, mixed by hand, and the mixture was ground through a 1/8" plate (Biro Model 822, Biro Manufacturing Co., Marble Head, OH). Patties were formed with a hand-held patty former. Each patty weighed approximately 113 g, with a diameter of 11.0 cm and a thickness of 1.0 cm. The patties were frozen in a blast freezer at -40°C (Vollrath Refrigeration Inc., River Falls, WI) for 12 hr.

Pork sausage was manufactured following 14, 60, 120 and 180 days of frozen storage for both the air-blast and still-air frozen MDP; finished sausage product evaluations were conducted at each of these sampling times.

Evaluations

Proximate analysis

Raw meat mixtures from each treatment and for each freezing method were analyzed for fat, moisture, protein and ash content using AOAC methods (AOAC, 1990, 1993). Moisture was determined by weight loss after 8-12 hr of drying at 100°C in a vacuum oven (Precision Scientific Model 524 A, Precision Scientific Co., Chicago, IL). Fat was determined by weight loss after 16 hr of extraction in a Soxhlet apparatus with petroleum ether, and protein was analyzed by using a nitrogen analyzer (Model FP 428, LECO Corp., St. Joseph, MI), a combustion method used to determine nitrogen released at high temperature and measured by thermal conductivity. A nitrogen-to-protein conversion factor of 6.25 was used. Ash was measured by heating the sample to 525°C using a muffle furnace. Analyses were conducted in triplicates.

Cooking loss

Weight losses during cooking were measured for each treatment and for each freezing method. Cooking losses were calculated as a percentage of the raw uncooked product weight. Six patties from each treatment combination were used for the cooking loss measurements.
Instrumental color evaluation

Instrumental color determinations were measured on the cooked sausage patties using a Hunter Lab Labscan 6000 Spectrocolorimeter (Model JB-1201 M (A), Hunter Asso. Laboratory Inc., Reston, VA) which was standardized using the white LS-12029 standard plate (X=81.60, Y=86.68, Z=91.18). Samples were measured for Hunter Lab color "L", "a" and "b" values. Patties were wrapped in saran before measurement and packaging material was compensated for during instrument standardization by covering the white LS-12029 standard plate with saran wrap before standardization.

Measurements were taken directly on the surface of the wrapped pattie. Mean value of each sample was obtained from four readings at two locations on each pattie surface.

Oxidative rancidity measurements

Cooked pork sausage from each treatment and for each freezing method was tested for oxidative rancidity using the TBA (2-thiobarbituric acid) test, as described by Tarladgis et al., (1960). TBA values for each sample were determined as an average of three measurements.

Instrumental texture evaluation

Texture was evaluated on the cooked sausage patties using an Instron Universal Testing Machine (Model 4502, Instron Corp., Canton MA) equipped with a 9-mm-diameter probe and a cyclic computer software program (Cyclic 2, Instron Corp., Canton MA). A "first bite" penetration test was used to determine the hardness of samples. A preconditioning program (20 g preload) was used to correct for small differences in height of the cooked sausage patties. The patties were first cooled to room temperature and measurements were taken perpendicular to the surface of each patty in three separate locations on each patty. The probe was set at 30 mm from the bottom of the plate and moved downward at a speed of 300 mm/min., stopping when it sensed the sample. It was then permitted to puncture the sample completely. Peak load expressed as Kg (force required to puncture the sample), peak energy (total work expended in puncturing until peak load was reached), and total energy (total work expended in completely puncturing the sample) were registered. The values obtained were an average of nine readings from three patties for each treatment combination.

Sensory evaluation

Frozen pork sausage patties were first weighed and then placed on a Wolf gas grill with a surface temperature of 176.6°C. The patties were cooked on one side for 10 min. and then turned on the other side. Total cooking time averaged 20 min. and cooking end-point temperature (internal) was monitored until 70°C was reached. Six patties per treatment, for each freezing method were prepared. They were then wrapped in aluminum foil and placed in an oven at 51.6°C to keep them warm.

Cooked pork sausage was evaluated by a 20-24 member untrained panel which included graduate students and staff who had participated in previous sensory evaluations of sausage products. Samples (cut into triangular wedges) from the eight treatments were placed in random order on a sample plate, and labeled with three-digit random numbers. The samples were presented simultaneously to all the panelists. Panelists were isolated in individual booths under fluorescent light. The panelists consumed unsalted crackers and water at room temperature (25°C) between samples. Samples from all seven treatments for each freezing method were evaluated separately during each session. Five attributes were evaluated using a seven-point hedonic scale: color (1=extremely light to 7=extremely dark), tenderness (1= extremely tender to 7= extremely tough), juiciness (1=extremely juicy to 7=extremely dry), flavor desirability or off-flavor (1=extremely desirable to 7=extremely undesirable) and flavor intensity (1=extremely strong to 7=extremely bland).

Statistical analysis

The experiment was analyzed using a completely randomized design (Cochran and Cox, 1992) and consisted of 7 treatments (6 treatments and a control) each with 2 freezing methods (rapid and slow) giving a total of 14 treatments which were replicated twice. The Statistical Analysis System (SAS Institute Inc. 1991) was used to determine means, standard errors and analysis of variance. Least Significance Difference (P<0.05) was used to test differences between means.

RESULTS AND DISCUSSION

The raw pork sausage showed a range in composition for all samples of 17.9-27.7% fat, 41.4-60.8% moisture, 14.0-16.4% protein and 2.5-3.2% ash but formulations did not significantly (P>0.05) differ within any one sampling time (Appendix C, Tables C1-C4). Pork sausage formulations at the 14 day and 60 day sampling points had a fat content of approximately 20% and approximately 27% at the 120 and 180 day sampling time. This probably had an effect on the cooking loss values which were higher for the 120 and 180 day samples and lower for the 14 and 60 day samples. The level or type of maltodextrin or corn syrup solid used however, did not have a significant effect (P>0.05) on the cooking loss. There were no significant differences (P>0.05) in cooking loss between the rapid and slow frozen samples, although the cooking losses were slightly higher for the slow frozen samples at all sampling times (Appendix C, Table C5). Nusbaum et al (1983) observed a significantly lower cooking loss in beef patties frozen using a faster freezing rate compared to the patties frozen using a slower freezing rate.

Light reflectance of the cooked pork sausage, as measured, quantitated diffuse surface reflectance, which is an indication of lightness or darkness of surface color. There were no significant differences (P>0.05) in the L values for pork sausage from any of the MDP samples for up to 180 day frozen storage. Although the formulation containing 8% 20 DE corn syrup solids had the lowest L value throughout the storage period, it was not significantly different (P>0.05) from the other treatments (Appendix C, Table C6).

It was expected that with higher dextrose levels in a product, greater non-enzymatic browning would be likely during heating due to reducing sugars. Sometimes this can result in unwanted browning in a meat product (Shand et al., 1990). However, no significant darkening of cooked samples occurred in this study.

It has been shown that lipids present in frozen meat are likely to undergo oxidative deterioration during storage, as indicated by the TBA test. TBA values, a measure of malonaldehyde and rancidity are shown in Table 1. TBA values showed an increasing trend over the period of frozen storage. There were significant differences in TBA values between treatments. TBA values were lowest for pork sausage treatments containing 8% 10 DE maltodextrin and 4% and 8% 20 DE corn syrup solids after 14 days of frozen MDP storage. The highest TBA values were observed for the pork sausage treatment containing 4% 5 DE maltodextrin, however, this was not significantly different (P>0.05) from the same maltodextrin used at the 8% level or the control.

After 60 days of frozen MDP storage, the pork sausage treatment containing the 8% 20 DE corn syrup solids had the lowest TBA value compared with any of the maltodextrins or corn syrup solids used at the 4% level or the control (P<0.05). However, the pork sausage containing the 20 DE corn syrup solids was not significantly different from the pork sausage treatments containing 8% 5 DE or the 8% 10 DE maltodextrins (P>0.05). After 120 days of frozen MDP storage, the pork sausage containing the 4% 5 DE maltodextrin treatment was significantly higher (P<0.05) in TBA values than the rest of the

treatments and the control. After 180 days of frozen MDP storage, the maltodextrins used at the 8% level and corn syrup solids used at the 4% and 8% level significantly (P<0.05) lowered the TBA values compared to the 4% level or the control. The freezing method (rapid vs. slow) did not have a significant effect (P>0.05) on the TBA values except after 180 days of frozen MDP storage when the rapid frozen samples had significantly lower TBA values than the slow frozen samples (P<0.05).

Cross sectional peak force required to puncture the pork sausage is shown in Table 2. The peak force values did not show any significant trend with storage time. However, there were significant differences in the peak force registered between treatments. Treatments containing the 8% 20 DE corn syrup solids and the control resulted in significantly higher (P<0.05) peak force values throughout the frozen storage period.

Sensory evaluation of the pork sausage showed the most notable quality differences to be color, tenderness and flavor desirability (Tables 3, 4 and 5). Other sensory characteristics (juiciness and flavor intensity) did not differ significantly (P>0.05) for any of the treatments (Appendix C, Tables C7 and C8).

Sensory color scores are shown in Table 3. Panelists found the pork sausage treatments containing 8% 20 DE corn syrup solids or 4% 10 DE maltodextrin to be significantly darker than 4% 20 DE corn syrup solids or 4% 5 DE maltodextrins after 14 days of frozen MDP storage. However, after 120 days of frozen MDP storage, panelists found treatments containing 8% 20 DE corn syrup solids to be significantly darker (P<0.05) than treatments containing 4% 5 DE, 4% 10 DE maltodextrins or 4% 20 DE corn syrup solids. After 180 days of frozen MDP storage, the pork sausage treatments containing 8% 10 DE maltodextrin or 8% 20 DE corn syrup solids were considered significantly darker (P<0.05) than the treatments containing the 4% 5 DE and the 4% 10 DE maltodextrins. Due to the higher dextrose content in the pork sausage at the 8% level of maltodextrins and corn syrup solids, more non-enzymatic surface browning probably occurred upon cooking. This could have a negative impact on the appearance of the cooked product with the higher level of dextrose. There were no significant differences (P>0.05) in color between the rapid and slow frozen samples.

Panelists found pork sausage containing maltodextrins and corn syrup solids to be significantly more tender (P<0.05) than the control when manufactured after 60 days of frozen MDP storage (Table 4). However, there were no significant differences for tenderness as a result of the level or the type of maltodextrins or corn syrup solids used. Similar results were observed at the 120 and 180 day sampling times.

Flavor desirability scores are shown in Table 5. Panelists were able to detect off-flavors in the pork sausage treatment containing 4% 5 DE maltodextrin after 60 days of frozen storage of MDP. This trend continued throughout the entire period of frozen storage. This flavor change corresponded to the higher TBA values observed for the pork sausage treatment containing the 4% 5 DE maltodextrin throughout the frozen storage period (Table 1). Panelists found pork sausage containing 8% 10 DE maltodextrin and 8% 20 DE corn syrup solid to be most desirable in terms of

flavor. The freezing method (rapid vs. slow) did not have a significant impact on the flavor desirability or the tenderness of products made with MDP regardless of the entire frozen storage time.

CONCLUSIONS

Results of this study suggest that 10 DE maltodextrin and 20 DE corn syrup solids may be most effective in improving the storage stability of mechanically deboned pork used in the manufacture of pork sausage. This was reflected by lower TBA values for pork sausage from MDP treatments containing 10 DE maltodextrins and 20 DE corn syrup solids used at the 8% level. Sensory evaluation of the pork sausage showed significantly higher color and tenderness scores in treatments containing the cryoprotectants for mechanically deboned pork. The pork sausage containing MDP treated with 5 DE maltodextrin at 4% adversely affected the TBA values and also resulted in a pork sausage with lower flavor desirability scores. The method of freezing (rapid vs. slow) did not have any effect on any of the quality or sensory attributes evaluated.

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	Days of frozen storage of MDP					
Treatments	14	60	120	180		
Control	1.39 ^{a,b}	1.14 ^b	1.68 ^b	2.34 ^a		
$4\%5{ m DE}^1$	1.55 ^a	1.90 ^a	2.77 ^a	2.46 ^a		
8% 5 DE	1.35 ^{a,b}	1.06 ^{b,c}	1.39 ^b	1.60^{b}		
4% 10 DE	$1.42^{a,b}$	1.21 ^b	1.28 ^b	2.29 ^a		
8% 10 DE	1.04 ^b	1.06 ^{b,c}	1.16 ^b	1.67 ^b		
4% 20 DE	0.98 ^b	1.22^{b}	1.22 ^b	1.63 ^b		
8% 20 DE	0.80 ^b	0.88 ^c	0.94 ^b	1.32 ^b		
SEM^2	0.15	0.07	0.26	0.16		
Freezing method				_		
Rapid freezing	1.17 ^a	1.22^{a}	1.43^{a}	1.53 ^b		
Slow freezing	1.64 ^a	1.19 ^a	1.56 ^a	2.27^{a}		
SEM ²	0.08	0.04	0.14	0.08		

Table 1.	Thiobarbituric	e acid (TBA) values	in pork	sausage	manufactur	ed with	mechanically
	deboned pork	previously	treated w	vith mal	todextrin	ns and corn s	syrup so	lids.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

	Days of frozen storage of MDP				
Treatments	14	60	120	180	
Control	1.83 ^{a,b}	2.01 ^{a,b}	1.64 ^a	1.64 ^a	
$4\% 5 \mathrm{DE}^1$	$1.62^{a,b}$	$1.88^{a,b}$	1.64^{a}	$1.47^{a,b}$	
8% 5 DE	$1.63^{a,b}$	1.73^{b}	$1.32^{b,c}$	$1.27^{\mathbf{b}}$	
4% 10 DE	1.54^{b}	$1.75^{a,b}$	$1.40^{\mathrm{a,b,c}}$	$1.45^{a,b}$	
8% 10 DE	$1.64^{\mathrm{a,b}}$	1.89 ^{a,b}	$1.33^{b,c}$	$1.47^{\mathrm{a,b}}$	
4% 20 DE	1.71 ^{a,b}	$1.97^{\mathrm{a,b}}$	$1.57^{a,b}$	1.64 ^a	
8% 20 DE	1.97 ^a	2.03^{a}	1.64 ^a	1.64 ^a	
SEM ²	0.14	0.09	0.08	0.11	
Freezing method					
Rapid freezing	1.66 ^a	1.93 ^a	1.33 ^b	1.46 ^a	
Slow freezing	1.75 ^a	1.86 ^a	1.57 ^a	1.39 ^a	
SEM ²	0.07	0.05	0.05	0.06	

Table 2.	Peak load (Kg) by Instron for pork sausage manufactured with mechanically
	deboned pork previously treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean. ^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

	Days of frozen storage of MDP					
Treatments	14	60	120	180		
Control	4.71 ^{a,b}	3.76 ^a	5.26 ^a	4.56 ^{a,b}		
$4\% 5 \mathrm{DE}^2$	4.35 ^b	4.61^a	$4.54^{b,c}$	$3.65^{\mathbf{c}}$		
8% 5 DE	$5.00^{\mathrm{a,b}}$	4.64^a	5.07 ^{a,b}	$4.67^{a,b}$		
4% 10 DE	5.34 ^a	4.55^{a}	4.05 ^c	3.96 ^{b,c}		
8% 10 DE	4.99 ^{a,b}	4.55^{a}	5.00 ^{a,b}	4.83 ^a		
4% 20 DE	4.36 ^b	5.03 ^a	4.41 ^{b,c}	$4.52^{a,b}$		
8% 20 DE	5.20 ^a	4.95 ^a	5.49 ^a	5.26 ^a		
SEM ³	0.27	0.48	0.19	0.26		
Freezing method						
Rapid freezing	4.80 ^a	4.68^{a}	4.66 ^b	4.55 ^a		
Slow freezing	4.89^a	4.49^a	5.00^{a}	4.43 ^a		
SEM ³	0.15	0.26	0.10	0.14		

Table 3. Sensory color scores¹ for pork sausage manufactured with mechanically deboned pork previously treated with maltodextrins and corn syrup solids.

¹Color scores based on a 7 point hedonic scale. Color 1=extremely light to 7=extremely dark. ²Dextrose equivalent. ³Standard error of the mean.

^{a, b, c}Means with different letters within the same column are significantly different (P<0.05).

Days of frozen storage					
Treatments	14	60	120	180	
Control	2.29 ^a	3.01 ^a	3.29 ^a	3.48 ^a	
4% 5 DE [*]	2.10 ^ª	2.31	2.69	2.39	
8% 5 DE	2.14^{a}	2.46	2.29 ^b	2.11 ^b	
4% 10 DE	2.02^{a}	2.17 ^D	2.26 ^b	2.26 ^b	
8% 10 DE	1.95 ^a	2.23^{b}	2.39^{b}	2.33^{b}	
4% 20 DE	2.04^{a}	2.66^{b}	2.49^{b}	2.48^{b}	
8% 20 DE	2.04^{a}	2.25^{b}	$2.46^{\mathbf{b}}$	2.03 ^b	
SEM ³	0.21	0.29	0.17	0.21	
Freezing Method					
Rapid freezing	2.04^{a}	2.36 ^a	2.41 ^b	2.14^{a}	
Slow freezing	2.13 ^a	2.51^{a}	2.69 ^a	2.43 ^a	
SEM ³	0.11	0.15	0.09	0.11	

Table 4.	Tenderness scores	for pork sausage	manufactured	with mechanical	ly
	deboned pork previ	ously treated with	maltodextrins	and corn syrup s	olids

¹Tenderness scores based on a 7 point hedonic scale. Tenderness 1=extremely tender, 7=extremely tough. ²Dextrose equivalent. ³Standard error of the mean. ^{a, b}Means with different letters within the same column are significantly different (P<0.05).

		Days of frozen storage			
Treatments	14	60	120	180	
Control	3.23 ^a	3.24 ^c	3.28 ^{b,c}	3.23 ^{a,b}	
$4\% 5 \mathrm{DE}^2$	3.27 ^a	4.32 ^a	3.87 ^a	3.77 ^a	
8% 5 DE	3.09 ^a	3.73 ^b	3.53 ^{a,b}	3.03 ^b	
4% 10 DE	3.31 ^a	3.37 [°]	3.35 ^{b,c}	3.25 ^{a,b}	
8% 10 DE	3.46 ^a	3.35 [°]	2.98 ^c	2.96 ^b	
4% 20 DE	3.45 ^a	3.40 ^{b,c}	3.01 ^c	3.01 ^b	
8% 20 DE	3.39 ^a	3.50 ^{b,c}	3.09 ^c	3.09 ^b	
SEM ³	0.16	0.12	0.14	0.20	
Freezing method		_	_		
Rapid freezing	3.31 ^ª	3.74 ^a	3.28 ^ª	3.05 ^a	
Slow freezing	3.32 ^a	3.38 ^a	3.32 ^a	3.34^a	
SEM ³	0.09	0.06	0.08	0.11	

Table 5.	Flavor desirability scores	¹ for pork sausage manufactured with mechanically
	deboned pork previously t	treated with maltodextrins and corn syrup solids.

¹Flavor desirability based on a 7 point hedonic scale. Flavor desirability 1=extremely desirable, 7=extremely undesirable. ²Dextrose equivalent. ³Standard error of the mean.

^{a, b, c} Means with different letters within the same column are significantly different (P<0.05).

GENERAL SUMMARY

Three experiments were conducted to study the functionality of hydrocolloids in meat systems. The first experiment evaluated the effects of starch and carrageenan on the quality characteristics of hams containing high levels of added water. The second experiment investigated the cryoprotectant effects of maltodextrins and corn syrup solids for freeze-thaw stability in mechanically deboned pork. The third experiment was done to study the quality and sensory characteristics of pork sausage made from frozen mechanically deboned pork containing these cryoprotectants and stored for various times.

The first part of this investigation was a study of the functionality of starch and carrageenan as water-binding and texture modifying agents in hams containing 55% added water. Eight treatments were formulated: kappacarrageenan (0% or 1.5%) x starch (0%, 2%, 3.5% or 5%). Hams were evaluated for cooking yields, purge, color, texture and sensory characteristics. Results from this experiment revealed that incorporation of carrageenan at a level of 1.5% increased yields, decreased purge and resulted in a sensory perception of reduced juiciness. Increasing levels of starch increased perception of juiciness. Combinations of starch and carrageenan. There was no synergistic effect on moisture retention due to a combination of starch and carrageenan. Microscopic studies were conducted to determine the location and distribution

of starch and carrageenan in ham. Starch and carrageenan were randomly distributed in localized areas with no evidence of an interaction between them.

The second part of this investigation was a study of the cryoprotectant effect of corn maltodextrins and corn syrup solids on frozen mechanically deboned pork. The experiment included 7 treatments: control, 4% and 8% each of 5 DE, 10 DE maltodextrins and 20 DE corn syrup solids blended with mechanically deboned pork followed by freezing with two freezing methods (rapid and slow). Samples were evaluated for color, thaw drip, protein solubility, gel strength, emulsifying capacity and oxidative rancidity.

Drip loss was significantly affected by the method of freezing (P<0.05) however, it was not affected by the type or level of maltodextrins or corn syrup solids used. TBA values, a measure of oxidative rancidity was also affected by the method of freezing but not by the maltodextrins or the corn syrup solids up to 180 days of frozen storage. However, after 240 days, 4% 5 DE, 4% 10 DE maltodextrins and 4% and 8% 20 DE corn syrup solids were significantly lower for TBA values than the control (P<0.05).

The amount of extractable salt-soluble protein after 60 days of frozen storage decreased by 31% in the control and by 7%-12% in the treatments containing the cryoprotectants. The 4% and 8% levels of both 10 DE maltodextrins and 20 DE corn syrup solids were effective for preventing losses of protein solubility. The treatment containing the 4% 5 DE developed limited solubility of the protein after 240 days of frozen storage.

A significant decrease (P<0.05) in gel strength was observed between the control and treatments containing 8% 10 DE maltodextrin and 8% 20 DE corn

syrup solids after 60 days of frozen storage. Rapidly frozen samples exhibited a significantly higher (P<0.01) emulsifying capacity compared to the slow frozen samples during the entire period of frozen storage. The maltodextrins and corn syrup solids used at the 8% level caused a significant increase in emulsifying capacity, followed by that used at the 4% level, while the lowest emulsifying capacity was presented by the control. The rapidly frozen samples exhibited a significantly higher emulsifying capacity compared to the slow frozen samples during all sampling times.

The third part of this investigation was a study of the quality and sensory characteristics of pork sausage in which 50% of the pork was replaced with frozen mechanically deboned pork containing cryoprotectants. The experiment included 7 treatments: control, 4% and 8% each of 5 DE, 10 DE maltodextrins and 20 DE corn syrup solids. The mechanically deboned pork with cryoprotectants was frozen using two freezing methods (rapid and slow). Pork sausage samples containing mechanically deboned pork were evaluated for cooking loss, color, texture, oxidative rancidity and sensory characteristics.

Cooking loss and the light reflectance values were unaffected by the freezing method, the type or the level of maltodextrins and corn syrup solids used throughout the period of frozen storage. TBA values, a measure of oxidative rancidity, increased over time, but were lowest for treatments containing 4% 10 DE maltodextrins and 4% and 8% 20 DE corn syrup solids after 2 weeks. Maltodextrins and corn syrup solids used at the 8% level were effective in lowering the TBA values compared to those used at the 4% level or

the control. The freezing method did not have a significant effect on the TBA values.

Peak force values for measurements of the pork sausage texture indicated that the treatments containing 8% 20 DE and the control required greatest penetration force throughout the period of frozen storage. Sensory evaluation of pork sausage showed the most notable quality differences to be color, tenderness and flavor desirability.

The research indicates that hydrocolloids impart several functional properties to meat systems. The starch and carrageenan used in this study were effective as water-binders and texture modifying agents in hams containing large quantities of added water. Freeze-induced protein denaturation of frozen mechanically deboned pork was reduced effectively by the use of 10 DE maltodextrin and 20 DE corn syrup solids. Future research should focus on the understanding of the molecular mechanism by which high molecular weight carbohydrates like maltodextrins and corn syrup solids prevent losses in meat protein functionality during frozen storage. APPENDIX A

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Treatment	Fat (%)	Moisture (%)	Protein (%)
Starch (%)	<u> </u>	—	·····
0	2.02^{a}	75.40 ^a	17.99 ^a
2	2 .17 ^a	74.00 ^{a,b}	17.85 ^a
3.5	2. 88 ^a	73.41 ^b	17.48 ^{a,b}
5	2.89 ^a	72.85 ^b	16.37 ^b
SEM^1	0.35	0.28	0.40
Carrageenan (%)			
0	2.21^{a}	74.19 ^a	17.70 ^a
1.5	2.77 ^a	73.64 ^a	17.14 ^a
SEM^1	0.49	0.40	0.56
Probabilities			
Replication	0.3544	0.0487	0.6305
Starch	0.7246	0.0146	0.0491
Carrageenan	0.4353	0.2780	0.1793
Starch*Carrageenan	0.4090	0.3811	0.2089

Table 1A: Fat, moisture and protein values for hams treated with kappa-carrageenanand modified corn starch

^{a, b}Means with different letters are significantly different (P<0.05).

¹Standard error of the mean.

Peak Load (N)	Peak Energy	Total Energy
<u></u>		
2.29 ^a	0.15 ^a	0.29 ^a
2.25 ^a	0.15 ^a	0.28^{a}
2.17 ^a	0.14 ^a	0.28^a
1.96 ^a	0.13 ^a	0.25 ^a
0.09	0.03	0.01
2.12^{a}	0.14 ^a	0.27 ^a
2.22 ^a	0.14 ^a	0.28 ^a
0.12	0.04	0.02
0.4276	0.6183	0.2842
0.2740	U.6389 0.7947	0.3145
0.1333	0.5135	0.2138
	Peak Load (N) 2.29 ^a 2.25 ^a 2.17 ^a 1.96 ^a 0.09 2.12 ^a 2.22 ^a 0.12 0.4276 0.2740 0.4418 0.1333	Peak Load (N) Peak Energy 2.29 ^a 0.15 ^a 2.25 ^a 0.15 ^a 2.17 ^a 0.14 ^a 1.96 ^a 0.13 ^a 0.09 0.03 2.12 ^a 0.14 ^a 2.22 ^a 0.14 ^a 0.12 0.04 0.4276 0.6183 0.2740 0.6385 0.4418 0.7347 0.1333 0.5135

Table 2A: Peak Load (N), peak energy and total energy values for hams treated with kappa-carrageenan and modified corn starch

^aMeans with same letters are not significantly different (P>0.05).

¹Standard error of the mean.

Treatment	L	a	b
Starch (%)		· · · ································	
0	54.68 ^a	8.91 ^a	5.91 ^a
2	56.51 ^a	7.76^{a}	5.16 ^a
3.5	55.59 ^a	7.87 ^a	5.19 ^a
5	53.41 ^a	8.36 ^a	5.01 ^a
SEM ¹	1.07	0.35	0.23
Carrageenan (%)			
0	55.14 ^a	8.43 ^a	5.54 ^a
1.5	54.96 ^a	8.02 ^a	5.10 ^a
SEM ¹	1.52	0.49	0.33
Probabilities			
Replication	0.6241	0.0139	0.0007
Starch	0.5353	0.3645	0.2651
Carrageenan	0.9065	0.4128	0.2042
Starch*Carrageenan	0.0703	0.4826	0.3129

Table 3A: L, a and b values for hams treated with kappa-carrageenan and modified corn starch

^aMeans with same letters are not significantly different (P>0.05).

¹Standard error of the mean.

Treatment	toughness	cohesiveness	flavor intensity	flavor desirability	color	overall acceptabi	lity
Starch (%)	<u></u>						
0	7.34 ^a	8.01 ^a	7.66 ^a	8.49 ^a	9.02^{a}	7.81 ^a	
2	9.07 ^a	7.93 ^a	8.09 ^a	8.15 ^a	6.35 ^a	7.9 3 ^a	
3.5	8.75 ^a	8.42^{a}	8.46 ^a	7.97 ^a	8.37 ^a	7.83 ^a	
5	8.40 ^a	7.07^{a}	7.93 ^a	8.16 ^a	7 .23 ^a	7.60^{a}	
SEM ²	3.34	2.52	2.54	2.51	7.94	1.90	
Carrageenan (%)						
0	8.71 ^a	7.74 ^a	8.10 ^a	8.24 ^a	7.84 ^a	8.02^{a}	16
1.5	8.07^{a}	7.99 ^a	8.01 ^a	8.15 ^a	7.64 ^a	7.57 ^a	-
SEM^2	4.73	3.57	3.59	3.55	11.22	2.69	
Probabilities							
Replication	0.0409	0.5188	0.1010	0.0399	0.9754	0.0404	
Starch Carrageanan	0.0987	0.4939	0.4773	0.8033	0.3749	0.8555	
Starch*Carrag.	0.0891	0.2918	0.7676	0.5499	0.3455	0.3864	

Table 4A: Sensory evaluation¹ scores for hams treated with kappa-carrageenan and modified corn starch

^aMeans with same letters are not significantly different (P>0.05). ²Standard error of the mean.

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1Sensory measurements using a 15-cm line scale. Toughness 0=very tough; 15=very tender; Cohesiveness 0=not cohesive; 15= very cohesive; Flavor intensity 0=not flavorful; 15=very flavorful; Flavor desirability 0=very undesirable; 15=very desirable; Color 0=light; 15=dark; Overall acceptability 0=not acceptable; 15=very acceptable

APPENDIX B

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	Days of frozen storage						
Treatments	0	2	60	120	180	240	
Control	36.55 ^a	40.70 ^a	41.63 ^a	46.13 ^a	40.76 ^a	43.76 ^a	
$4\% 5 \mathrm{DE}^1$	42.67^{a}	41.68^a	41.15^a	44.72 ^a	41.19 ^a	42.95 ^a	
8% 5 DE	41.63 ^a	40.83 ^a	35.91^{a}	43.54^a	43.10 ^a	42.93 ^a	
4% 10 DE	40.87 ^a	40.40 ^a	42.43 ^a	45.32^{a}	$43.40^{\rm a}$	42.01^{a}	
8% 10 DE	40.02^{a}	40.22^{a}	40.71^{a}	42.65^{a}	40.75 ^a	41.59 ^a	
4% 20 DE	41.47^{a}	41.44 ^a	43.88 ^a	44.92 ^a	43.23 ^a	43.14 ^a	
8% 20 DE	38.21 ^a	36.77 ^a	40.09 ^a	41.49 ^a	39.63 ^a	38.99 ^a	
SEM ²	1.88	1.10	2.49	1.11	1.16	1.17	
Freezing method	l						
Rapid freezing		40.91 ^a	42.87^{a}	44.29^a	$42.20^{\rm a}$	42.35^{a}	
Slow freezing		39.96 ^a	39.64 ^a	43.96 ^a	41.81 ^a	41.11 ^a	
SEM ²		0.59	1.33	0.59	0.57	0.63	

Table B1. "L" values for mechanically deboned pork treated with maltodextrins and corn syrup solids.

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	Days of frozen storage					
Treatments	0	2	60	120	180	240
Control	17.19 ^a	13.55 ^a	14.43 ^a	11.28 ^a	10.70 ^a	9.40 ^a
$4\% 5 \mathrm{DE}^1$	18.13 ^a	13.56 ^a	14.84 ^a	11.65^{a}	12.64 ^a	11.42 ^a
8% 5 DE	17.81^{a}	14.55^{a}	14.59^{a}	11.93^{a}	11.75^{a}	12.04^{a}
4% 10 DE	17.86 ^a	14.31^{a}	15.86^{a}	$12.17^{\mathbf{a}}$	12.87 ^a	$12.52^{\rm a}$
8% 10 DE	17.13 ^a	13.62^{a}	15.02^{a}	11.79^{a}	12.62^{a}	12.38^{a}
4% 20 DE	17.58^{a}	14.61 ^a	· 14.90 ^a	11.87^{a}	12.03^{a}	11.30 ^a
8% 20 DE	17.96 ^a	14.78 ^a	16.39 ^a	13.69 ^a	12.79 ^a	13.07
SEM ²	0.50	0.68	0.57	1.11	0.96	1.05
Freezing method		_	_	_		
Rapid freezing		14.13^{a}	15.75 ^a	12.55^{a}	12.60^{a}	12.21^{a}
Slow freezing		14.15 ^a	14.55 ^a	11.56 ^a	11.13 ^a	11.26 ^a
SEM^2		0.36	0.30	0.35	0.54	0.56

Table B2. "a" values for mechanically deboned pork treated with maltodextrins and corn syrup solids.

Treatments	0	2	60	120	180	240
Control	10.99 ^a	9.52 ^a	9.31 ^a	9.35 ^a	10.09 ^a	8.70 ^a
4% 5 DE ¹	11.99 ^a	9.56^{a}	9.30 ^a	9.24^a	9 .70 ^a	9.23 ^a
8% 5 DE	11.69 ^a	10.32^{a}	10.09^{a}	9.48^a	9.81 ^a	10.18^{a}
4% 10 DE	11.43 ^a	9.95^a	9.88 ^a	9.50 ^a	10.09 ^a	9.92 ^a
8% 10 DE	11.12 ^a	9.37 ^a	9.42 ^a	9.06 ^a	9.46^a	9.75 ^a
4% 20 DE	11.33^a	10.35^{a}	9.29 ^a	9.46^a	9.88^a	10.11^{a}
8% 20 DE	10.81 ^a	9.15 ^a	9.42 ^a	8.91 ^a	8.66 ^a	9.32^a
SEM ²	0.44	0.41	0.37	0.19	0.27	0.32
Freezing method			-			
Rapid freezing		9.77 ^a	9.86 ^ª	9.50 ^ª	9.55 ^a	9.80 ^a
Slow freezing		9.72 ^a	9.20^b	9.06 ^b	9.62 ^a	9.40^a
SEM ²		0.24	0.20	0.10	0.14	0.17

Table B3.	"b" values for mechanically of	deboned pork trea	ated with maltodextrins	and corn
	syrup solids.			

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APPENDIX C

	Days of frozen storage of MDP				
Treatments	14	60	120	180	_
Control	20.59 ^a	18.19 ^a	29.62 ^a	27.73 ^a	
$4\% 5 \mathrm{DE}^1$	19.41 ^a	18.65 ^a	$27.92^{\mathbf{a}}$	28.40^{a}	
8% 5 DE	19.50 ^a	15.96 ^a	27.18^{a}	28.93 ^a	
4% 10 DE	19.76 ^a	$20.62^{\mathbf{a}}$	27.25 ^a	25.53^{a}	
8% 10 DE	19.42 ^a	18.54 ^a	26.72^{a}	27.27^{a}	
4% 20 DE	19.59 ^a	16.83 ^a	27.69 ^a	27.76 ^a	
8% 20 DE	19.71 ^a	16.15 ^ª	27.57 ^a	27.74 ^a	
SEM ²	0.21	1.29	0.87	1.01	
Freezing method	0	9	9	2	
Rapid freezing	19.83	16.99°	28.34	28.53 [°]	
Slow freezing	19.60^a	18.81 ^{°°}	27.07 ^a	26.71 ^ª	
SEM ²	0.11	0.69	0.46	0.54	

Table C1.	Fat content (%) for pork sausage manufactured with mechanically
	deboned pork previously treated with maltodextrins and corn syrup solids.

	Days of frozen storage of MDP				
Treatments	14	60	120	180	
Control	59.19^a	61.48 ^a	44.15 ^a	53.92 ^ª	
$4\% 5 \text{ DE}^1$	59.25^{a}	60.57 ^a	41.52^a	53.38^a	
8% 5 DE	58.25^{a}	61.79 ^a	40.76^a	51.44^{a}	
4% 10 DE	5 9 .03 ^a	59.11^a	40.82^{a}	54.73 ^a	
8% 10 DE	58.52 ^a	59.88 ^a	40.58^{a}	52.51^{a}	
4% 20 DE	5 9 .08 ^a	61.29 ^a	41.16^a	52.85^a	
8% 20 DE	46.95^a	61.26 ^a	40.53 ^a	51.97 ^a	
SEM ²	4.23	1.88	0.50	0.75	
Freezing method	L				
Rapid freezing	58.66 ^a	61.48 ^a	42.02^{a}	54.04^{a}	
Slow freezing	55.70 ^a	60.05 ^a	40.70 ^a	53.62 ^a	
SEM ²	2.26	0.64	0.27	0.40	

Table C2.Moisture content (%) for pork sausage manufactured with mechanically
deboned pork previously treated with maltodextrins and corn syrup solids.

	Days of frozen storage of MDP					
Treatments	14	60	120	180		
Control	16.00 ^a	16.70 ^a	14.04 ^a	14.72 ^a		
$4\%5\mathrm{DE}^1$	16.04^{a}	16.31 ^a	$14.15^{\mathbf{a}}$	14.24 ^a		
8% 5 DE	15.57^{a}	16.57 ^a	13.97^a	13.55^{a}		
4% 10 DE	15.84 ^a	15.85^{a}	14.10 ^a	14.77 ^a		
8% 10 DE	15.69 ^a	16.22^{a}	13.96^a	14.33 ^a		
4% 20 DE	15.98 ^a	16.70^a	14.05 ^a	14.44 ^a		
8% 20 DE	15.67 ^a	16.33 ^a	13.54 ^a	13.98 ^ª		
SEM ²	0.10	0.31	0.15	0.27		
Freezing method						
Rapid freezing	15.84 ^a	16.63 ^a	13.89^a	14.04 ^a		
Slow freezing	15.81 ^a	16.14^a	14.05^{a}	14.54 ^a		
SEM ²	0.05	0.16	0.08	0.14		

Table C3.Protein content (%) for pork sausage manufactured with mechanically
deboned pork previously treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean.

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	Days of frozen storage of MDP				
Treatments	14	60	120	180	M
Control	3.20 ^a	2.83 ^a	2.52 ^a	2.65 ^a	
$4\% 5 \text{ DE}^1$	3.05^{a}	2.70^{a}	$2.73^{\mathbf{a}}$	2.83^{a}	
8% 5 DE	3.01 ^a	2.66^{a}	$2.67^{\mathbf{a}}$	2.59 ^a	
4% 10 DE	$2.85^{\mathbf{a}}$	2.71^{a}	2.62^{a}	2.59 ^a	
8% 10 DE	2.80^{a}	$2.45^{\mathbf{a}}$	2.55^{a}	2.66 ^a	
4% 20 DE	2.99 ^a	$2.67^{\mathbf{a}}$	2.76^{a}	2.82^{a}	
8% 20 DE	3.02 ^a	2.77^{a}	2.52^{a}	2.66 ^a	
SEM ²	0.10	0.13	0.07	0.07	
Freezing method Rapid freezing Slow freezing	2.97 ^a 3.00 ^a	2.73 ^a 2.64 ^a	2.65^{a} 2.60^{a}	2.71^{a} 2.66 ^a	
SEM ²	0.54	0.07	0.04	0.04	

Table C4.Ash content (%) for pork sausage manufactured with mechanically
deboned pork previously treated with maltodextrins and corn syrup solids.

	Days of frozen storage of MDP				
Treatments	14	60	120	180	
Control	19.96 ^a	16.67 ^a	30.31 ^a	20.93 ^a	<u> </u>
4% 5 DE ¹	18.33 ^ª	15.54 ^a	28.12 ^a	18.66 ^a	
8% 5 DE	18.14 ^a	16.14^{a}	$27.54^{\mathbf{a}}$	20.32^{a}	
4% 10 DE	18.43 ^a	15.12^{a}	24.82^{a}	16.98^{a}	
8% 10 DE	18.75 ^a	16.92^{a}	26.48^{a}	21.19 ^a	
4% 20 DE	18.43 ^a	16.67^a	24.96^a	20.24^{a}	
8% 20 DE	19.21 ^a	16.34 ^a	28.65 ^a	20.63^a	
SEM ²	2.31	1.47	2.00	1.75	
Freezing method			2		
Rapid freezing	19.04 ^a	16.56 ^a	25.64 ^a	17.80 ^a	
Slow freezing	21.60 ^a	17.84 ^a	28.04 ^a	20.19 ^a	
SEM ²	1.24	0.78	1.07	0.94	

Table C5. Cooking loss values for pork sausage manufactured with mechanically
deboned pork previously treated with maltodextrins and corn syrup solids.
Days of frozen storage of MDP					
Treatments	14	60	120	180	
Control	38.07 ^a	35.44 ^a	28.65 ^a	30.27 ^a	
$4\% 5 \mathrm{DE}^1$	33.86 ^a	37.08^a	32.40^a	35.38 ^a	
8% 5 DE	33.13 ^a	36.45^a	33.28 ^a	31.68 ^a	
4% 10 DE	39.03 ^a	$36.74^{\mathbf{a}}$	36.17^a	33.93 ^a	
8% 10 DE	33.99 ^a	33.10 ^a	33.42^{a}	34.43^a	
4% 20 DE	36.19 ^a	34.32^{a}	35.91 ^a	31.37 ^a	
8% 20 DE	30.47 ^a	32.26 ^a	30.82 ^a	29.64 ^a	
SEM ²	2.81	1.65	2.31	1.51	
Freezing method	0	9	a		
Rapid freezing	34.73 ^ª	34.24	34.47 [°]	32.89 ^a	
Slow freezing	35.20 ^a	35.88 ^ª	31.43 ^a	31.87 ^a	
SEM ²	1.50	0.88	1.24	0.81	

Table C6. "L" values for pork sausage manufactured with mechanically
deboned pork previously treated with maltodextrins and corn syrup solids.

¹Dextrose equivalent. ²Standard error of the mean.

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^aMeans with the same letters within the same column are not significantly different (P>0.05).

	Days of frozen storage of MDP				
Treatments	14	60	120	180	
Control	3.71 ^a	3.69 ^a	3.81 ^a	2.77 ^a	
$4\%5\mathrm{DE}^2$	3.78 ^ª	3.53 ^ª	4.12^{a}	3.53 ^a	
8% 5 DE	4.07^{a}	4.10 ^a	3.47 ^a	2.76 ^a	
4% 10 DE	3.67 ^a	3.56 ^a	3.40^a	3.45^{a}	
8% 10 DE	3.87 ^a	3.50 ^a	3.17 ^a	3.17 ^a	
4% 20 DE	3.58 ^a	3.81 ^a	3.51^{a}	3.43 ^a	
8% 20 DE	3.64 ^a	3.27 ^a	3.08 ^a	3.55 ^a	
SEM ³	0.20	0.22	0.22	0.26	
Freezing method					
Rapid freezing	3.74^{a}	3.64 ^a	3.48^{a}	3.00 ^a	
Slow freezing	3.78 ^a	3.63 ^a	3.54 ^a	3.18 ^a	
SEM ³	0.10	0.11	0.12	0.14	
Slow freezing	3.74 ^a 0.10	3.63 ^a 0.11	3.54 ^a 0.12	3.18 ^a 0.14	

Table C7. Juiciness scores¹ for pork sausage manufactured with mechanically deboned pork previously treated with maltodextrins and corn syrup solids.

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¹Juiciness scores based on a 7 point hedonic scale. Juiciness 1=extremely juicy to 7=extremely dry. ²Dextrose equivalent. ³Standard error of the mean.

^aMeans with the same letters within the same column are not significantly different (P>0.05).

	Days of frozen storage of MDP				
Treatments	14	60	120	180	
Control	2.86 ^a	3.20 ^a	3.05 ^a	3.06 ^a	
$4\% 5 \mathrm{DE}^2$	3.20^{a}	3.00^{a}	3.26 ^a	$3.47^{\mathbf{a}}$	
8% 5 DE	3.07^{a}	3.37^{a}	2.99 ^a ,	2.95^{a}	
4% 10 DE	2.82^{a}	3.32^{a}	3.37^{a}	3.48^{a}	
8% 10 DE	2.92^{a}	3.39 ^a	3.09^{a}	3.15 ^a	
4% 20 DE	3.07 ^a	3.30^{a}	2.93^{a}	3.17^{a}	
8% 20 DE	2.81 ^a	3.25 ^ª	3.24 ^a	3.03 ^a	
SEM ³	0.14	0.15	0.14	0.15	
Freezing method	٩	8	9	9	
Rapid freezing	2.79 [°]	3.23	3.17	3.10"	
Slow freezing	3.14"	3.29"	3.09 [°]	3.27	
SEM ³	0.08	0.08	0.08	0.08	

Table C8.	Flavor intensity scores	¹ for pork sausa	age manufactured v	vith mechanically
	deboned pork previous	ly treated with	maltodextrins and o	corn syrup solids.

¹Flavor intensity based on a 7 point hedonic scale. Flavor intensity 1=extremely strong, 7=extremely bland. ²Dextrose equivalent. ³Standard error of the mean.

^aMeans with the same letters within the same column are not significantly different (P>0.05).

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor Dr. J. G. Sebranek for his guidance, support and encouragement. He readily responded to my appeal for expert advice and gave me generously of his time whenever I needed help. I gratefully record my obligation to him for his helpful suggestions and pertinent comments. Thank you Dr. Joe, you are the best advisor anyone could ever have.

I also thank Dr. D. G. Olson, Dr. F. C. Parrish Jr., Dr. C. L. Knipe and Dr. C. T. Wright Jr. for serving on my committee.

I am obliged to Jerry Knight and the meat lab crew for their assistance in the processing room. A special thanks to Jerry Knight for "lighting up the place" with his smile. I am grateful to Marcia King-Brink and her staff for assisting with the analyses and to Lisa Mayberry for taking care of all the paper work.

The Meat Science section of the Animal Science department at Iowa State University provides a stimulating research environment and I would like to thank the faculty, staff and students for their valuable ideas and insights that have immensely broadened my education.

I am grateful to Dr. Joseph George of AMPC Inc. for recognizing my abilities and making me a part of his research team even before getting this degree.

I will never forget the love and friendship of Angie. She has been there for me during the good and bad times and our friendship has grown stronger over the years. I thank my friend Mark Christensen from Brazil for showing me "the light at the end of the tunnel" and Yanyun Zhao for her friendship and help with the statistical analyses.

My heartfelt thanks goes out to my parents, brothers, and their families and to my husband's family for sending words of encouragement and support from across the sea.

Finally, I would like to thank my husband for being a "mother" and a "father" to the kids while I was working towards this goal.

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