EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF SHOULDER CLODS

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CHAPTER I

INTRODUCTION

Over the past several years, major national health organizations have made recommendations regarding the consumption of saturated fat and cholesterol, since the overconsumption of these substances has been linked to the occurrence of different health disorders (National Research Council, 1988).

Besides satisfying appetite, meat and other animal products are good sources of nutrients. Through the years, the industry has responded to the consumer demands for flavor, appearance, keeping quality, safety and, more recently nutritional quality (National Research Council, 1988).

The development of low-fat and/or low-cholesterol products has been approached by a number of researchers. Several studies investigated the replacement of animal fat with oils from plant origin (Marquez et al., 1989; Park et al., 1989), whereas other studies suggest that the reduction of the amount of fat can be accomplished through a dilution effect of the animal fats, by the addition of non-meat proteins (Sofos and Allen, 1977; Anonymous, 1990a,

Central Soya, 1990) and through the use of vegetable gums (Anonymous, 1990c).

Other studies suggest that reduction of fat levels in meat products formulations can be accomplished through the use of certain processing technologies (i.e., preblending) (Hand et al., 1987), and by changes in the formulation (Claus et al., 1989; 1990).

The interest in the development of new formulations that contain less fat followed the approval in 1988 of the USDA "40 percent rule". This new regulation allows the manufacture of frankfurters and similar products containing a combination of fat and added water up to 40 percent, and the fat level is restricted to no more than 30 percent (Federal Register, 1988).

Several low-fat meat products are already found in the market. The manufacture of 95% fat-free ham and low-fat bacon are examples of this. Nevertheless, the frankfurter-type products available today as low fat contain 22.5% fat, a reduction of 25% of the original level. "Lite" beef frankfurters containing less than 22.5% fat are not, yet, popular in the market. To successfully manufacture products of lower fat content, a better understanding of the role of the meat proteins in the emulsifying process, and the mechanism of water binding in low fat systems is of primary importance.

Processed meat products (batter-type and sectioned and formed products) depend very much on the formation of a

continuous matrix throughout the product. This property is contributed mostly by the soluble myofibrillar proteins, which have been regarded as the functionally more important meat proteins (Kinsella, 1982; Wilding et al., 1984).

In order to determine the effect of environmental and processing factors on the performance of meat proteins in low-fat formulations, a detailed study of the functional properties of the meat components during different conditions has to be carried out. This would lead to the establishment of the optimal conditions of pH, temperature and, salt and phosphate concentrations, required to obtain good quality low-fat products.

This study investigates the effect of temperature on the water binding and solubility properties of proteins from beef shoulder clods and from formulations prepared at different fat and added water levels.

CHAPTER II

REVIEW OF LITERATURE

The Development of Low-Fat Meat Products and Previous Experimental Work

The relationship between food consumption and health has been one of the most important issues concerning all implicated sectors: consumers, processors and food producers, and health-related governmental agencies. In recent years, attention has turned from the nutrient deficiency to concern for overconsumption of calories, fat and cholesterol (Gormley et al., 1987; National Research Council, 1988). This diet-health awareness represents one of the main issues facing the red meat industry today; animal products have been implicated as major sources of fat-related food components (National Research Council, 1988).

In order to help consumers meet their dietary goals, the industry has responded in two ways: a) formulating existing product lines with reduced fat alternatives and b) developing new products that emphasize leanness and wholesomeness (Bischoff, 1990). Several types of new reduced-fat meat products were introduced to the American market during the past three years. Since 1978, 73 new or

improved variations of already existing frankfurter formulations have been introduced to the market. The same trend away from calories and fat is evident for other products like smoked sausage, bacon, salami, ham and bologna (Bush, 1990; Dornblaser, 1990).

The development of these products and/or changes to existing formulations was made possible after the creation of the "40 percent rule" this rule states that a maximum combination of 40 percent of fat and added water can be used, however, fat is limited to no more than 30 percent of the finished product (Federal Register, 1988). In view of these new developments, water binding may become as important as fat binding in comminuted meat products (Rust and Olson, 1988).

Fat substitution has been approached in different ways:

Use of Non-Meat Ingredients

Use of Carbohydrate-Based Replacements. For more than a decade, gums, polydextrose, and corn or potato starch maltodextrins have been used to partially or totally replace fats or oils in a wide variety of food products (Anonymous, 1990b). Gums or plant hydrocolloids, mainly carrageenan, are commonly used in order to modify the texture of many products. Among the claimed benefits of carrageenan in reduced fat meat systems are: assistance in retaining moisture, stabilization of the emulsion and enhancement of mouthfeel. Also, it has been reported that carrageenan

allows for a 50% fat reduction while maintaining product quality (Foegeding and Ramsey, 1986; Anonymous, 1988).

In preventing water loss from a low-fat meat emulsion, xanthan gum has been reported to be more effective than carrageenan (Wallingford and Labuza, 1983). Lin et al. (1988) studied the effect of four types of carboxymethyl cellulose (CMC) in low-fat frankfurters. Their results indicated that as the molecular weight of the CMC decreased, the emulsion stability also decreased. However, the processing yield and proximate composition of the products were not affected.

Use of Oils of Plant Origin. Since not only the amount, but also the type of fat in the diet is becoming increasingly important for consumers, several studies have dealt with the incorporation of unsaturated oils to replace the fat from animal products.

Park et al. (1989) reported results using high-oleic sunflower oil (HOSO) and omega-3 polyunsaturated oil from fish. Frankfurters with 5% fish oil received very low sensory scores. The incorporation of maximum amounts of HOSO into low fat beef-pork and all-beef frankfurters increased oleic acid by 34 and 62%, respectively. Texture profile analysis and sensory evaluation showed that the reduction in total fat caused texture problems, especially increased firmness and decreased juiciness. Townsend et al. (1971) investigated the effects of varying types and levels

of fat on emulsion and frankfurter characteristics. Shrinkage was inversely related to fat content and occurred to a extent in frankfurters containing cottonseed oil when compared with beef or pork fat. Peanut oil was the replacement ingredient in a study dealing with the stability and sensory quality of beef frankfurters containing different levels of final fat (Marquez et al., 1989). Less emulsion stability, lower smokehouse yield and lower sensory juiciness scores were noted as final fat content of beef frankfurters was lowered to 12%. Firmness, darkening of external color, and flavor intensity were enhanced in the low fat product. Frankfurters with 60% fat as peanut oil were equally acceptable to sensory panels as 29% beef fat frankfurters.

Use of Plant Proteins. The addition of plant proteins has been shown to improve product texture, as the fat level of meat decreases and the moisture level increases. These proteins would also improve the products' nutritional value (Bischoff, 1990). High levels of textured soy protein (45%) did not adversely affect emulsion stability of formulations low in fat (Sofos and Allen, 1977).

Decker et al. (1986) conducted studies to determine the effect of using isolated soy protein (ISP) as a meat replacement in frankfurter formulations of different fat levels. As the fat level was reduced the values increased with respect to color and texture. Replacement of meat with

ISP resulted in lower texture scores than using leaner meats. Ma et al. (1989) used acid-hydrolized oat protein isolates in a wiener-type product. This study reported significantly higher firmness and cohesiveness for products manufactured using isolates.

Torgensen and Toledo (1977) studied the physical properties of various protein preparations that could be related to their functional properties in comminuted meat products. The impact of using whey protein concentrate, peanut flour, single cell protein and chicken meat protein in a luncheon loaf formulation was evaluated. Significant correlation coefficients were noted between fat binding and solubility at any temperature ranging, from 4.5 to 72°C, solubility at 100°C, textural mechanical properties, and water absorption capacity at 90°C and water binding. The pattern of solubility change over a range of temperatures was a better index of performance than solubility at a single temperature.

Changes in Formulations and Use of Different Processing Techniques

Under this system, the reduction of fat could be accomplished by either using leaner meats (with the subsequent increase in cost), or by a dilution effect from substances like water.

The physical, chemical and organoleptic properties of beef-pork bologna formulated with different combinations of

fat and added water were studied by Claus et al. (1989). The results indicated that textural parameters increased with higher protein content. Color scores were increased as water level increased and uncooked batters had lower shear values and higher purge losses (when cooked) as added water increased.

The effect of preblending in combination with reduced levels of fat and salt, was determined with frankfurters prepared from beef and pork trimmings (Hand et al., 1987). Regarding preblending, this study suggested that textural properties were not affected by preblending; however, with alterations in the formulations, low fat, low sodium frankfurters could be manufactured.

The effects of massaging, preblending, and time of addition of water and fat on several characteristics of bologna were studied by Claus et al. (1990). Massaging increased Instron texture profile analysis (TPA) values compared with preblended and non-preblended products. However, none of the processing regimes produced the desired water-binding capacity.

The results from these studies suggest that it is possible to manufacture low fat, high added water products, but the role of the different components of meat batters in low fat formulations still has to be determined. Product water-binding capacity appears to be one of the new limitations replacing fat-binding ability, in production (Rust and Olson, 1988). Purge losses could be reduced

through a better understanding of the performance properties of proteins, their types, and their interactions with water and fat in meat batters.

Muscle Proteins

With the exception of water, proteins are the major component of lean meat. On the basis of solubility, they may be broadly classified into water-soluble, salt soluble, and insoluble fractions (Tarrant, 1982). Classifications can also be made on the basis of distribution, organization and function in the living muscle. For example, intracellular and extracellular proteins are those that reside, respectively, inside and outside the sarcolemmal membrane (Asghar et al., 1985).

Intracellular Proteins

These can be further subdivided into two main groups: sarcoplasmic and myofibrillar proteins.

The soluble proteins of the sarcoplasm are referred to as "sarcoplasmic proteins". About 100 different proteins are known to be present in the sarcoplasmic fraction constituting 30 to 35% of total muscle protein or about 5% of the weight of muscle (Asghar et al., 1985). Some of the main characteristics of sarcoplasmic proteins are: very low water-binding capacity, form low-viscosity solutions, to interact slightly with lipids in emulsion formation, and to not have the ability to form gels (from protein-protein

interactions). From the solubility standpoint, water or low-concentration salt solutions can be utilized (Acton et al., 1983).

Studies on the effect of pH suggest that there is a dramatic decrease in solubility, as pH is lowered to approximately 5.6. Further, the amount of soluble protein increases, at constant pH, as the temperature is increased from 18 to 40°C. No effect was observed between heat treatments; the most significant effect was in pH (Trautman, 1967).

Proteins which compose the myofibrils within muscle fibers are collectively defined as "myofibrillar proteins". They constitute about 55 to 60% of total muscle protein, or 10% of the weight of muscle. These can be subdivided into contractile and regulatory proteins.

The myofibrillar proteins myosin and actin are directly involved in the contraction-relaxation cycle of living muscle and are termed "contractile proteins" (Asghar et al., 1985). Actomyosin is formed by the reversible linking of myosin and actin during pre-rigor muscle contraction and by cross-linking which characterizes post-rigor meat (Tarrant, 1982). Tropomyosin and troponins are major regulatory proteins which impart calcium sensitivity to the contractile protein (actomyosin) system in muscle (Asghar et al., 1985).

In recent years, more information has become available regarding the nature and chemistry of intermediate filaments. Chemically, they differ from contractile and

regulatory proteins in many aspects. Morphologically they resemble collagen fibrils which exist extracellularly.

These proteins are believed to strengthen the architecture of the myofibrillar system in muscle by acting as "scaffold" or "backbone" proteins. They were isolated from the KI-insoluble residue of skeletal muscle and exhibited very high molecular weights.

The main fractions identified are known as: titin, nebulin and desmin. Recently, fractions of these proteins have been prepared without the use of denaturing solvents. The availability of these preparations should enable properties relevant to processed meat products (i.e., heat-induced protein-binding ability) to be evaluated (King and MacFarlane, 1986; Asghar et al., 1985).

Extracellular Proteins

The interstitial space of muscle cells contains three proteins fibrillar in nature: collagen, reticulin and elastin.

The fibrils of collagen consist of a triple helix, that shorten to about one third of their original length when heated to about 70° C. At temperatures above 80° C, collagen is converted into gelatin. Reticulin, which resembles collagen in structural aspects, does not produce gelatin upon heating. Elastin is not decomposed by heat and has a very limited swelling ability. This protein is responsible for the elastic properties of binding tissue

(Schut, 1976; King and MacFarlane, 1986; Asghar et al., 1985).

Functionality of Meat Proteins

Proteins are structural components of foods that, through their, contributed properties, make products attractive to consumers (Mattil, 1971). In processed meats, proteins have been mentioned as the principal functional and structural components. These also determine the characteristic handling, texture and appearance of the products (Smith, 1988).

Besides having satisfactory intrinsic properties (i.e. nutritional) or acceptable flavor, color and texture, they must also possess additional functional properties: solubility, swelling, emulsifying and foaming, as well as curdling, flavor, binding, and thermal stability. All these properties make proteins compatible with, while possibly enhancing, the food to which they are added (Kinsella, 1976). The relative importance of each functional property varies with the product, processing method and stage of processing.

Bone-in hams and corned beef, for example, retain muscular structure and water binding which are of primary importance. The proteins in frankfurters must bind water and fat, to form a firm, elastic gel.

Protein Functionality

Pour-El (1981) defined functionality as "any property of a food ingredient except its nutritional ones that affects its utilization." He also described the terms functional evaluation, functional value, model system (or model test), and utility system, also associated with functionality, useful to quantify functional attributes of proteins and other food ingredients.

Extensive studies on the functionality of food proteins have been conducted by Kinsella (1976, 1982, 1983) and by Kinsella and Fox (1986). Broadly, the functional properties of proteins denote any physicochemical property which affects the processing and behavior of proteins in food systems, as judged by the quality attributes of the final product (Kinsella, 1976). The influence of proteins on the physical properties of foods has been regarded as more important than their nutritional properties in determining the acceptability of foods (Parker, 1987).

Table 2.1 contains several general classes of functional properties of proteins important in food applications. These functions vary with factors affecting the environmental conditions, such as pH, temperature, protein concentration, protein species, and ionic strength of the medium. They are also affected by the presence of other macromolecules in the medium (carbohydrates and lipids) (Kinsella, 1976) and by processing methods, which

TABLE 2.1

GENERAL CLASSES OF FUNCTIONAL PROPERTIES OF PROTEINS IMPORTANT IN MEAT PRODUCTS APPLICATIONS

General Property	Specific Functional Term
Organoleptic	Color, flavor, odor, texture, mouthful.
Hydration	Solubility, dispersability, water absorption, rheological, water holding capacity, viscosity.
Surface	Emulsification, foaming, protein/lipid film formation.
Structure	Elasticity, cohesion.
Textural	Viscosity, network cross-binding.
Rheological	Gelation, extrudability, cohesivity.

may include solubilization and extraction, hydrolysis, heat treatment, freezing, dehydration and microbial fermentation (Kilara and Sharkasi, 1986). Regenstein and Regenstein (1984) proposed a standard methodology for determining various functional properties of proteinaceous materials. The properties covered are, among others, heat gelation, emulsification, foaming, viscosity, film formation and sensory evaluation.

Solubility has been pointed out as one major property that controls the overall functionality of protein-containing products (Trautman, 1967; Smith, 1988; Whiting, 1988b; Regenstein and Regenstein, 1984). Water holding capacity has also been mentioned as another important property, which affects quality and composition (Kauffman et al., 1986; Hamm, 1960).

Among all the proteins found in muscle, myosin is known to play the most important role in the contractile mechanism in living muscle, and imparts indispensable binding properties and water holding capacity to meat products (Samejima et al., 1981). It has also been related to such important organoleptic properties as juiciness and tenderness (Hamm, 1960; Bouton et al., 1971).

Protein Solubility

The terms protein solubility and protein extractability have been used interchangeably in the literature.

Extractable protein can be defined as the amount of protein that can be removed from the muscle tissue, under certain environmental conditions, by means of a suitable medium, the extracting solution, which is in direct contact with the proteinaceous material. Solubility has been regarded as the percentage of the total protein that is retained in the supernatant after centrifugation of a protein solution at a specific speed and period of time (Regenstein and Regenstein, 1984).

As reported by Sayre and Briskey (1963), the solubility of sarcoplasmic and myofibrillar proteins has been used to characterize protein changes during animal maturity and to compare protein composition of different muscles.

Special emphasis will be placed on the description of factors that affect the solubility of myofibrillar proteins, since they have been reported as most important from a functionality view point.

With the purpose of establishing a method of protein extraction, Helander (1957) reviewed a number of factors havind impact on protein recovery. The main effects of the following techniques were evaluated: muscular tissue storage at different temperatures before and after sectioning, effect of repeated muscular contractions on protein recovery, effect of pH level, different types of salts, various additives, varying ionic strength, solvent volume used, and repeated extraction on protein yield. As a result of those experiments, a general method for protein

extraction was developed, which consists of the utilization of 0.03 M potassium phosphate, pH 7.4 for the extraction of sarcoplasmic protein and 1.1 M KI and 0.1 M Phosphate buffer at pH 7.4 for the extraction of myofibrillar proteins. The fractions are analyzed for nitrogen content or for protein content. This procedure or variations of it have been used widely by many researchers (Sayre and Briskey, 1963; Mac Bride, 1986). However, since this technique is intensive and time consuming, less rigorous methods have been developed.

Many researchers have studied the effects examined by Helander (1957) along with others that are also considered of importance. Saffle and Galbreath (1964) determined the effects of fat, pH, freezing and post-mortem aging on the amount of salt-soluble protein extracted from four different areas of beef carcasses. The rate of recovery of the total proteins increased with higher pH extracting solutions.

Trautman (1967) observed the influence of simulated carcass temperatures and pH conditions on the soluble protein components from ham muscle. He did not find a significant effect of temperature, but determined that a value of pH 6.4 tended to increase the concentration of salt soluble proteins.

Helander (1957) tested the effect of a number of salts (KCl, NaCl, $MgCl_2$, $CaCl_2$, LiCl, KI and KBr) on the extractability of muscle proteins. KI solutions 1.1 M proved to be the most efficient on the extractability of

muscle proteins. More recently, King and Earl (1988) found that neutral and alkaline polyphosphates combined with sodium chloride extracted more protein than other combinations of salts.

Wagner and Añón (1986) found that the solubility and viscosity of myofibrils decreased as a result of frozen storage. Previous studies have shown that similar alterations are observed in the proteins of frozen meat. Sarcoplasmic protein, total extractable protein and actomyosin extractability decreased with frozen storage (Awad et al., 1968).

Miller et al. (1980) tested the effect of frozen storage on several functional properties of lean and fat samples from beef and pork. They found that low storage temperatures had detrimental effects on extractable proteins from lean sections and on the emulsifying capacity of those fractions.

Studies conducted by Nakai (1983) and by Li-Chan et al. (1985) focussed on the importance of using solubility as well as hydrophobicity in order to predict functional properties. The later study suggests that involving both hydrophobicity and solubility parameters in regression models describe emulsifying capacity, better correlation coefficients are obtained than with solubility alone. Studies by Bigelow, as reported by Nakai (1983) indicate that the higher the charge frequency and the lower the hydrophobicity, the higher the solubility.

In his experiments, Helander (1957) used different extraction times and found that even with long extraction periods, the amount of total protein extracted was limited. He reported that 5 hours was the optimal extraction time for myofibrillar proteins. Gillet et al. (1977) showed that shorter extractability periods also yield satisfactory results. Gumpen and Fretheim (1983) reported that the extractability increased after two hours of agitation and then stabilized.

The need for a standard method for the determination of solubility of proteins has been recognized by a number of researchers. Morr et al. (1985) suggested a modification of the nitrogen solubility index procedure. Protein content and soluble protein were determined by micro-Kjeldahl or Biuret procedures. The latter, exhibited considerable error and variability for some of the proteins tested.

Asghar and Yeates (1974) presented a procedure for the fractionation of muscle protein into sarcoplasmic and myofibrillar components. This method represents an improved version of Helander's method of protein separation. The main features of the proposed procedure are simplicity, reproducibility and accuracy.

A method for the isolation of actomyosin and myosin from post-rigor turkey meat was developed by Dudziak and Foegeding (1988). Gel filtration data indicated that myosin, unassociated with actin was the predominant protein extracted from post-rigor tissue.

Water Holding Capacity

Water-holding capacity (WHC) of raw and cooked meat has been related to important organoleptic properties, mainly, juiciness and tenderness (Hamm, 1960). However, regarding cooked meat or meat products, other researchers have used the term water binding capacity (Trout and Schmidt, 1983). The terms cook yield, cooking loss (inversely), water binding value and water binding ability are currently used in the literature to describe WHC (Poulanne and Terrell, 1983; Regenstein, 1984).

Similarly to protein solubility, the content and physical state of water in foods influences the physical, chemical, quality and functional characteristics of food components (Kinsella and Fox, 1986).

Water is present in muscle in three forms: bound, immobilized and free. Bound water is associated with proteins by means of hydrogen bonds. The layers of water molecules that are attached to the bound water constitute immobilized water. The third type is held only by surface forces and is known as free water (Forrest et al., 1975).

With the interest in the manufacture of low-fat meat products containing higher amounts of added water, the importance of increasing the water holding capacity of meat has emerged as a matter of significant importance (Rust and Olson, 1988). The most important factors that influence water holding capacity of meat and meat products are pH, fat

content, freezing, post-mortem age, presence of salts (mainly phosphates and sodium chloride) and temperature.

The positive effect of NaCl on the WHC of meat is well known. However, recent market trends that indicate consumer preferences for low-sodium meat products have increased. As the amount of sodium chloride used in meat formulations is lowered, WHC of processed meat products may become an important issue regarding textural and microbiological properties as well as from the economic view point (lower yield).

The effect of temperature on WHC has been reported to occur at temperatures far below those where coagulation of proteins starts. Wisman-Pedersen, as reported by Schut (1976), found a linear decrease in WHC with increasing temperature from 0 to 25°C, whereas according to Wierbicki et al. (1958) the swelling of meat increased between 10 and 25°C with a maximum at 25°C.

The influence of fat was studied by Swift et al. (1954). They found that up to a fat : protein ratio of 2.8:1, the water retention of sausages mixes increased with increased content of fat, for fat should not contribute to the binding of water. At higher contents of fat (3.4:1), the water retention decreased. Hamm (1960) suggested that with increasing fat contents, there is also an increase in the amount of salt per unit weight of lean meat because the addition of salt is calculated in total amount of lean meat plus fat with fat taking much less salt than muscle tissue.

Changes in WHC have been shown to be closely related to pH, and to be a sensitive indication of variations in the changes and structure of muscle proteins. Bouton et al. (1971) studied the effect of pH on the WHC of mutton and found that the amount of juice expressed from the cooked meat and cooking loss generally increased with increasing pH. Changes in WHC due to variations in the pH of post-mortem muscle are explained by Hamm (1960). He indicates that as muscle undergoes post-mortem processes, there is a significant reduction in the pH accompanied by a decrease in the amount of water bound. This is due mainly to the fact that as pH lowers, the proteins approach the isoelectric point leaving insufficient interfilament spacings for the water to bind the proteins.

Phosphates are added to cured meats in order to control the loss of natural juices that occurs from the time of slaughter to packing reducing juiciness and susceptibility to freezer burns (Dziezak, 1990).

When used in combination with salts, phosphates control fluid loss by increasing the water binding or retention of natural juices. They accomplish this by increasing pH, ionic strength and by complexing with protein-bound magnesium and calcium so that the actomyosin present in the muscle can dissociate and expose more binding sites for hydration (Dziezak, 1990).

The combined effect of sodium chloride and added phosphates has been studied by several researchers (Offer

and Trinick, 1983; Barbut, 1988; Barbut et al., 1988; Kijowski and Mast, 1988; Paterson et al., 1988). These studies have shown that the inclusion of phosphates enhances the protein extractability of NaCl and improves the WHC.

The effect of salt, phosphate and massaging on cooking loss in sectioned ham was examined by Siegel et al. (1978a, 1978b). They found that the presence of both phosphates and massaging was beneficial for the production of an overall superior product.

Several methods have been proposed for the determination of WHC, however, there is a large magnitude of variation among the available procedures. An extensive review of several common laboratory techniques was performed by Kauffman et al. (1986a). The tests were conducted using three types of muscle samples, normal, DFD (dark, firm, dry) beef and PSE (pale, soft and exudate) pork under several experimental procedures. The results indicated that the most reliable methods are drip loss, swelling of homogenized samples by added water, and absorption of surface fluids on filter paper.

The effectiveness of the use of filter paper to estimate drip loss of pork was studied by Kauffman et al. (1986b). The results obtained suggest that this method may be useful to the meat industry, especially for practical applications.

A simple centrifugal method for measuring expressible moisture was developed by Jauregui et al. (1981). The

procedure seemed to be highly sensitive to factors that affect the water-binding properties of muscle foods. It basically measures the amount of liquid squeezed out of a sample with the application of centrifugal force by measuring the weight gain of a filter paper surrounding the sample.

Emulsion Formation and Role of the Proteins during Heating

An emulsion has generally been described as a mixture of two inmiscible liquids, one of them being dispersed into the other in the form of fine droplets (Schut, 1976). This is accomplished by the use of a third substance, the emulsifying agent, which is structurally compatible with the immiscible liquids.

Sausage meat batters have been referred to as emulsions, although these systems consist of a solid (the fat particles) dispersed in an external or continuous phase, which is a solution of salts and proteins, and, at the same time is a medium of dispersed muscle fibers and connective tissue (Schut, 1976). The salt soluble proteins myosin and actomyosin are generally considered the emulsifying agent.

Brown and Toledo (1975) proposed to discontinue the use of the word "emulsion" and replace it by the term "batter". The batter has also been described as a high viscosity protein sol with suspended fat particles (Whiting, 1988a). During grinding or chopping of meat and fat with the

addition of salt and water, a matrix is formed in which the fat is dispersed.

Heating meat batters causes structural changes in muscle proteins which promote aggregation first and then progresses to gelation (Foegeding, 1988a, 1988b). In order to preserve the matrix structure, fat binding ability and water holding capacity of proteins are of primary importance.

The strength of the interfacial protein film and its resistance against external forces has been known to determine the stability of the meat batter (Schut, 1976; Jones, 1984). In order to attain the most stable condition, the protein molecules orient themselves at the interface between the two inmiscible phases. This orientation occurs such that a multimolecular membrane (the matrix) is formed, where the hydrophobic side chains of the myosin molecules are attracted toward the lipid phase and hydrophilic sides (heads of myosin) are attracted toward the aqueous phase (Schut, 1976; Jones, 1984).

The condition of the meat proteins and consequently, their ability to form a rigid matrix can be expressed in terms of solubility, rate of hydration and emulsifying ability. These quality parameters are strongly influenced by those that affect the solubility and WHC, mainly: pH, temperature, presence of phosphates and NaCl, ionic strength, and, species and age of meat (Schut, 1976).

A detailed study of the emulsifying properties of meat protein was presented by Swift (1965). He proposed a method for the determination of the amount of oil that can be emulsified by proteins.

The effects of chopping temperature on the stability of sausage emulsions was studied by Helmer and Saffle (1963). It was found that the emulsions were stable at chopping temperatures of 15.5°C, but emulsion breakdown occurred when the emulsion was chopped to 32°C. Jones and Mandigo (1982) examined the microstructural differences that occur within a meat emulsion from formation to breakdown through a sequential increase in endpoint chopping temperature. Significantly lower yields, strong evidence of emulsion breakdown and higher cookout losses were observed as the chopping temperature of the treatments increased from 10 to 28°C.

Brown and Toledo (1975) studied the relationship between chopping temperatures and fat and water binding in comminuted meat batters. Their results indicated that, in the particular system studied, there was an inverse relationship between fat and water binding. As fat binding increases, water binding decreases, and vice versa. Thus, fat and water binding appear to be in competition with each other for binding sites in the system. The temperature range attained by the batter during chopping, where binding was maximum, ocurred at 15 to 22°C, but was only true the

first time a stable batter was produced from the original unchopped meat.

A detailed rheological analysis of the effects of heating rates and sugars on frankfurter batters was performed by Saliba et al. (1987). From structural failure tests, heating rate was shown to have much greater effect on texture than type of sugar utilized (glucose or sucrose). The development of rigidity and elasticity were both time and temperature dependent.

All these studies suggest that a better understanding of the protein-protein, protein-water and protein-fat interactions is required to obtain high-quality products. By combining processing factors and ingredients, it is feasible to predict the behavior of non-traditional meat products such as low-fat frankfurters and similar products (Foegeding, 1988a). Given the importance placed on solubility and water holding capacity, several aspects concerning their quantification, as well as factors affecting them will be examined in the following sections.

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CHAPTER III

THE EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF SHOULDER CLODS

Abstract

The effect of temperature on the solubility of beef shoulder clod proteins was evaluated in the range of -2 to 26°C, 2°C intervals. Samples were treated with 2% NaCl for the extraction of total protein and with 0.03 phosphate and with 1.1 M KI for the extraction of sarcoplasmic and myofibrillar fractions, respectively. Regression models ($P<0.01$) were obtained to characterize the solubility of total salt extractable, sarcoplasmic and myofibrillar proteins. Maximum amounts of solibilized extracted protein were obtained for total salt extractable at 14°C, sarcoplasmic at 26°C and myofibrillar at 6°C. Temperature did not have an effect on WHC ($P>0.05$).

Introduction

With the increased interest in the consumption of low-fat, low-sodium meat products, additional studies addressing processing conditions effects on the functionality of meat proteins have become a matter of major importance.

The term protein functionality has been used by a number of researchers to describe the ability of food proteins to impart certain characteristics in products. Many sensory, textural, physical and chemical properties of foods are attributed to the presence of proteins. Their role in a number of food products and the factors that influence their performance have been studied by Kinsella (1976, 1982, 1983) and by Mattil (1971).

In emulsion-type meat products, three functional properties of meat proteins are important: emulsion capacity and stability, water retention, and protein solubility (Regenstein and Regenstein, 1984). Temperature, pH, presence of salts, ionic strength, particle size, processing techniques, and postmortem age of meat have been mentioned as the principal factors influencing the performance of proteins during emulsion formation and heating.

With the increased amount of added water present in low fat products due to the USDA "40 percent rule" (Federal Register, 1988), the problems of adequate water binding have become a main issue in processing (Rust and Olson, 1988). Water binding potential (expressed as water holding capacity, WHC) and protein solubility may be considered, the two most important functional properties that define the characteristics of the final product. The objective of this study was to determine the effect of temperature on the water holding capacity and solubility of beef shoulder clod proteins.

Materials and Methods

Sample Preparation

Three USDA choice beef shoulder clods (all or parts of deltoides, triceps, brachii, brachialis and infraspinatus muscles), approximately 72 hr post-mortem, where obtained from a local supplier. The clods were frozen (-29±2°C for 72 hr) and one clod was randomly selected (one replicate) and thawed (3±1°C, 72 hr).

The clod muscles were separated and trimmed of all visible fat. The weight of the clods after fat removal was 5.4±0.1 kg. The meat was ground (grinder, model 5424852, Biro Mfg. Co., Marblehead, OH) using a 2.54 cm plate and reground through a 5 mm plate before mixing (Leland ribbon-paddle mixer) for 1 min. The ground meat was divided into 16 portions (335±5 g each) and vacuum packaged (Multivac, Kansas City, MO). One sample portion was used for the determination of protein, fat and moisture content (AOAC, 1984). The proximate composition was protein 20.21% (SE 0.388), fat 5.82% (SE 1.03) and moisture 72.67% (SE 0.81). Each sample was randomly assigned to one of 15 temperature treatments (from -2 to 26°C at 2°C intervals) and frozen (-29±2°C) until time for analysis. Each sample was thawed (3±1°C) for eighteen hours prior to the start of protein extraction.

Protein Extraction

Total proteins were extracted (quadruplicate) using a modified version of the method of Hand et al. (1985). The samples were weighed (50±0.1 g) in 250 ml Nalgene bottles and allowed to equilibrate to the required temperature. Then, a 100±0.1 ml NaCl 2% w/v solution (also at the required temperature) was added to each sample. The bottles were placed in a covered shaker water bath (model BKS-350, Gallenkamp and Co., Sussex, England) and shaken at 120 rpm for 10 min. The temperature of the water bath (±0.1°C) was regulated by a refrigerated circulator (Lauda model RMS-20, Brinkmann Instruments, Westbury, NY). After the extraction period, samples were centrifugated at 1860 x G for 10 min (model J-6M Beckman Instruments, Palo Alto, CA) at the same temperature of extraction, and then filtered through #1 Whatman paper. The protein content of the filtrates was determined by the Biuret method (Gornall et al., 1949).

Sarcoplasmic and myofibrillar proteins were extracted using a 0.03 M potassium phosphate solution at pH 7.4 and 1.1 M potassium iodide in 0.1 M phosphate solution at pH 7.4, respectively, according to a modified version of the methods of Helander (1957) and Sayre and Briskey (1963). The extracting solutions were prepared and the pH was adjusted as described by McBride (1986).

Samples weighing 5±0.05 g were placed in 50 ml polycarbonate tubes (quadruplicate) and temperature was

equilibrated to that required by each treatment. Sarcoplasmic proteins were extracted first with 30 ml of potassium phosphate 0.03 M at pH 7.4 (also at the same temperature). The samples were shaken at 90 rpm for 2 hr. After the extraction period, the tubes were centrifuged at 3640 x G for 15 min at the temperature of extraction. After separation of the supernatant, 30 ml of KI 1.1 M solution (pH 7.4) were added to each tube. The samples were shaken for 4 hr at 90 rpm and centrifuged at 3640 x G for 15 min. The supernatant containing the extracted myofibrillar proteins was separated by filtration. Sarcoplasmic and myofibrillar extracts were analyzed for protein content using the Biuret method (Gornall et al., 1949).

Determination of the Water Holding Capacity (WHC)

WHC was determined by a modified method of Jauregui et al. (1981). The temperature of operation was varied, as needed for each of the treatments, and the centrifugation time and rotor speed were changed to 45 min and 3640 x G, respectively. This experiment was performed in triplicate.

Statistical Analysis

The results from this experiment were analyzed using linear regression (Steel and Torrie, 1980) and the analysis of variance was performed with the SAS program (SAS Institute, Inc., Version 6.1), with temperatures as treatments.

Results and Discussion

The effect of temperature on the total salt extractable protein is shown in Figure 3.1. The best fit was obtained with a fourth degree polynomial regression ($P<0.05$). The regression line indicated that a maximum amount was solubilized at 14°C, within the range studied. Reports from other studies have indicated that the optimal temperature for the extraction of total protein from beef and pork combination sources is 7.2°C, using a 7.5% NaCl solution (Gillet et al., 1977).

Figure 3.2 shows the effect of the temperature on the solubility of sarcoplasmic proteins. The significant model indicated that the point of maximum solubility is 26°C. However, there were small magnitudes of change in the range of 6° and 16°C before increasing to the maximum at 26°C.

The effect of temperature on myofibrillar protein solubility is shown in Figure 3.3. The regression curve was a fourth degree polynomial ($P<0.05$). This indicates that the optimum temperature for extraction of myofibrillar proteins is 6°C. These results compare with those of Rust (1977), which suggest that the optimum temperature for the extraction of contractile proteins appears to be in the range of 4.4-7.2°C. The extent of protein recovery achieved in the total soluble, sarcoplasmic and myofibrillar solubility methods is comparable to the results of Helander

(1957) and Ashgar and Yeates (1974), utilizing similar methodology.

Temperature had no ($P>0.05$) effect on water holding capacity (WHC). The overall mean (0.437) nevertheless, indicates that the method used for the determination of WHC is adequate, in view of the low variance (0.02) and high repeatability of the results, which is in agreement with the findings of Jauregui et al. (1981).

This experiment characterized the significant effect of temperature on the solubility of total, sarcoplasmic and myofibrillar proteins. In addition to temperature other factors (species, postmortem age, frozen storage, pH, particle size, salt, mechanical action) have been found to influence protein solubility (Helander, 1957; Sayre and Briskey, 1963; Li-Chan et al., 1985; Mac Bride, 1986; Paterson et al., 1988) and consequently affect the manufacture of processed meat products.

From this study it can be concluded that maximum amounts of total salt extractable proteins are solubilized at 14°C. Sarcoplasmic solubility has little change from 4° to 16°C and then increases to a maximum at 26°C. Maximum myofibrillar protein extraction occurred at 6°C.

Figure 3.1. Effect of Temperature on the Total Soluble Protein.

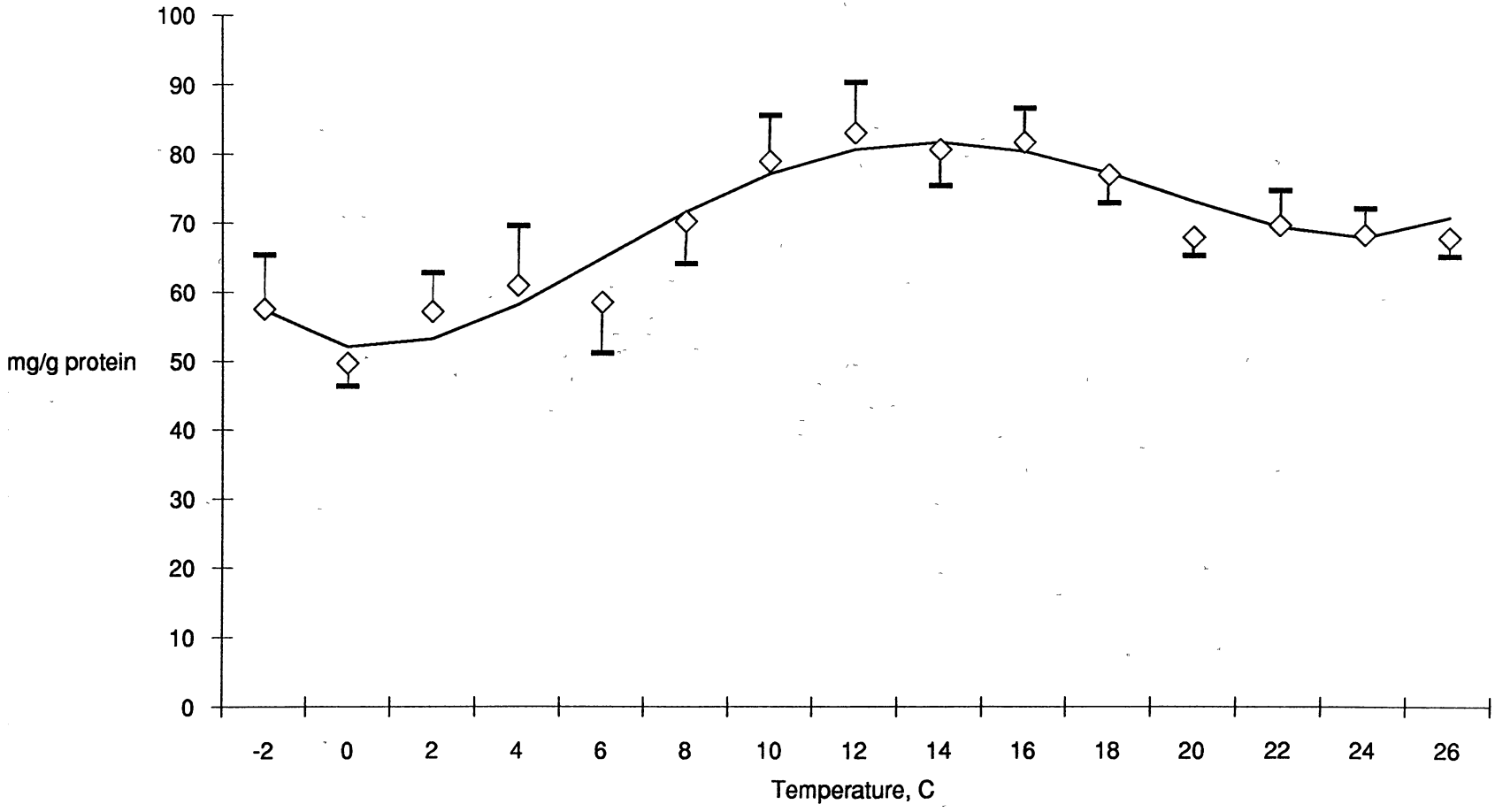


Figure 3.2. Effect of Temperature on the Solubility of Sarcoplasmic Protein.

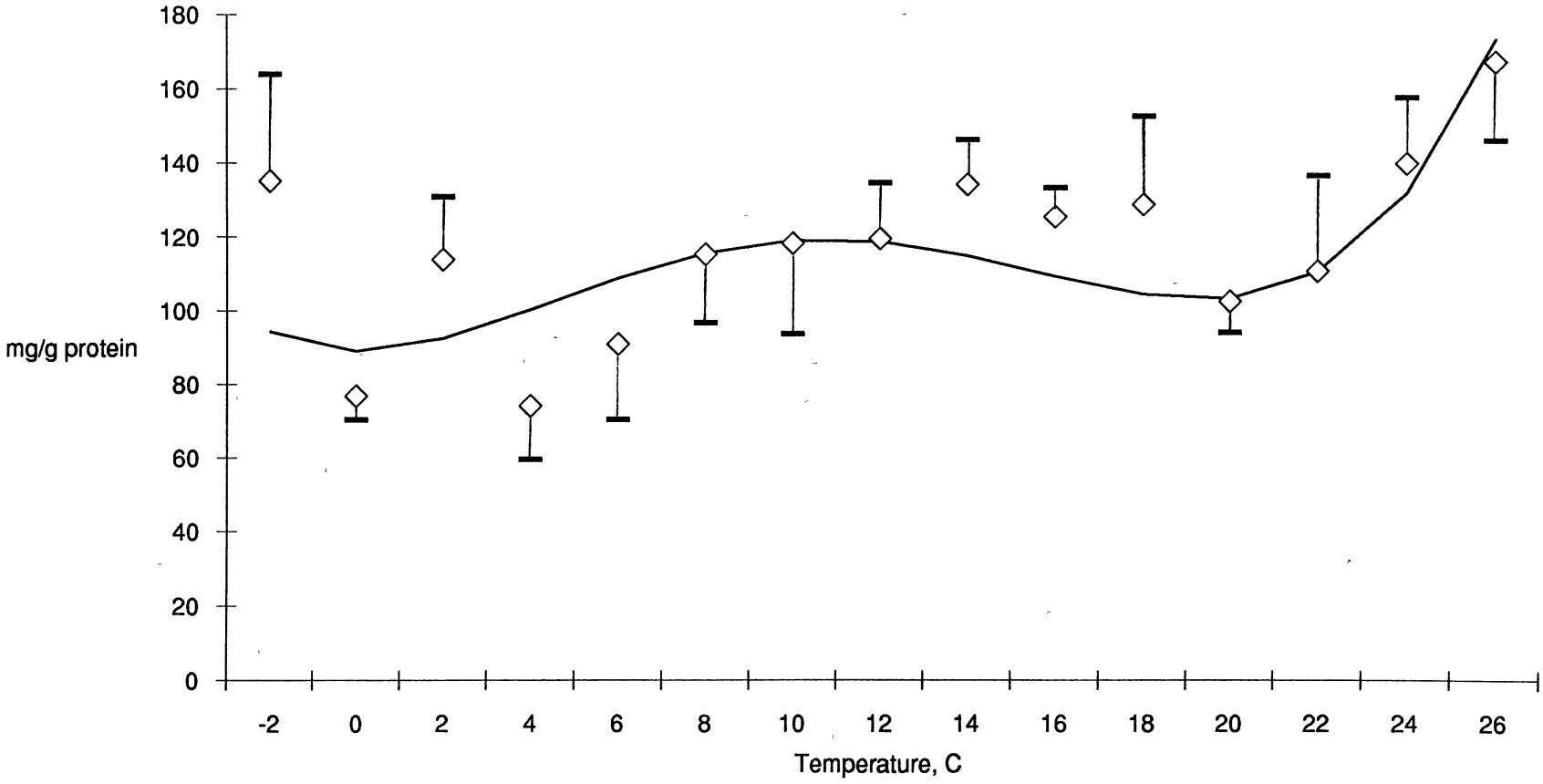
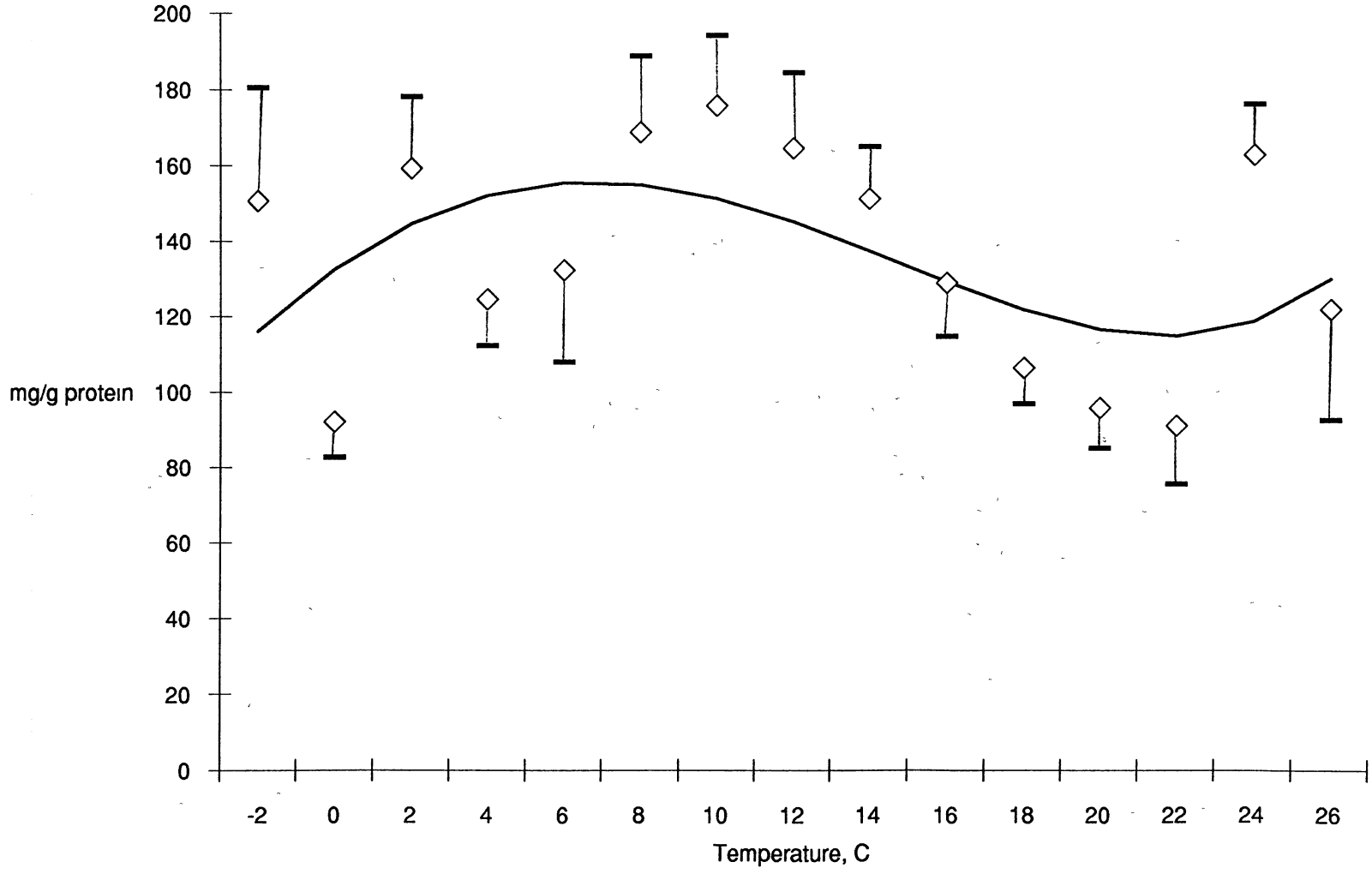


Figure 3.3. Effect of Temperature on the Solubility of Myofibrillar Protein.



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CHAPTER IV

THE EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY AND WATER HOLDING CAPACITY OF BEEF FORMULATIONS CONTAINING DIFFERENT FAT AND ADDED WATER LEVELS

Abstract

The effect of temperature on the protein extraction and WHC of samples prepared at different fat and added water (AW) levels was evaluated in the range -2 to 26°C. Samples were formulated from beef shoulder clods, beef fat and water to contain 5% fat-35% AW, 13.5% fat-26.5% AW, 21.5% fat-18.5% AW and 30% fat-10% AW. Salt extractable proteins showed maximum extractability at 12°C ($P<0.01$). Myofibrillar proteins showed maximum solubility at 10° C. Temperature had no ($P>0.05$) effect on the solubility of sarcoplasmic proteins. The highest value of WHC was obtained at 2°C. Total protein solubility reached a maximum at 28% fat-12% AW. The solubility of myofibrillar proteins reached a maximum at 25% fat - 15% AW.

Introduction

During the past several years, major national health organizations have recommended reduced consumption of saturated fat and cholesterol since the overconsumption of these substances has been linked to the occurrence of health disorders (National Research Council, 1988). Consumers have responded by demanding lower calorie convenience foods. Recognizing this increasing trend toward the consumption of low calorie convenience foods, the industry is responding by formulating and producing new food products that meet those criteria (Kinsella, 1987).

The development of low-fat, low-cholesterol products has been approached by a number of researchers. Several studied the replacement of animal fat by oils from plant origins (Marquez et al., 1989; Park et al., 1989), whereas others suggest that the reduction of the amount of fat can be accomplished through a dilution effect of the animal fats by the addition of non-meat proteins (Sofos and Allen, 1977; Anonymous, 1990a) and through the use of vegetable gums (Anonymous, 1990b).

The reduction of fat levels in meat products through the use of processing technologies (i.e., preblending) has also been recommended (Hand et al., 1987), as well as by changes in the formulation (Claus et al., 1989; Claus et al., 1990). The industrial interest in the development of low-fat meat products increased following the approval in

1988, of the USDA "40 percent rule". This new regulation allows the manufacture of frankfurters and similar products that contain a combination of fat and added water up to 40 percent where the fat level is restricted to no more than 30 percent (Federal Register, 1988).

Several low-fat meat products are already found in the marketplace. The manufacture of 95% fat-free ham and low-fat bacon are examples. Nevertheless, the frankfurter-type products available today as low-fat contain 22.5% fat, a reduction of 25% of the original level. "Lite" beef frankfurters containing less than 22.5% fat are not, yet, popular in the market. To successfully manufacture products of lower fat content, the functional roles of major macromolecules (proteins in the case of animal products) need to be determined (Kinsella, 1987).

Processed meat products (batter-type and sectioned and formed products) depend upon the formation of a continuous matrix through the product. This property is contributed mostly by the soluble myofibrillar proteins, which have been regarded as the functionally important proteins (Wilding et al., 1984). The importance of the temperature control during emulsion formation and heating of the product has been studied by Helmer and Saffle (1963) and by Jones and Mandigo (1982). The effect of protein extraction temperature upon protein solubility and emulsifying efficiency was determined by Gillet et al., 1977. The optimum temperature for the extraction of total protein from

beef and pork was 7.2°C, using 7.5% NaCl extracting solution.

The objective of this study was to investigate the effect of temperature on the water binding and solubility properties of beef proteins from formulations prepared at different fat and added water levels.

Materials and Methods

Sample Preparation

Three USDA choice shoulder clods (all or parts of deltoideus, triceps brachii, brachialis and infraspinatus muscles), approximately 72 hr post-mortem, were obtained from a local supplier. The clods were frozen (-29±2°C for 72 hr), and one clod was randomly seleected (one replicate) and thawed (3±1°C, 72 hr). Beef fat was obtained (frozen) from the OSU meat Laboratory and handled in the same manner.

The clod muscles were separated and trimmed of all visible fat. The weight of the clods after fat removal was 5.1±0.1 kg. The lean meat and the required amount of fat were ground separately (Grinder, Biro Mfg., Marblehead, OH), first through a 2.54 cm plate and then reground through a 5 mm plate. The meat was then mixed (Leland ribbon-paddle mixer) for 1 min for homogeneity. One portion of the beef lean was separated for the determination of protein, fat and moisture content (AOAC, 1984). A similar treatment was given to the beef fat. The proximate composition of the

lean fraction was moisture 73.11% (SE 0.80), protein 20.24% (SE 5.30) and fat 5.28% (SE 1.03). The proximate composition of the fat fraction was moisture 38.74% (SE 0.77), protein 10.84% (SE 1.16) and fat 48.96% (SE 0.98).

The lean meat and beef fat were combined to give the following fat and added water (AW) targets: 5% fat-35% AW, 13.5% fat-26.5% AW, 21.5% fat-18.5% AW and 30% fat-10% AW. A protein target of 12% was fixed for all samples.

The proportions of the lean, beef fat and water components of the sample were determined using Least Cost Formulator, LCF (Least Cost Formulator, Ltd., Virginia Beach, VA). The amounts required for each of the fat targets were mixed for 1 min. Each fat-lean mixture was divided into 16 portions (340±5 g, each) and vacuum packaged (Multivac, Kansas City, MO). One portion was used for the verification of the target proximate compositions of the samples.

Samples from each of the fat and AW targets were randomly assigned to one of 15 temperature treatments (-2 to 26°C at 2°C intervals) and were frozen (-29±2°C for 72 hr) analyzed. Samples were thawed (3±1°C) 18 hr prior to protein extraction.

Protein Extraction

Total proteins were extracted (triplicate) using a modified version of the method of Hand et al. (1985). The samples were weighed (50±0.1 g) into 250 ml Nalgene bottles

and allowed to equilibrate to the required temperature. Then, a 100±0.1 ml NaCl 2% w/v solution (also at the required temperature) was added to each sample. The bottles were placed in a covered shaker water bath (model BKS-350, Gallenkamp and Co., Sussex, England) and shaken at 120 rpm for 10 min. The temperature of the water bath (±0.1°C) was regulated by a refrigerated circulator (Lauda model RMS-20, Brinkmann Instruments, Westbury, NY). After the extraction period the samples were centrifugated at 1860 x G for 10 min (model J-6M Beckman Instruments, Palo Alto, CA) at the same temperature of extraction, and then filtered through #1 Whatman paper. The filtrates were used for the determination of the protein content by the Biuret method (Gornall et al., 1949).

Sarcoplasmic and myofibrillar proteins were extracted using a 0.03 M potassium phosphate solution, pH 7.4 and a 1.1 M potassium iodide in 0.1 M phosphate solution, pH 7.4, respectively, according to a modified version of the methods of Helander (1957), and Sayre and Briskey (1963). The extracting solutions were prepared and the pH adjusted as explained by McBride (1986).

Samples weighing 5±0.05 g were placed in 50 ml polycarbonate tubes (triplicate) and the temperature was equilibrated to that required by the treatment. Sarcoplasmic proteins were extracted first, with 30 ml of potassium phosphate 0.03 M, pH 7.4 (also at the same temperature). The samples were shaken at 90 rpm for 2 hr.

After the extraction period, the tubes were centrifuged at 3640 x G for 15 min, at the same temperature of extraction. After extraction the supernatant, 30 ml of KI 1.1 M solution (pH 7.4) were added to each tube. The samples were shaken for 4 hr at 90 rpm and centrifuged at 3640 x G for 15 min. The supernatant, that contained the extracted myofibrillar proteins was separated by filtration. Sarcoplasmic and myofibrillar extracts were analyzed for protein content using the Biuret method (Gornall et al., 1949).

Determination of the Water Holding Capacity (WHC)

WHC was determined according to the method of Jauregui et al. (1981), that was modified in the following manner: the temperature of operation was varied, as needed for each of the treatments, and the centrifugation time and rotor speed were changed to 45 min and 3640 x G, respectively. This experiment was performed in triplicate.

Statistical Analysis

The statistical analysis of the experimental data was performed according to the linear regression model (Steel and Torrie, 1980), using the GLM procedure of the SAS program (SAS Institute, 1985). The temperature-fat interaction was not significant ($P>0.05$), and the effects of those variables were analyzed independently.

Results and Discussion

The proximate composition of the formulated samples used in the analyses is shown in Table 4.1. Fat targets were achieved within reasonable deviations. However, the added water target was much more difficult to achieve. This was attributed to sampling error as samples were ground and not homogenized.

The effect of temperature on total soluble protein is shown in Figure 4.1. A fourth degree polynomial regression model ($P<0.05$) was obtained for the data. In this model, the point of maximum solubility was 12°C, which differs from the results of Gillet et al. (1977), who obtained a value of 7.2°C as the optimum temperature for the extraction of salt soluble protein. However, their experiments were conducted only at 5 different temperatures, using three beef and two pork sources.

The solubility of the sarcoplasmic proteins was not ($P>0.05$) affected by temperature in the interval studied. The average sarcoplasmic protein extracted was 129 mg/g of protein in sample (SE 8.20).

Figure 4.2 shows the effect of temperature on myofibrillar protein solubility. A regression model of fourth degree ($P<0.01$) was used to represent the experimental data. The point of maximum solubility was 10°C. Rust (1977) indicates that the optimum temperature for the extraction of contractile proteins in the presence

TABLE 4.1

PROXIMATE COMPOSITION OF SAMPLES

Fat Target (%)	AW Target	Moisture[a] (%)	Protein[b] (%)	Fat[c] (%)	AW[d] (%)
5.0	35.0	81.78	13.07	4.82	29.50
13.5	26.5	73.36	13.61	13.32	18.92
21.5	18.5	67.91	12.05	18.61	19.72
30.0	10.0	57.71	13.02	29.03	5.63

[a]SE = 0.8939.
[b]SE = 0.4305.
[c]SE = 1.1418.
[d]Added water (AW) = Moisture (%) - 4(Protein %).

of salt (during batter formation) appears to be in the range of 4.4 to 7.2°C. The extent of protein recovery achieved in the total soluble, sarcoplasmic and myofibrillar solubility methods is comparable to the results of Helander (1957) and Ashgar and Yeates (1974), utilizing similar methodology.

The effect of temperature on the water holding capacity of samples is presented in Figure 4.3. This curve was obtained as the best fit polynomial regression curve ($P<0.01$). This indicated a maximum value of WHC at 2°C, after which there was a decline through the temperature interval studied.

Figure 4.4 shows the effects of fat and added water level on total salt extractable protein. The cubic regression model ($P<0.01$) showed a maximum protein solubility at 27.5% fat and 12.5% added water. Since the protein content of the samples was the same, this may be explained in terms of the added water. As the fat level increases, the AW of the samples decreases, and the salt solution added to the sample becomes more concentrated, and therefore, more efficient in terms of protein extractability. The significant positive effect of salt concentration on protein solubility has been reported by Gillet et al. (1977) and by Li-Chan et al. (1985).

The cubic polynomial regression model ($P<0.05$) for the solubility of the myofibrillar proteins is shown on Figure 4.5. This curve shows maximum myofibrillar protein extraction at 25% fat - 15% AW.

The effect of the temperature on the WHC of the samples is represented in Figure 4.6. A significant ($P<0.05$) reduction in the value of WHC was observed as fat and added water level increased. These results do not agree with the findings of Claus et al. (1989, 1990) which indicated that for low-fat frankfurter formulations, the purge (water loss) was higher than for those prepared at higher fat contents. However, the samples used in this study were not cooked and thus protein denaturation changes that significantly influence water binding ability do not apply.

From the results of these experiments it can be concluded that maximum amounts of total salt extractable proteins are obtained at 12°C, while myofibrillar protein is obtained at 10°C. The solubility of sarcoplasmic proteins is not affected by the temperature. Therefore, for optimal salt extractable protein solubility in a range of 10 to 12°C would achieve the best overall results.

Maximum amounts of total salt extractable protein, myofibrillar protein and water binding were achieved in the higher fat (24-30%) and lower added water (10-15%) formulations. This may be attributed to efective salt concentration. Processors might utilize the effective salt concentration by processing procedures that maximize protein extraction at low water concentration before adding the entire water to the system. As these temperature and protein solubility relationships were established in a model

system, further research must be conducted using the relationships in a cooked sausage product.

Figure 4.1. Effect of Temperature on the Total Soluble Protein.

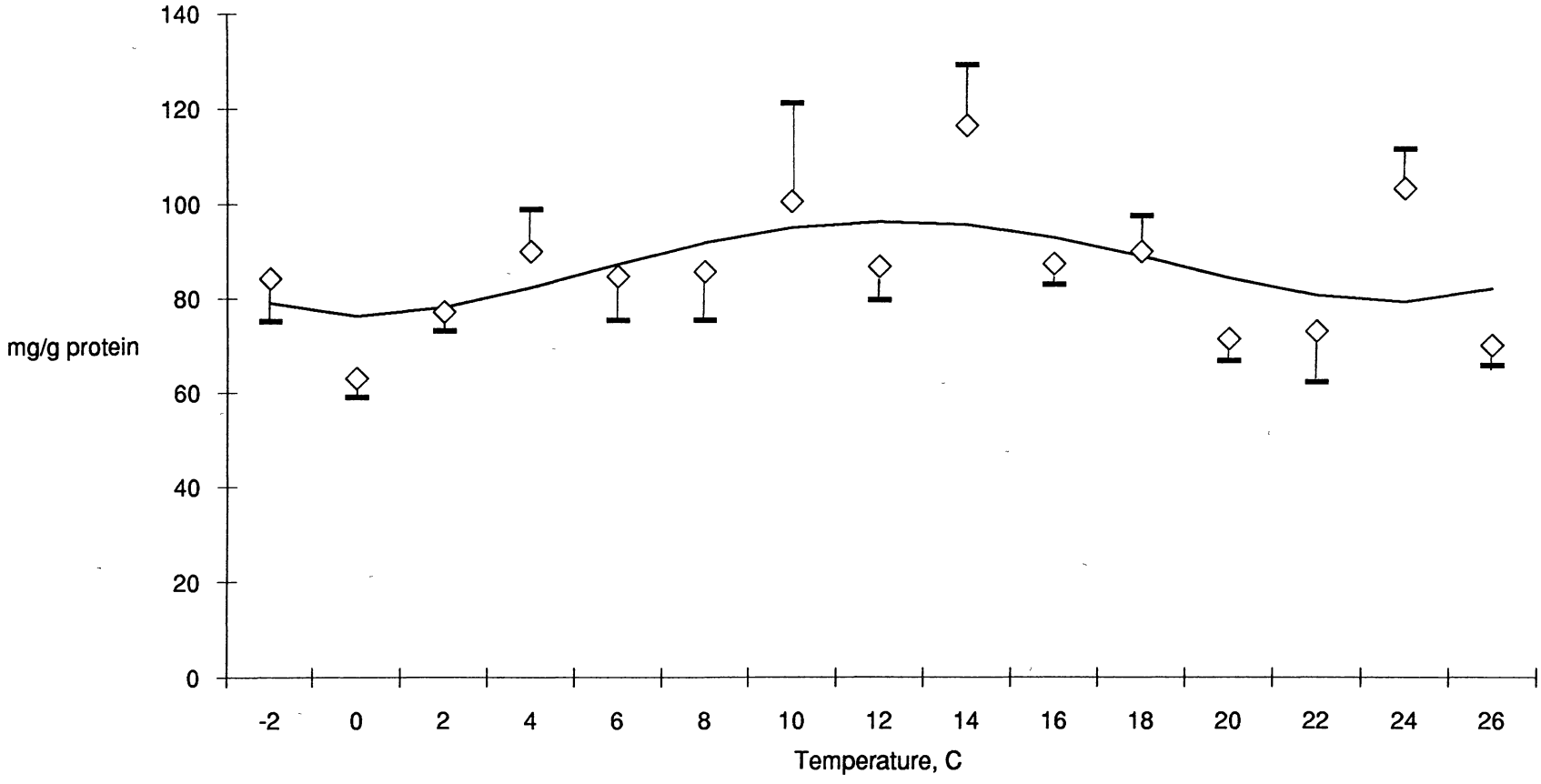


Figure 4 2. Effect of Temperature on the Solubility of Myofibrillar Protein.

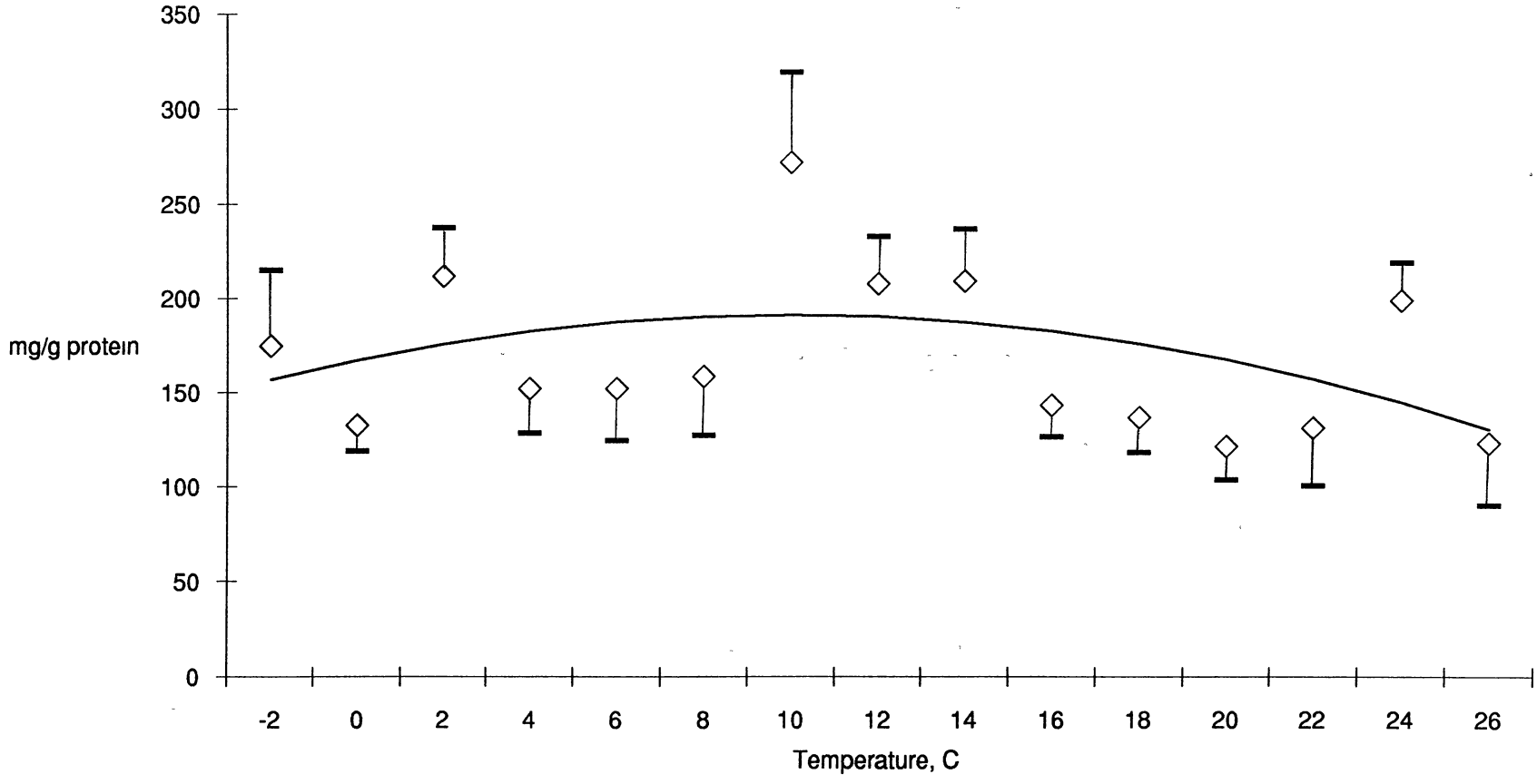


Figure 4.3. Effect of Temperature on the Water Holding Capacity.

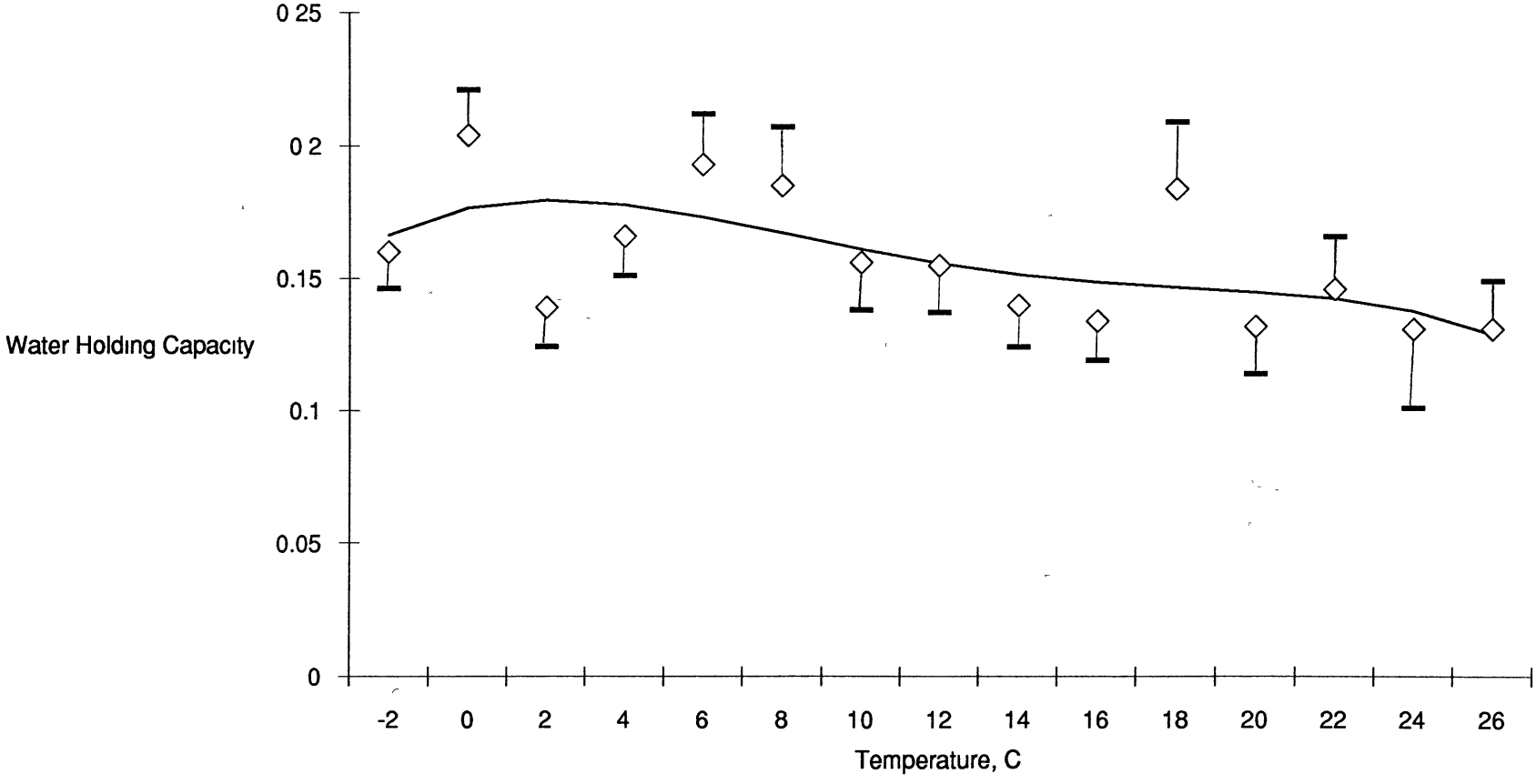


Figure 4.4. Influence of Fat and Added Water Level on the Total Protein Solubility.

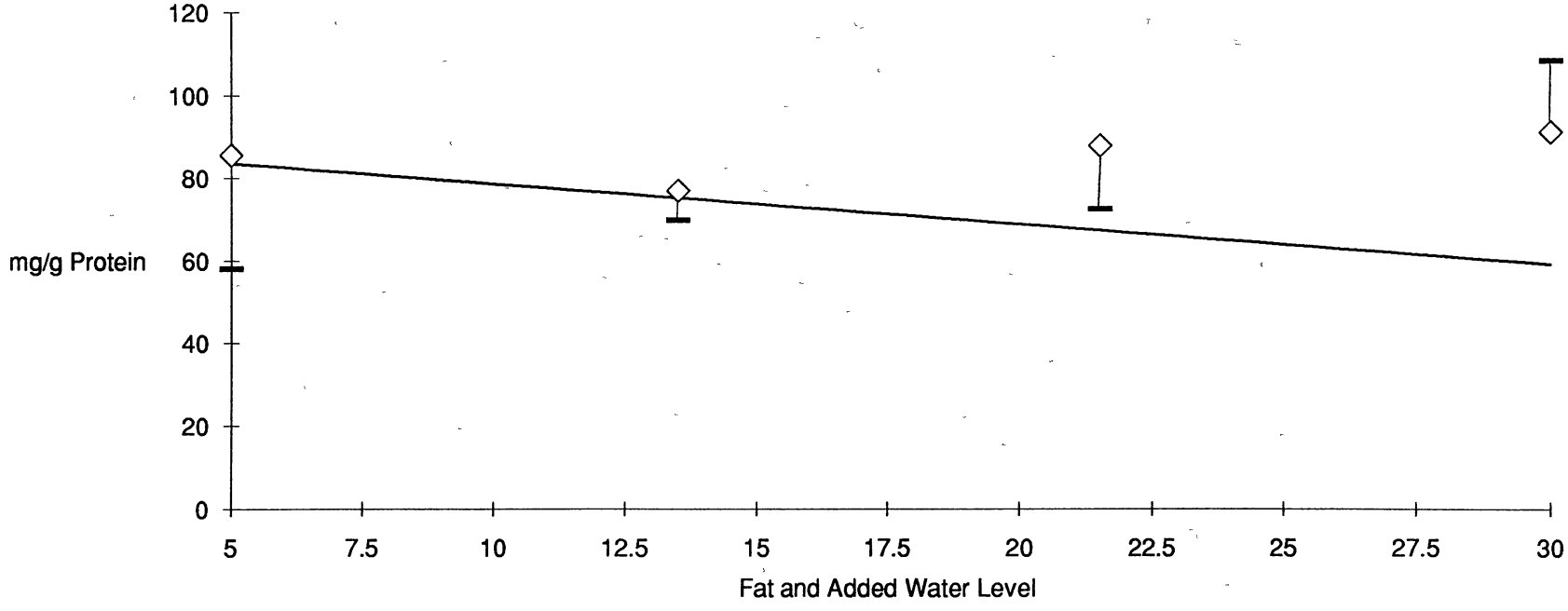


Figure 4.5 Influence of Fat and Added Water Level on the Solubility of Myofibrillar Protein.

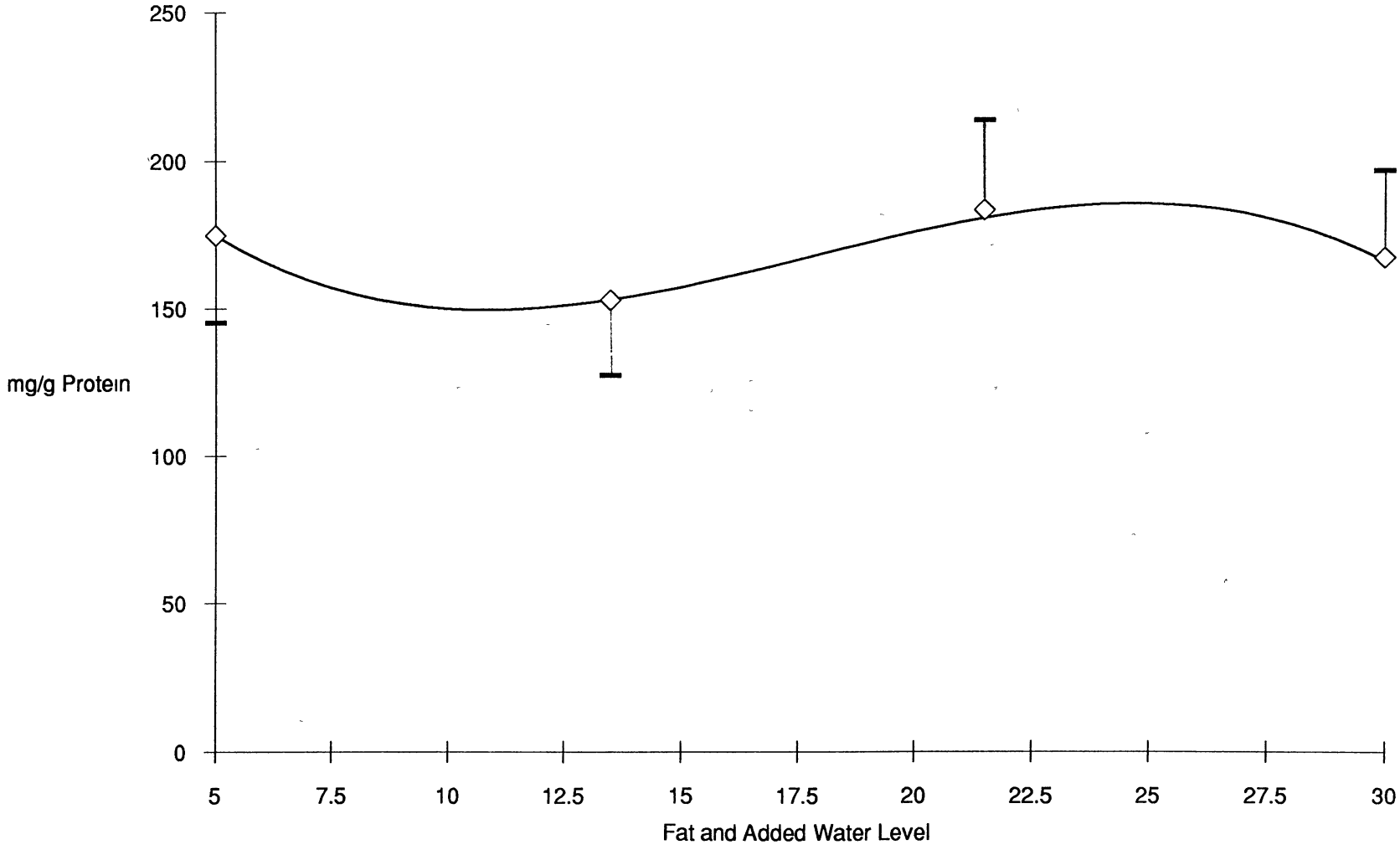
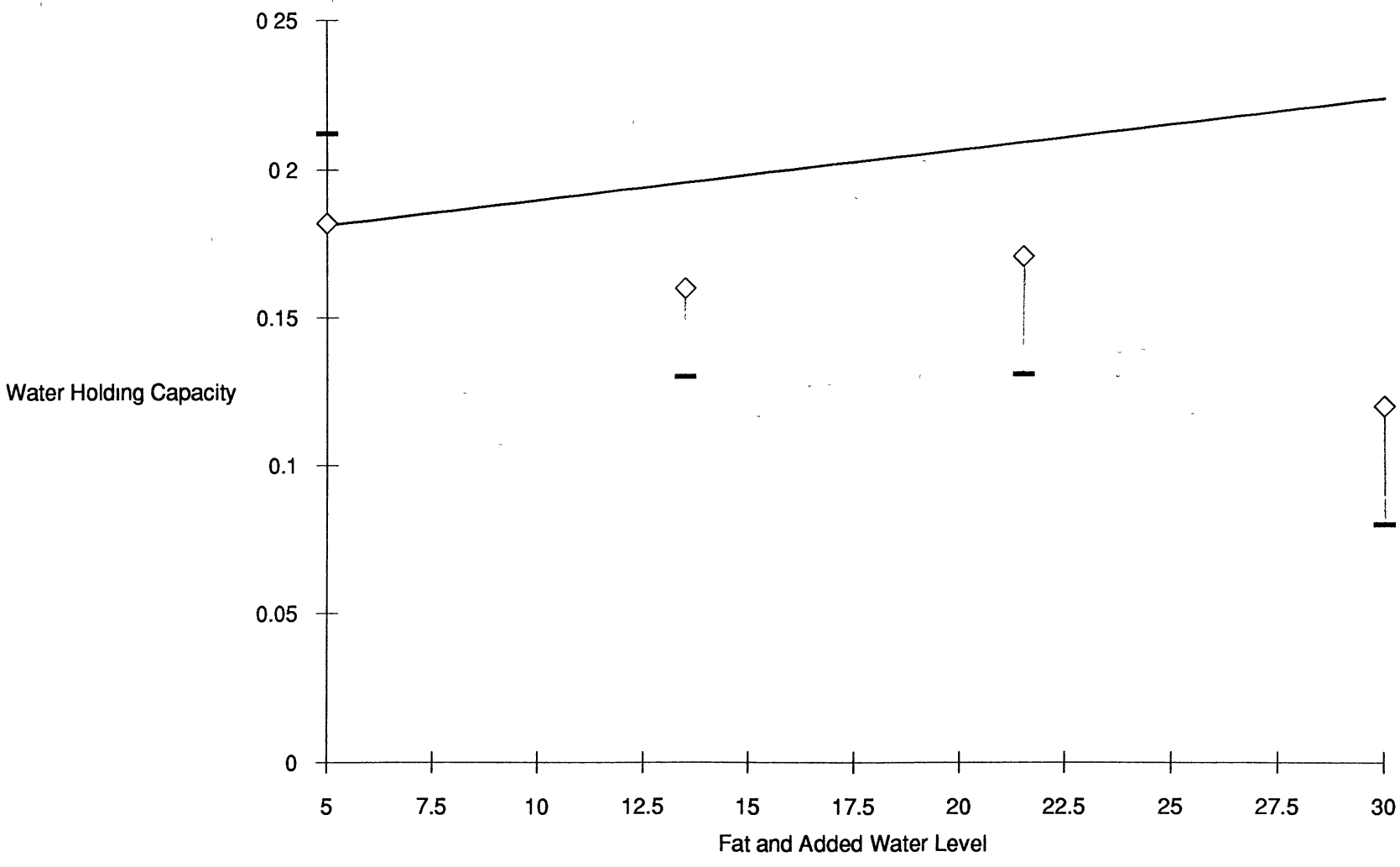


Figure 4.6. Influence of Fat and Added Water Level on the Water Holding Capacity



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APPENDIXES

APPENDIX A

EXTRACTION OF TOTAL SOLUBLE PROTEINS

Weigh 50±0.1 g of sample in a 250 ml Nalgene bottle.

|

Add 100 ml of 2% w/v NaCl solution

|

Shake at 120 RPM during 10 min

|

Centrifuge at 1860 x G during 10 min

|

Filter through Whatman paper #1

| |

Analyze filtrate for protein content.

Residue may be used the measurement of water uptake.

The total soluble protein (TSP) and the sarcoplasmic and myofibrillar protein present in the sample were calculated by means of the following expression:

$$\text{Protein Content (mg/g of protein)} = \frac{\text{S. R. mg/ml} * 5 \text{ ml} * \text{V.E.S. ml}}{\text{V. extract} * \text{S. weight}}$$

Where:

S.R. = Spectophotometer reading for the sample, mg/ml.

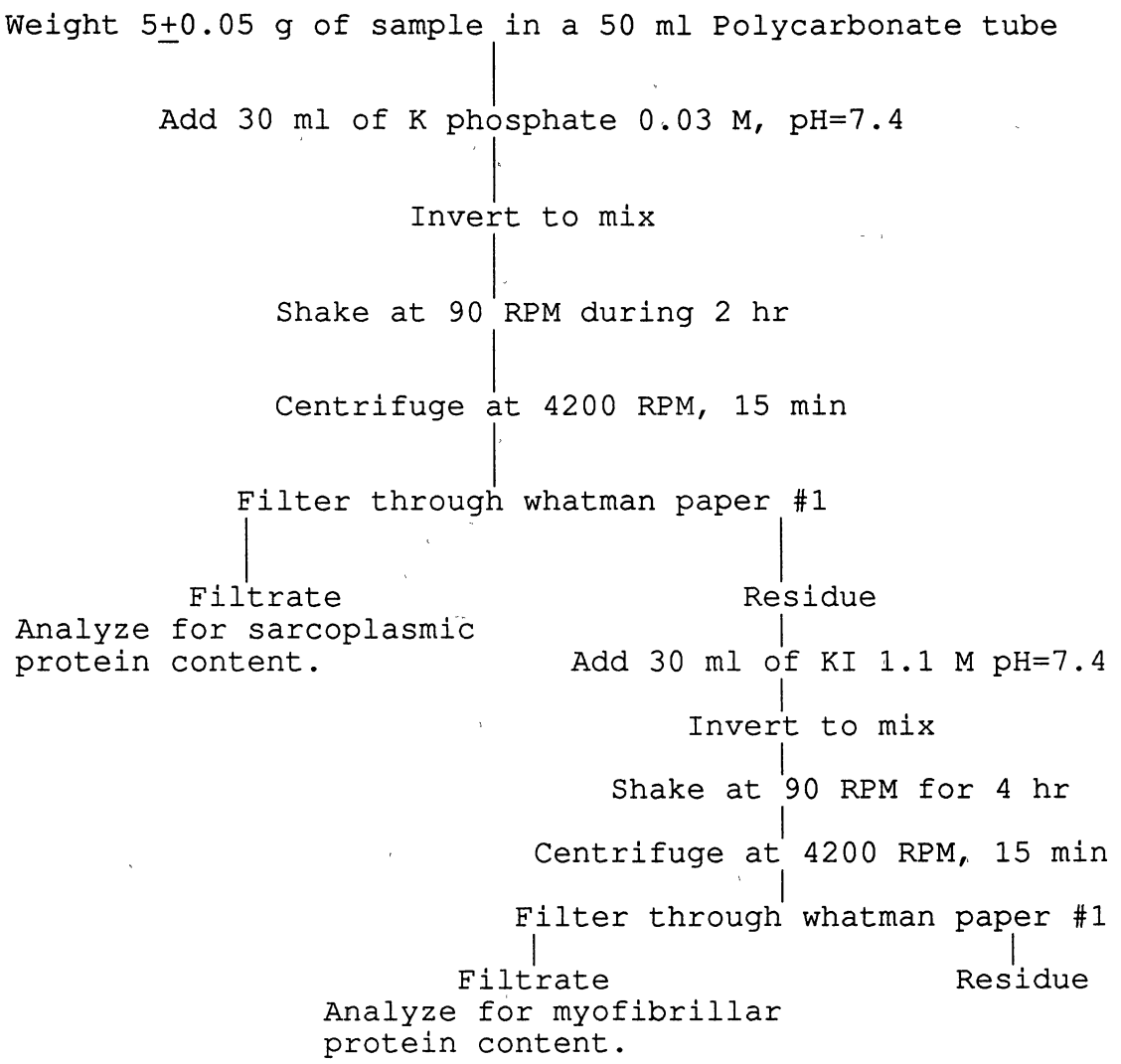
V.E.S. = Volume of extracting solution, ml (100 ml for total soluble protein, 30 ml for sarcoplasmic and myofibrillar extraction).

V. extract = Volume of protein extract used for the Biuret determination, ml.

S. weight = Sample weight, g.

APPENDIX B

EXTRACTION OF SARCOPLASMIC AND MYOFIBRILLAR PROTEINS



APPENDIX C

DETERMINATION OF THE WATER HOLDING CAPACITY

Fold two pieces of Whatmen paper #3 (5.5 cm dia.)
1 piece of whatman paper #50 (7.0 cm dia.)
into a thimble shape (with paper #50
as internal surface)

|

Weigh papers

|

Weigh again after the addition of
1.5±0.2 g of sample

|

Centrifuge at the required temperature
during 45 min, 3640 x G

|

Remove meat from paper

|

Weigh papers

The WHC of samples was calculated with the following formula:

$$WHC = 1 - \left(\frac{\text{moisture loss}}{\text{(original moisture content of sample)}} \right)$$

Where:

Moisture loss, g = (weight of paper + sample) before centrifugation - (weight of paper + sample) after centrifugation.

APPENDIX D

BIURET METHOD OF PROTEIN DETERMINATION

1. Biuret Reagent.

   1.58 g $CuSO_4$.
   6 g Na-K Tartrate (Rochelle Salt) in 500 ml of water.
   add 300 ml of NaOH (10% solution) and complete volume, with water, to 1 L.

2. Preparation of Standards.

   BSA (Bovine serum albumin, 10 mg/ml).

   To make standards, combine according to the following chart:

BSA (ml)	Biuret reagent (ml)	Water (ml)
0.0	4.0	1.0
0.1	4.0	0.9
0.2	4.0	0.8
0.3	4.0	0.7
0.4	4.0	0.6
0.5	4.0	0.5

3. Sample Preparation.

   Add 4ml of Biuret reagent to tube, add protein extract (0.2 ml for total soluble protein, 0.4 ml for sarcoplasmic or myofibrillar determinations), make volume to 5 ml with water, vortex and incubate during 30 min. Read concentration (at 550 nm).

APPENDIX E

TABLE E.1

MEANS AND STANDARD ERROR OF SIGNIFICANT VARIABLES IN CHAPTER III

T (°C)	TSP[a] Mean	TSP[a] SE[d]	S.P[b] Mean	S.P[b] SE[d]	M.P[c] Mean	M.P[c] SE[d]
-2	57.5	7.86	135.2	28.6	150.7	29.6
0	49.7	3.39	298.3	6.5	92.0	9.4
2	57.2	5.61	113.9	16.9	159.3	18.7
4	61.0	8.64	74.1	14.6	124.5	12.3
6	58.5	7.42	150.9	20.6	132.2	24.4
8	70.3	6.18	115.3	18.6	168.9	20.0
10	79.0	6.56	118.4	24.7	175.9	18.3
12	83.1	7.28	84.2	15.0	164.7	19.7
14	80.7	5.21	134.4	11.9	151.4	13.6
16	81.8	4.83	84.7	7.7	129.1	14.3
18	77.1	4.11	139.0	23.7	106.5	9.7
20	68.1	2.77	86.7	8.6	95.8	10.8
22	69.8	5.04	110.9	25.8	91.2	15.6
24	68.4	3.82	140.1	17.7	163.2	13.2
26	67.9	2.74	167.4	21.3	122.0	29.3

[a]Solubility of total soluble protein, mg protein/g of protein in sample.
[b]Solubility of sarcoplasmic protein, mg protein/g of protein in sample.
[c]Solubility of myofibrillar protein, mg protein/g of sample.
[d]Standard error.

TABLE E.2

MEANS AND STANDARD ERROR OF SIGNIFICANT VARIABLES IN CHAPTER IV, BY TEMPERATURE

T (°C)	TSP[a] Mean	TSP[a] SE[d]	M.P[b] Mean	M.P[b] SE[d]	WHC[c] Mean	WHC[c] SE[d]
-2	84.1	9.04	174.6	40.20	0.160	0.014
0	63.1	4.15	132.9	14.00	0.204	0.017
2	77.2	4.20	211.8	25.77	0.139	0.021
4	89.9	8.90	152.0	23.65	0.166	0.015
6	84.7	9.40	152.3	27.91	0.193	0.019
8	85.6	10.25	158.6	31.42	0.185	0.022
10	100.5	20.73	272.2	47.53	0.156	0.018
12	86.8	7.23	207.9	25.04	0.155	0.018
14	116.6	12.72	209.4	26.93	0.140	0.016
16	87.3	4.41	143.4	16.96	0.134	0.015
18	90.0	7.51	137.0	18.82	0.184	0.025
20	71.4	4.61	121.5	17.69	0.132	0.018
22	73.1	10.9	131.4	30.6	0.146	0.020
24	103.3	8.3	199.0	20.1	0.131	0.030
26	70.0	4.31	123.2	32.9	0.131	0.018

[a]Solubility of total soluble protein, mg protein/g of sample.
[b]Solubility of myofibrillar protein, mg protein/g of sample.
[c]Water holding capacity.
[d]Standard error.

TABLE E.3

MEANS AND STANDARD ERROR OF SIGNIFICANT VARIABLES IN CHAPTER IV, BY FAT LEVEL EFFECT

FL[a] (%)	AW[b] (%)	TSP[c] Mean	TSP[c] SE[f]	MP[d] Mean	MP[d] SE[f]	WHC[e] Mean	WHC[e] SE[f]
5.0	35.0	85.5	27.10	174.8	29.60	0.182	0.03
13.5	26.5	77.0	7.18	153.0	25.73	0.160	0.03
21.5	18.5	88.0	15.45	180.7	60.25	0.171	0.04
30.0	10.0	91.2	17.1	165.3	59.30	0.120	0.04

[a]Fat level.
[b]Added water.
[c]Solubility of total soluble protein, mg/g of protein in sample.
[d]Solubility of myofibrillar protein, mg/g of protein in sample.
[e]Water holding capacity.
[f]Standard error.

TABLE E.4

REGRESSION EQUATIONS OF SIGNIFICANT VARIABLES IN CHAPTER III

Variable	Parameter estimate		R^2
TSP[a]	Intercept	52.110	0.16
	T	-0.7950	
	T2	0.7940	
	T3	-0.05771	
	T4	0.0011	
SP[b]	Intercept	8.583	0.064
	T	3.923	
	T2	0.814	
	T3	0.054	
	T4	0.0011	
MP[c]	Intercept	125.201	0.062
	T	-1.897	
	T2	1.819	
	T3	-0.164	
	T4	0.004	

[a]Total soluble protein, mg/g of protein in sample.
[b]Solubility of sarcoplasmic protein, mg/g of protein in sample.
[c]Myofibrillar protein, mg/g of protein in sample.

TABLE E.5

REGRESSION EQUATIONS OF SIGNIFICANT VARIABLES IN CHAPTER IV, FOR TEMPERATURE EFFECT

Variable	Parameter estimate		R^2
TSP[a]	Intercept	76.3208	0.036
	T	-0.0340	
	T2	0.0542	
	T3	-0.0444	
	T4	0.00092	
MP[b]	Intercept	156.5671	0.037
	T	-1.3812	
	T2	2.0111	
	T3	-0.1750	
	T4	0.0039	
WHC[c]	Intercept	0.1766	0.038
	T	0.0031	
	T2	-0.8988×10^{-3}	
	T3	0.0529×10^{-3}	
	T4	-0.0010×10^{-3}	

[a]Solubility of total protein, g of protein/g of protein in sample.
[b]Solubility of myofibrillar protein, g of protein/g protein in sample.
[c]Water holding capacity.

TABLE E.6

REGRESSION EQUATIONS OF SIGNIFICANT VARIABLES IN CHAPTER IV, FOR FAT LEVEL EFFECT

Variable	Parameter estimate		R^2
TSP[a]	Intercept	112.0091	0.018
	FL	-7.4603	
	FL2	4.7310	
	FL3	-0.0082	
MP[b]	Intercept	251.8743	0.0082
	FL	-22.0240	
	FL2	1.4580	
	FL3	-0.0273	
WHC[c]	Intercept	0.2527	0.095
	FL	-0.0203	
	FL2	0.0014	
	FL3	-0.00003	

[a]Solubility of total protein, mg/g of protein in sample.
[b]Solubility of myofibrillar protein, mg/g of protein in sample.
[c]Water holding capacity.

VITA

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Candidate for the Degree of

Master of Science

Thesis: EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF SHOULDER CLODS

Major Field: Food Science

Biographical:

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