THE EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF TRIM FROM SIX DIFFERENT CARCASS SOURCES

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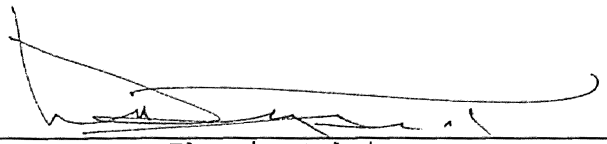

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DEDICATION

This thesis is dedicated to those who are far from my eyes, yet are close to my heart; my father Taha and my mother Kabole.

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CHAPTER I

INTRODUCTION

The nutritionally related health problems experienced by a large section of the world population have been attributed to the over-consumption of fat, in particular saturated fat and cholesterol (National Research Council, 1988). Medical and nutritional evidence has linked the development of coronary heart diseases to the intake of high amounts of dietary fat and cholesterol. Reducing dietary intake of saturated fat, cholesterol, salt and sugar to obtain enough energy and to maintain ideal body weight were recommended by several health organizations as a means to improve health.

Meat and its products are excellent sources of high quality protein, B-complex vitamin and certain minerals, especially iron. They are easily digested, and when cooked, lean meat supplies essential nutrients that contribute significantly to dietary balance. At the same time, meat and meat products provide 36% of the total caloric content of the food supply (National Research Council, 1988). Due to this fact and the consumer's new demand for low fat and low calorie foods, the meat industry has responded by producing leaner cuts of meat,

and developing and formulating new low fat, low calorie products (Claus, et al., 1990).

Development of a low fat, low cholesterol products have been investigated by several researchers. Huffman, et al., (1991) indicated that the simple and most efficient method of producing a low fat meat product is by reducing fat and thereby replacing the fat with other food ingredients. A wide range of ingredients has been suggested as fat substitutes, with the major categories being water, protein, carbohydrates and synthetic compounds (Keeton, 1991).

A new approach to a reduced fat level in meat products has been recommended by Hand, et al., (1987) by using processing technologies (i.e., preblending) as well as through modifications of processed meat formulations (Keeton, 1991; Claus et al., 1990). The meat industry's chance to develop low fat products increased with the advent of the "40% rule", a recent USDA cooked sausage labeling modification. This regulation permits fat and added water to substitute for one another provided that their total dose not exceed 40%, and that the total fat does not exceed 30% (Federal Register, 1988).

The replacement of fat with water in low fat meat products increases pressure on the proteins of a beef frankfurter system, for example, to bind or immobilize the added water. The role of proteins in low fat meat

products has thus become more important. Of the proteins found in meat products, the myofibrillar proteins play an important role in holding the added water in a high moisture system. However, several factors influence the ability of these proteins to function in such a system. Some of the major factors are temperature, pH, ionic strength, particle size, and postmortem age (Regenstein, 1984). Of these major factors, temperature and ionic strength are more easily controlled in meat product manufacture.

The objective of this study was to evaluate the effect of temperature on the extraction of total salt soluble proteins of various beef trimmings, in particular, bull, blade, cow, heart, head, and plate trimmings.

CHAPTER II

GENERAL LITERATURE REVIEW

The Development of Low-fat Meat Products

In the industrial nations, improving agriculture through public funding of research, and applying scientific knowledge to the food industry, including enrichment, fortification as well as improving food quality and distribution, have almost ended the occurrence of nutritional deficiencies (Hand, 1981). However, in recent years, attention has turned from nutritional deficiencies to the concern for over-consumption of calories, fat, and cholesterol. It has been estimated that about one million deaths per year in the United States are caused by heart disease and 43 million adults are overweight (National Research Council, 1988). Huffman et al. (1991) reported that over two-thirds of consumers have some concern about their health with fat consumption accounting being a major concern. As consumers become too willing to link fat intake and health problems, their demand for lean meat becomes greater. Diet health awareness has become a potentially dangerous wide window from which red meat may be attributed to the degenerative

diseases, including cardiovascular (heart attacks), stroke and cancer (Briggs and Schweigert, 1990). Due to this fact as well as the consumer's new demand for low fat and low calorie foods, the red meat industry has responded by producing lean cuts of meat and by developing and formulating new low fat and low calorie products (Claus et al., 1990).

Due to the fact that at least some level of fat or fat-like material in processed meat is essential for desirable texture and flavor, the meat industry has reduced fat in meat products by using non meat ingredients, modifying raw materials, using added water or changing processing techniques. However, it may be for the reader's benefits to focus on each of these methods individually.

Use of Non-meat Ingredient

Use of Plant Proteins

Plant proteins have been used in processed meat products to achieve many advantages, including reducing fat content, increasing cooked yield, lowering cost, enhancing functional properties and improving nutritional value (Bischoff, 1990). Wheat flour, wheat gluten, soy protein, cottonseed flour, oat flour, and corn germ meal

have been mentioned as examples of protein additives (Keeton, 1991).

Sofos and Allen (1977) found that using a high level of textured soy protein (45%) did not adversely affect emulsion stability in low fat meat products. Soy proteins have traditionally been the most used product for extending ground beef, patties, pepperoni and other formulations. Decker et al. (1986) investigated the effect of using isolated soy protein as a meat extender in frankfurter formulations of different fat content. As the fat level was reduced, color and texture values increased. Hookgenkamp (1991) reported that soy protein acts very similar to lean meat and claimed benefit of soy protein as improving the functional and nutritional properties of a product. Keeton (1991) pointed out that the extension of ground beef meat up to 30% with dehydrated protein is possible, yet attention to their formulation is required to avoid altering sensory properties. With the recent emphasis on low fat meat products, animal and plant protein offer a potential fat substitute in processed products.

Use of Oils of Plant Origin

Not only is the reduction of fat important in low fat meat products but also the type of is becoming increasingly important from a nutritional point of view.

Many studies have been conducted to examine the replacement of saturated fat with unsaturated oil. Park et al. (1989) substituted (7.5%) of the fat in a low fat (15%) frankfurter with a fish oil having a high oleic acid content and found that fish oil imparted an undesirable fish flavor to the resulting frankfurters. In an ensuing study, Park (1990) increased the added water up to the maximum allowable amount and found that the frankfurters were as acceptable as 28% fat controls. Marquez et al. (1989) processed franks having 12% fat, 20% and 29% fat levels, yet with a 60% replacement of each fat level with peanut oil the 12% fat franks resulted in a less acceptable panel scores as well as a decrease in emulsion stability and lower smokehouse yields.

Keeton (1991) conducted an experiment using beef and pork trims in formulating frankfurters and found that as the mono-unsaturated/saturated fatty acid ratio increased, an increase in firmness and springiness occurred with a decrease in juiciness. Townsend et al. (1977) studied the effect of several types and levels of fat on emulsion and frankfurter properties. They found an inverse relationship between fat content and shrinkage in frankfurters containing cottonseed oil when compared with beef or pork fat.

Use of Carbohydrate-based Additives

Various carbohydrates have been used to partially or to totally replace fat or oil in several products. Gums, polydextrose, and corn and potato starch maltodextrines have been used for more than a decade. Most of the carbohydrates that are available for fat replacement in processed meat fall into three categories: gum (hydrocolloid), starch and cellulose-based derivatives. Among the claimed advantages of gums are to maintain viscosity, to stabilize emulsions and inhibit syneresis, and to encapsulate particulates (Keeton, 1991).

Plant gum carrageenan was selected for moisture retention on the basis of its ability to form a complex with water and protein. Among the claimed advantages are improved juiciness, tenderness, and mouth feel. Huffman et al. (1991) reported that there are three basic types of carrageenan: kapa, iota and lambda. Huffman et al. (1991) stated that unlike other binders that are used to bind meat particles, iota carrageenans also have other intrinsic binding properties that are beneficial in the production of low fat ground beef such as a cold solubility that enhances the machinability of low fat ground beef. However, not all iota carrageenans are alike and that a proper blend of iota carrageenans are important to achieve high quality, low fat meat products. Use of other gums such as alginate or xanthan gums enhanced water

binding in frankfurters but were found to be harmful to gel strength (Whiting, 1984).

Cellulose-derived components have been used in meat products. Lin et al. (1988) examined the utilization of four types of carboxymethyl cellulose at 0.25% in a low fat (~15%) high moisture frankfurter system and noted that cooked franks had a soft texture and low Instron texture values as compared to controls.

Carbohydrate-based fat replacement or its combination with gums, starch, and/or protein seems to offer the most cost-effective means of replacing a significant portion of fat in meat products while duplicating the textural and sensory properties of animal fat (Huffman et al., 1991).

Modifying Traditional Processing Methods

Use of Added Water

Reducing fat in processed meat can be achieved by either using leaner meat, which increases the cost, or by substituting fat with water or other ingredients having low calories (Keeton, 1991). According to the USDA rule 40, fat and added water can substitute for one another, provided that their total calories do not exceed 40% and the fat content does not exceed 30% (Federal Register, 1986). The meat industry's chance to develop low fat meat products has increased with the advent of this regulation.

Reducing the fat level in an emulsion type sausage while keeping added water constant (increasing lean content) affects organoleptic and texture properties of the original product. The product becomes more rubbery, less juicy, darker in color, and results in less cooked yield (Hand et al., 1987; Rongey and Bratzler, 1966).

Claus et at. (1990) replaced fat with added water in a bologna formulation using a range from 30% fat/10% added water to 5% fat/35% added water, and found that low fat/high added water bologna to be less firm, less cohesive, less juicier, and darker, with less cooking yield when compared to a 30% fat/10% added water control. In another study, Claus et al. (1990) reported that neither massaging of raw materials nor pre-blending at 10% fat/30% added water in the production of bologna was enough to reduce cooking losses compared to the control (30% fat-10 added water).

Increased cooking losses were also noted for pork sausage having either a 15%, 25%, 35% fat content with 3% or 13% added water as added water increased (Ahmed et al., 1990). Taste panel scores for overall palatability of low fat/added water sausages were equivalent to scores for a 35% fat formulation without added water.

Replacement of fat with added water decreases caloric intake in addition to altering organoleptic and textural properties of low fat meat products. An increased water

content also reduces cooking yield, increases vacuum purge and softens texture, besides affecting product shelf life and flavor (Keeton, 1991).

Modifying Raw Materials

Changing fat content and fatty acid composition can be done by reformulating meat products and by replacing more saturated fats with less saturated fats (Keeton, 1991). The fat level of meat products may be reduced biologically by manipulating the composition of the raw meat ingredients either by altering an animal's genetics or by manipulating feed composition. Changing the lipid composition of an animal diet can dramatically change the fatty acid composition of meat trims from non-ruminants. Shackelford et al. (1990) produced pig carcasses that had a 10% to 40% less saturated fatty acid content by modifying feed composition. They found that sausage made from the high oleic acid tissues had lower sensory panel scores for springiness, cohesiveness, texture, and overall palatability. Pork trim from pigs fed a diet containing 10% or 20% canola oil produced a low fat frankfurter having 20% less saturated fat (St. John et al., 1986). However, altering a non-ruminant's diet can cause the carcass to be more oily and to have less trim in addition to having lipid that is more susceptible oxidative rancidity (Keeton, 1991).

Modifying Processing Methods

Pre-blending has been suggested as a new approach to reduce fat and salt levels in meat products by Hand et al. (1987). Results revealed that pre-blending had no effect on textural properties but had a limited effect on color (dark color). Pre-blending also enhances protein binding, color and water holding capacity (Acton and Saffle, 1969).

The effect of massaging, pre-blending and time of addition of water and fat on physical and sensory characteristics was studied by Claus et al. (1990). Results revealed that physical manipulation via massaging may offer a possible alternative to increase water binding and protein to protein interaction. Other advantages for massaging were improved color, texture, fat distribution, and the binding quality of sectioned and formed meat products. However, even though massaging has been successfully used in cured pork products, limited benefits are obtained in sausage manufacturing.

The results from these studies indicate that the manufacture of low fat, high added water products may be achieved. However, as the fat level decreased, the lean component became a dominant factor in affecting the textural properties of meat products. Further research on the role of the different the components of meat is needed, in particular, the protein components of the lean

tissue. Regardless of the methods used to substitute fat either totally or partially, there is a clear-cut relationship between the amount, type and functional properties of the proteins utilized.

Muscle Protein

The proteins of muscle can be divided into three main groups based on their relative solubility at different ionic strengths: (1) sarcoplasmic, (2) myofibrillar, and (3) connective tissue (Lawrie, 1979). They may be broadly classified into water-soluble, salt-soluble, and insoluble fractions (Tarrant, 1982).

Myofibrillar Proteins

Myofibrillar proteins are the proteins of the myofibril, the basic structural unit responsible for contraction in living animals and involved in the development of rigor mortis after death (Pearson and Young, 1989). It may be further subdivided into three subgroups: (1) the major contractile proteins (actin and myosin), (2) the regulatory proteins that play an important role in initiation and control of contraction, and (3) the cytoskeletal or scaffold proteins that provides structural support for the manifestation of molecular actions into useful work. Myofibrillar protein constitutes about 55 to 60% of the total muscle protein,

or 10% of the muscle protein (Asghar et al., 1985). The two major contractile proteins, myosin and actin, compose 65% of the myofibril while the regulatory proteins, troponin and tropomyosin compose 10% (Yates and Greaser, 1983).

Myosin in either skeletal or cardiac muscle makes up more than one-third of the total protein (Pearson and Young, 1989). Among all the proteins found in muscle, myosin plays an 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).

Actin, approximately 23% of the mass of the myofibril, is the major structural unit of the thin filament (Gordon and Yates, 1992). Other proteins of the thin filament include troponin and tropomyosin; the main proteins regulating the interaction between myosin and actin (Greaser and Gergely, 1973; Cummins and Perry, 1974).

Actomyosin is formed by the interaction between actin and myosin that characterizes post-rigor meat (Tarrant, 1982). It is this interaction and the events leading to the development of rigor mortis that determines much of the value and utility of meat and how it is used in meat products.

Sarcoplasmic Proteins

The sarcoplasmic proteins comprise about 30-35% of total muscle protein. There are over 100 different sarcoplasmic proteins, consisting mostly of the enzymes involved in metabolism. The sarcoplasmic proteins are believed to occupy about 25% of the cytoplasmic space. Thus, they are important constituents of muscle (Pearson and Young, 1989). Some of the important properties of sarcoplasmic protein are low water binding capacity, an ability to form low viscosity solutions that interact slightly with lipids in emulsions, and little or no ability to form protein-protein gel upon heating (Acton et al., 1983). The sarcoplasmic proteins are removed from muscle by extraction with dilute solutions having an ionic strength of less than 0.1. Water is often used for this purpose (Pearson and Young, 1989).

Connective Tissue

The interstitial space between muscle cells contains three proteins, all fibrillar in nature: collagen, elastin, and reticulin. The connective tissue proteins are the most abundant group of proteins in the body. Collagen alone may comprise as much as 25% of the total body protein (Seifter and Gallop, 1966). The connective tissue proteins are relatively insoluble and not only impart toughness and shape, but also provide protection to

the skeletal muscles (Pearson and Young, 1989). When heated to about 70° C, collagen fibrils shorten to about one-third of their original length. At 80° C, collagen is converted into gelatin. Reticulin does not undergo gelation upon heating. Elastin is not decomposed by heat and has a very limited swelling ability. It has been reported that elastin is responsible for the elastic properties of connective tissue (Schut, 1976; Asghar et al., 1985).

Functional Properties of Muscle Protein in Meat Products

Proteins are the principal functional and structural components of processed meats that determine the characteristic handling, textural, and appearance of the final products (Hermansson et al., 1986). In processed meat, the functional properties of meat protein are usually related to both physical and chemical properties. Functional properties of proteins are of extreme importance because proteins can either be beneficial or detrimental in food systems containing them as a major ingredient (Hoogenkamp, 1991).

Functionality of food proteins was extensively studied by Kinsella (1976, 1982), and by Kinsella and Fox (1986). Broadly, they defined it by any physical, or chemical property that affects the behavior of proteins in

a food which in turn alters the quality of the final product (Kinsella, 1976).

Table I contains several general classes of functional properties of protein that are important in food applications. Environmental conditions such as pH, temperature, protein concentration, protein species, ionic strength, and presence of other macromolecules affect the functionality a protein (Kinsella, 1976). Processing methods, including solubilization and extraction, hydration, heating, freezing and dehydrating also affect a protein's functionality.

Solubility is a very important function of the protein that controls the overall functionality of protein-containing products (Trautman, 1967; Smith, 1988; Whiting, 1984; Hoogenkamp, 1991). Water-holding capacity has also been mentioned as an important property that affects quality and composition of a meat product.

The main functional property of a protein used in meat products is binding of fat and water, the degree of solubilization, and the degree of gelling. Other functional properties are important such as sensory and the organoleptic properties of color, taste, texture, and flavor. Hydration properties, also affecting the array of sensory and the organoleptic properties, include the interaction of proteins with water. From a sensory point of view, color, texture and the shape or form of a food

TABLE I

FUNCTIONAL PROPERTIES OF PROTEINS IMPORTANT IN MEAT PRODUCTS

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

product are most important to a consumer (Hoogenkamp, 1991).

Protein solubility is a very important property of protein functionality in protein-containing products (Trautman, 1967; Smith, 1988; Whiting, 1988; Hoogenkamp, 1991). The ability to replace fat with water in a low-fat meat product depends to some degree on the salt soluble proteins extracted. Therefore, emphasis should be placed on the solubility of proteins as well as the parameters that affect protein solubility.

Protein Solubility

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

The amount of sarcoplasmic protein in early embryonic stage may be as high as 70%, yet it declines to about 30-35% of the total extractable protein in adult mammalian skeletal muscle (Pearson and Young, 1989). The decrease in the proportion of sarcoplasmic proteins in muscle as

animals reach maturity is mainly due to the increase in the amount of myofibrillar protein as the embryo develops and the animal matures. Thus, the solubility of sarcoplasmic and myofibrillar protein has been used to characterize protein changes during animal maturity as well as to compare protein composition of different muscles (Sayre and Briskey, 1963).

Methods of Protein extraction

Several studies have attempted to quantitate the amount of sarcoplasmic and myofibrillar protein extracted from muscle. The following basic steps have been used to extract protein from muscle tissue:

1) mechanical or physical disruption of tissue;
2) solvent addition;
3) mixing, blending or other mechanical agitation;
4) separation of the soluble phase; and
5) determination of the protein content in the soluble phase and the residue.

Helander (1957) investigated various factors that affect protein extraction. The effects of several important factors that could affect protein extraction were evaluated: the effect of storage at different holding temperatures before and after different freezing methods, the effect of pH, the effect of different salts, ionic strength, solvent volume, and the effect of repeated

extraction. A general method for protein extraction was developed using 0.03 M potassium phosphate, pH 7.4 for the extraction of a sarcoplasmic protein and 1.1 M KI and 0.1 M phosphate buffer at pH 7.4 for extraction myofibrillar protein. Great difficulty in extracting all the myofibrillar protein from muscle tissue was reported since the extraction performance reached an equilibrium dependent on the amount of solvent used, the number of repeated extraction's as well as the extraction duration. This method or modifications of it have been used by many researchers (Sayre and Briskey, 1963), yet this procedure is generally tedious and time-consuming, and is unsatisfactory for routine work (Morr et al., 1985). Thus, similar methods have been developed and used for evaluating protein extraction.

The effect of fat, pH, freezing and post-mortem aging on the extraction of salt soluble proteins from different areas of beef carcasses was studied by Saffle and Galbreath (1964). The amount of total extracted protein increased when the pH of the extraction solutions became higher.

The effect of different salts (NaCl, KCl, LiCl, $MgCl_2$, $MgCl_2$, $CaCl_2$, KBr and KI) on the extractability of muscle protein was studied by Helander (1957). KI was clearly superior to others as an extraction agent. However, neutral and alkaline polyphosphates combined with

sodium chloride extraction have been reported to be superior to other combinations of salt on protein extraction (King and Earl, 1988).

Helander (1957) also reported that a pH of 7.4 resulted in the highest amount of extracted protein. Two extraction's were adequate for the extraction of sacroplasm proteins while three extractions were adequate for the extraction of myofibrillar proteins. Short extraction times were found necessary to prevent autolytic postmortem changes in muscle over long periods (days or months). Storage of frozen tissue below 0° C diminished protein extraction. Similar results were reported by Wanger and Añon (1986) and Awad et al. (1968). An increase in storage time decreased protein solubility and viscosity. Others parameters such as extraction temperature, extraction time and extracted meat source were reported to effect protein extraction Gillett et al. (1977) and Miller et al. (1980).

The effect of pre-rigor muscle temperature and post-rigor pH on protein solubility of ham muscles was studied by Trautman (1967). His results did not demonstrate an effect of temperature, yet a pH value of 6.4 resulted in high amount of protein being solubolized.

MacBride (1986) compared several methods of extracting muscle proteins to find how alterations in methodology affected protein solubility measurements.

Results indicated that protein solubility measurements were affected by variation in the methods of separating the soluble phase (centrifugation versus filtration) and the method of extraction (blending versus not blending). Blending and centrifugation resulted in a higher amount of extracted protein. A comparison of pre-rigor and post-rigor muscle demonstrated that pre-rigor muscle was superior for the extraction muscle protein.

Changes in the speed of centrifugation were also found to affect the amount of water expressed from muscle or protein suspensions (Jauregui et al., 1981; Miller et al., 1968). Thus, the amount of water obtained from the residue in turn affected the volume of the soluble phase and consequently protein concentration.

In summary, the replacement of fat with water in a low fat meat product results in the extracted proteins having a greater influence on the textural properties of the resulting meat product. These textural properties are dependent on the functionality of the extracted proteins and the temperature of extraction. As such, the objective of this study was to evaluate the effect of temperature and beef trimming type on the extraction of salt soluble protein.

CHAPTER III

THE EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF TRIM FROM SIX DIFFERENT CARCASS SOURCES

Abstract

The ability to replace fat with water in a low fat meat product depends to some degree on the salt soluble proteins extracted. The objective of this study was to evaluate the effect of temperature on the extraction of total salt soluble proteins of various beef trimmings. Three replications of six beef meat trimmings (bull, blade, cow, heart, head and plate) were evaluated in the range of -3 to 24° C in 3° C intervals. A 2% NaCl solution was used for extracting protein. A design with replication as blocks and meat trimming source and temperature as treatments was used. Type of beef trimming had significant effects on total salt extracted protein ($P < 0.05$) with blade trimmings having the largest amount of extracted protein ($P < 0.05$) and head trimmings having the least amount of extracted protein ($P<0.05$). Regarding efficiency of protein extraction, heart muscle trim had the highest protein extraction efficiency, 31.4 $\pm$ 8.5% (P

<0.05), while head trimmings had the lowest, 19.5 ± 8.5% (P<0.05). Protein extraction-temperature profiles exhibited several maximas and minimas with each meat trimming exhibiting a unique protein extraction-temperature relationship.

Introduction

In the past several years, various public health agencies such as the American Heart Association (1986) and the American Cancer Society (1984) recommended that consumers eat low fat and low salt meat products. Low fat and low salt meat products have been produced to meet consumers' desires for the perceived healthful benefits. However, maintaining organoleptic and textural properties of the original product have been a problem (Hand et al., 1987). Many of the changes in organoleptic and textural properties resulted from the impact of a compositional change on protein functionality. The salt soluble myofibrillar proteins' myosin and actomyosin of pre-rigor and post-rigor muscles, respectively, have a unique functionality regarding the organoleptic and textural properties of processed meat products (Wilding et al., 1984; Forrest et al., 1989).

The functionality of meat protein has become a matter of major priority. The functionality of a protein is related to its physical and chemical properties

(Hoogenkamp, 1991) which in turn impact the ability of the protein to influence the organoleptic and textural characteristics of end products (Kinsella, 1982).

The emulsion capacity and stability, water retention, and protein solubility are the three most important functional properties of meat proteins (Regenstein, 1984). Temperature, pH, ionic strength, particle size, and postmortem age are the principle factors influencing protein behavior during emulsion formation and heating. The replacement of fat with water in low fat products increases the pressure on frankfurter system and the salt soluble proteins of becom important for binding the added water. However, typical material sources from different areas of the carcass have not been comprehensively evaluated for protein extraction over a useful temperature range having sufficient resolution. Therefore, the overall objective of this study was to evaluate and characterize the effect of temperature on the extraction of salt soluble protein from the beef trimmings of six different carcass locations; bull, blade, cow, heart, head and plate.

Materials and Methods

Materials

Three replicates of six different beef trimmings from bull, cow, blade, head, heart and plate (30 kg each) were obtained from a local supplier (Ralph's Packing Company, Perkins, OK). Each trimming was approximately 10 days postmortem and had not been frozen. The beef trimmings were ground using a 2.0 cm plate at 3.8 ± 0.2° C, frozen at -28 ± 2° C for 96 hrs. and then thawed at 3 ± 1° C for 72 hrs. Each thawed trimming was then chopped at 2 ± 0.5° C (K64 Seydelmann Robert Riser Canton MA) for 45 seconds at low speed, followed by an additional 45 seconds at high speed to ensure homogeneity. The chopped trimming was divided into 11 portions, each 0.68 ± 0.1 kg, packaged using feezer pags (Ziploc®, DownBrands Inc, Indianapolis), randomly assigned to one of ten temperature treatments, and frozen at -29 ± 1° C until used. The eleventh portion was used for the determination of protein, fat, and moisture content (AOAC, 1984). The mean proximate composition of each beef trimming is presented in Table II.

Protein Extraction

Protein extraction was conducted in triplicate on each beef trimming using a modified version of the method

TABLE II

PROXIMATE MEAN COMPOSITION OF BEEF TRIMMINGS USED FOR EXTRACTION

Meat	% Protein*	% Fat	% Moisture
Bull	20.4 ± 1.9^{a}	3.6 ± 2.8^{d}	74.8 ± 1.7^{a}
Blade	20.5 ± 1.0^{a}	4.2 ± 1.5^{d}	74.4 ± 0.7^{a}
Cow	19.4 ± 0.3^{a}	9.9 ± 0.8^{cd}	69.7 ± 1.0^{a}
Heart	15.2 ± 1.0^{b}	11.9 ± 2.1^{c}	71.9 ± 1.6^{a}
Head	14.0 ± 0.4^{bc}	28.4 ± 7.1^{b}	56.5 ± 6.0^{b}
Plate	13.2 ± 0.3^{c}	39.6 ± 3.9^{a}	46.3 ± 4.9^{c}

* Each value represents the mean and standard deviation, n=3.

abcd Numbers within a column not followed by a common superscript are significantly different ($P < 0.05$).

of Hand et al. (1985). Samples were extracted at ten temperature treatments ranging from -3 to 24° C in 3° C increments. To reduce the possible day-to-day variation or an effect of freezing on protein extraction, triplicate samples were also extracted at a standard temperature of 24° C. Each sample was thawed at 3 ± 1° C for 18 hr before the start of protein extraction. Six samples of each trimming were weighed (10.0 ± 0.05 g) into 50 mL Nalgene polycarbonate centrifuge tubes. Three samples were allowed to equilibrate to the treatment temperature, whereas the other three to the standard temperature before 20.0 ± 0.1 mL of NaCl 2% (W/V), also temperature equilibrated, was added to each sample. Tubes containing the treatment extraction were placed in a covered shaker water bath (model BKS-350, Gallenkamp and Co., Sussex, England) equipped with a refrigerated circle. The temperature inside the water bath was maintained at the treatment temperature by using the heating coils of the water bath to warm water chilled by the coil of the refrigerated circulator (Lauda model RMS-20, Brinkmann Instruments, Westbury, NY). Standard samples were placed in adjacent covered shaker water bath (model 25, Precision Scientific Inc., Chicago, IL) having a temperature of 24° C. Possible variations in protein extraction resulting from different shaking speeds between treatment and standard shaking water baths were reduced by shaking both

treatment and standard samples by way of a connecting arm. Samples were shaken at 120 rpm for one hr. After the extraction period, treatment and standard samples were centrifuged at 1860 x g for 10 minutes (model J-6M, Beckman Instrument, Palo Alto, CA) at the same treatment temperature of the extraction. The supernatant was filtered through #4 Whatman paper. The protein content of duplicate 0.2 mL aliquots from treatment and standard filtrates was determined by Biuret (Gornall et al., 1949) using Human Serum Albumen and Globulin standard (Sigma Chem. Co., St. Louis, MO.).

Statistical Analysis

The day-to-day variation in protein extraction was reduced by using extraction triplicates and protein duplicates along with a standard extraction temperature (24° C) to control for the possibility of an effect of the amount of time a sample was frozen before the treatment extraction. To this end, regression was used to estimate the change in extraction due to the amount of time that the extraction samples were frozen. The mean protein extracted standard for each beef trimming replicate was regressed over time frozen for each trimming replicate (procedure REG, SAS Institute, Incorporated, Cary, NC). For significant linear relationships ($P < 0.1$), the mean standard protein extracted was subtracted from each

treatment protein extracted for a given beef trimming, beef trimming replicate, and temperature. This difference was then added to the intercept of the appropriate beef trimming replicate regression equation to estimate the protein extracted for a given treatment temperature at zero day frozen. Protein extraction efficiency was calculated by dividing the amount of extracted protein by the total amount protein in the sample that was extracted. A complete block design with meat trimming replicates as blocks, and meat and temperature as treatments was used to analyze extracted protein and extraction efficiency using Analysis of Variance (procedure ANOVA, SAS Institute, Cary, NC). Estimates of the variance components for protein extraction were determined using standard samples by ANOVA (procedure VARCOMP) using a mixed random model with trimming as a fixed effect.

Results and Discussion

Effect of Freezing

Under most experimental conditions as it is impossible to analyze fresh beef as well as utilize chemical, physical or organoleptic methods within such a time frame that prevents influential changes from taking place. Freezing is one of the most convenient and efficient means for sample storage and preservation (Smith

et al., 1969). However, the amount of time a sample has been frozen has been reported to affect protein extraction, possibly through protein denaturation (Saffle et al., 1964). Thus, control or standard extractions at 24° C were used to monitor the effect of frozen storage on the total salt soluble protein. Data revealed a decrease in protein extracted from bull, blade, cow and heart trimmings (replication 1 and 2, $P < 0.1$) due to freezing time. Figure 1 shows the effect of storage time on the total soluble protein of bull trimming (see the APPENDIX for similar figures for blade, cow and heart trimming). These results support the findings of Saffle et al. (1964), Miller et al. (1980) and Wagner (1986). These results exemplify the importance of controlling frozen meat inventories.

Sources of Variability in Extracting Protein

Few studies have assessed the sources of variation for extracting protein using salt solutions from meat trimmings. Accordingly, analyses were conducted to estimate the contribution of meat trimming replication, between days of extraction, i.e. over time, and within day of extraction and protein determination to the variation in protein extraction. The data were analyzed for random effects using a nested hierarchical model of meat trimming replication, day to day extraction, within day extraction

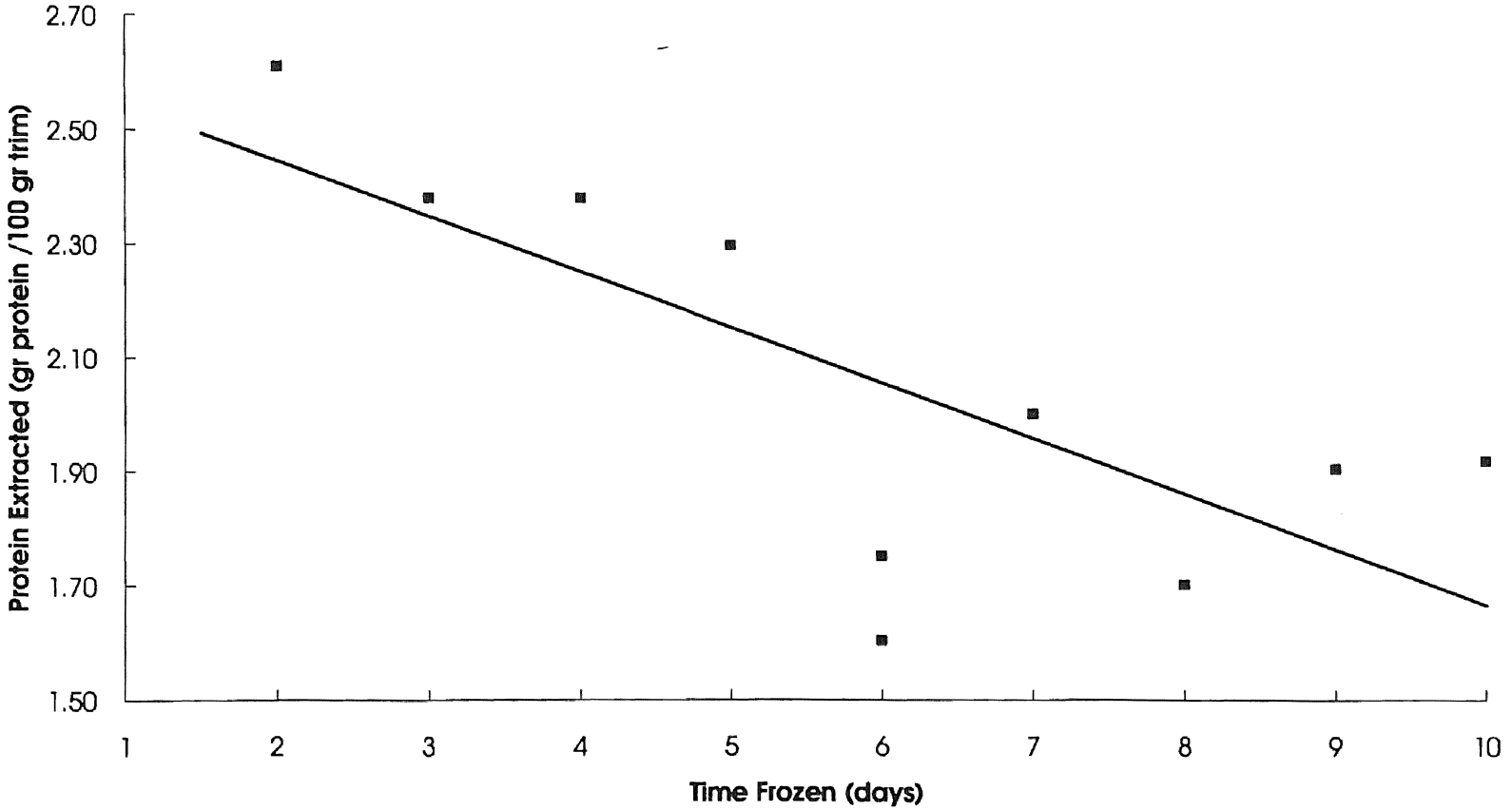

Figure 1. Effect of Frozen Storage of Bull Trimmings on the Protein Extracted.

and protein (the error term) using meat trimming as a fixed effect. All variance terms were greater than zero ($P < 0.05$). The largest component of the variance of the random effects, accounting for 45%, was attributed to the day to day extractions. As noted above, freezing samples of meat trimmings affected protein extraction and as such, would be a consequential component of the day-to-day variance. An additional component of the day to day variation might also result from any non-uniformity in protein composition of the chopped meat block (see Materials and Methods). The next largest source of variability, 35% of the variance, was attributed to the replication of the meat trims. Performing triplicate extractions and duplicate protein determinations for each trimming contributed 9 and 10%, respectively, to the variability of protein extraction. These results suggest that future studies on extracting salt soluble protein from meat trimmings would benefit from increases in the number of day to day extractions and in the number of trimming replications.

Effect of Meat Types

The effect of different beef trimmings on the total salt extractable protein is presented in Table III. As discussed above, meat sources were quite variable in protein extraction due to the variation in proximate

TABLE III

LEAST SIGNIFICANT DIFFERENCES AMONG TREATMENT MEANS FOR PROTEIN EXTRACTION AND PROTEIN EFFICIENCY OF BEEF TRIMMINGS FROM SIX DIFFERENT CARCASS SOURCES

Meat	Protein Extraction (g/100 g trimming)*	Protein Efficiency (%)
Blade	5.01[a]	24.46[b]
Heart	4.72[b]	31.41[a]
Bull	4.57[c]	21.98[d]
Cow	4.52[c]	23.61[c]
Head	2.83[d]	19.53[e]
Plate	2.73[d]	21.89[d]

* (n=3)

[abcde] Numbers within a column not followed by a common superscript are significantly different ($p<0.05$).

composition among meat trimming replicates. This latter point probably accounts for a significant effect ($P < 0.05$) of trimming re plication. The anatomical source of each beef trimming had significant effects on total salt extracted protein ($P < 0.05$). The largest amount of protein was extracted from blade trimming followed by heart trimming. The extraction of salt soluble protein was similar for bull and cow. The least amount of protein was extracted from head and plate trimmings as compared to the other beef sources with the amount extracted being almost half of the quantity extracted from blade trimming. Both of these trimmings had higher fat (Table II) and connective tissue contents (Wiley et at., 1979). Fat may have masked any differences or similarities either by interfering with protein dissolution or in binding the extracted protein. Anderson et al. (1963) reported that the addition of C_{18} fatty acids reduced the quantity of protein which could be extracted from fish muscle. However, Saffle and Galbreath (1964) found that fat had no effect on the percentage of protein that could be extracted. The relatively high amount of connective tissue in both trimmings may also explain the small amount of extracted protein. Beef head trimming has been found to contain 23.6 mg collagen/g and plate trimming contains 12.8 mg collagen/g (Wiley et al., 1979). Both of these values are considerably higher than cow meat trimming

(Wiley et at., 1979). The high amount of connective tissue in both of these trimmings will account for such differences since it are insoluble and contribute to the total amount of proteins pressented in muscles. Further more it may act as a barrier via preventing the breakup of cells during extraction or entrapping the soluble proteins within cells.

Protein Extraction Efficiency

The anatomical source of each beef trimming also had significant effects on protein extraction efficiency ($P < 0.05$). Heart trimming had the highest protein extraction efficiency (Table III) while having an intermediate protein content (Table II). This behavior may be explained by the relatively high level of sarcoplasmic proteins present in heart muscle. Head trimming had the lowest protein extraction efficiency. Surprisingly, significant differences ($P < 0.05$) in protein extraction efficiency where found between bull and cow trimming while no differences existed in the amount of protein extracted nor in protein content (Table II). Saffle and Gelbreath (1964) found that the extraction efficiency for three meat trimmings consisting of 100, 80, and 60% lean were 33.6, 30.0, and 30.4%, respectively, when 3% NaCl was used to extract protein. The difference in extraction efficiency between the present study and Saffle and Gelbreath (1964)

most likely reflects the higher ionic strength of the extraction solution used in the latter study.

Effect of Temperature

The effect of temperature upon the extraction of salt soluble protein from bull trimming is shown in Figure 2. The protein extraction-temperature profile of bull trimming exhibited several maximas and minimas when a 2% NaCl solution was used to extract protein. Three maximas were present: 0-6°, 12° and 18-24° C with the regions of 12° and 18-24° C having the highest amount of extracted protein. Minimas were located at -3°, 9°, and 15° C with -3° C exhibiting the lowest amount of extracted protein. The bull trimming protein extraction-temperature profile revealed that it may be practical to extract protein at 18-24° C during processing to obtain results similar to processing procedures occurring at 0-6° C. Other regions, namely -3°, 9° or 12° could be utilized to decrease the amount of protein extracted if such a situation would be desired.

Figure 3 shows the effect of temperature upon the extraction of 2% NaCl soluble protein from blade trimming. Protein extraction maximas existed at -3°, 9-12° and 21-24° C. Two minimas were apparent in the protein extraction-temperature profile. One occurred at 3° with the other occurring at 15° C. The minima at 3° C had the

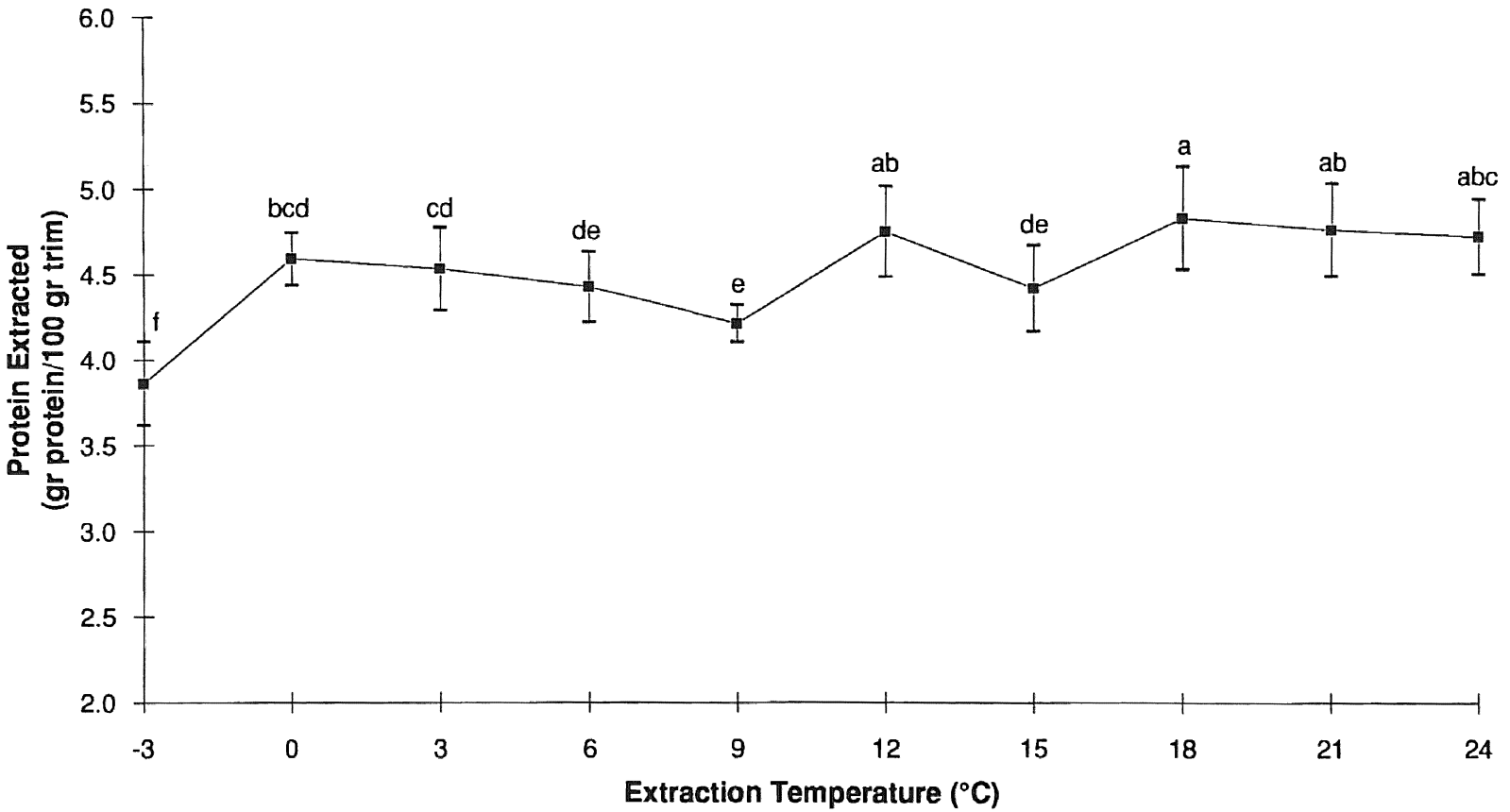

Figure 2. Effect of Temperature on the Protein Extracted from Bull Trimming. Means lacking a common superscript letter differ ($P < .05$)

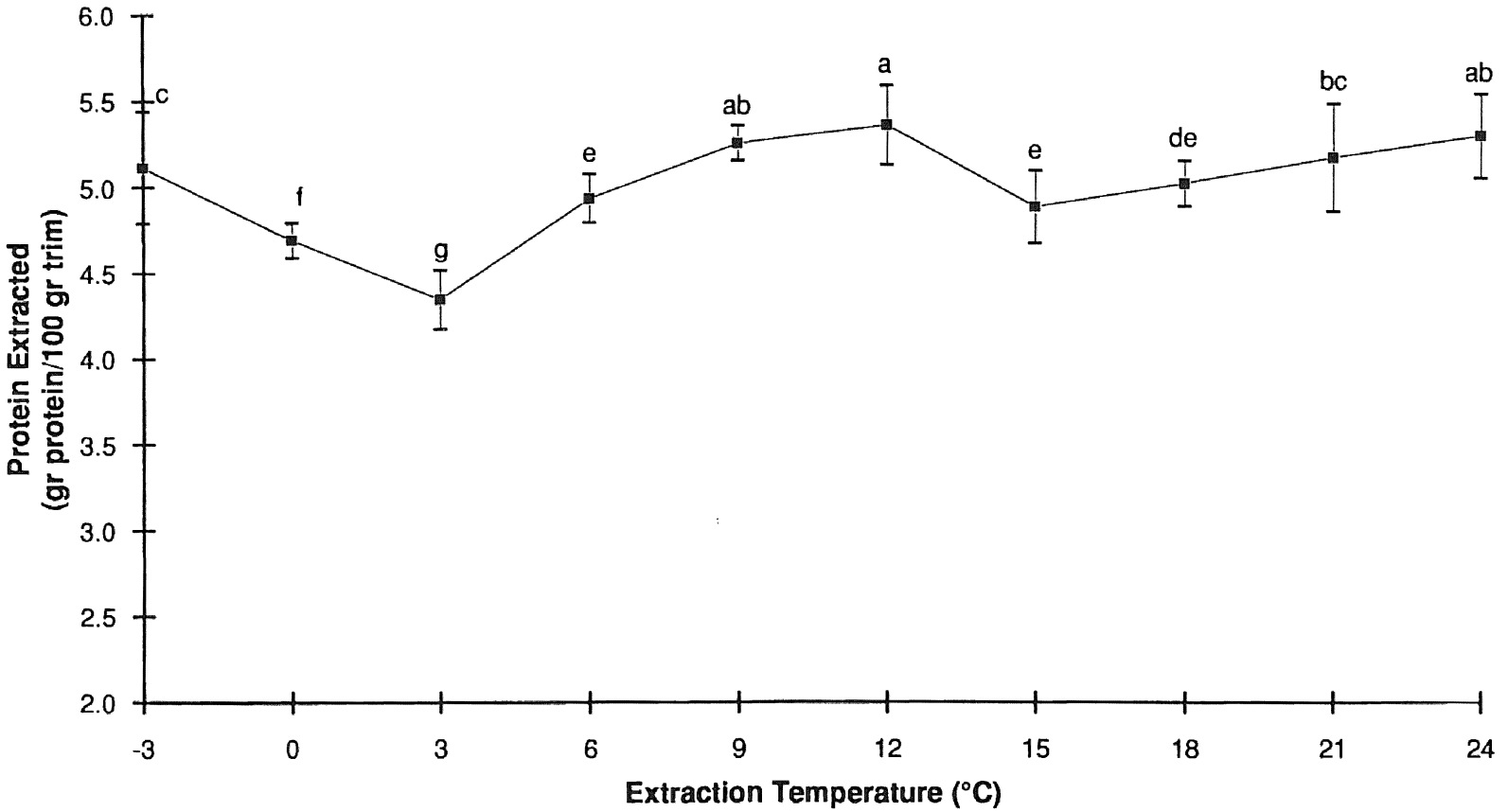

Figure 3. Effect of Temperature on the Protein Extracted from Blade Trimming. Means lacking a common superscript letter differ ($P < .05$)

lowest amount of extracted protein. These results suggest that to extract salt soluble protein from blade trimming, processors may take advantage of these variations to control protein extraction. Reports from other studies indicated that the optimal temperature for the extraction of the total protein from beef shoulder clods was 14° C using 2% NaCl (Gadea de Lopez, 1990). This study also indicated that the maximum solubility of sarcoplasmic and myofibrillar protein occurred at 26° and 6° C, respectively. Gillett et al. (1977) found that the optimal temperature for the extraction of total protein from beef and pork combination sources was 7.2° C using 7.5% NaCl over five different temperature treatments (-3.9°, 1.7°, 7.2°, 12.8° and 23.9° C). These results exhibited several broad extraction maximas that would be consistent with the results from both of these studies.

The effect of temperature on the extraction of protein from cow trimming by 2% NaCl is shown in Figure 4. The protein extracted from cow blade trimming displayed three maximas at 3-6°, 12° and 18-24° C with 18° and 24° C having the highest amount of extracted protein. Protein extraction minima occurred at temperatures of -3°,9° and 15° C with -3° and 9° C having the lowest amount of extracted protein. These results are qualitatively similar to the protein extraction-temperature profile of bull trimming (Figure 2).

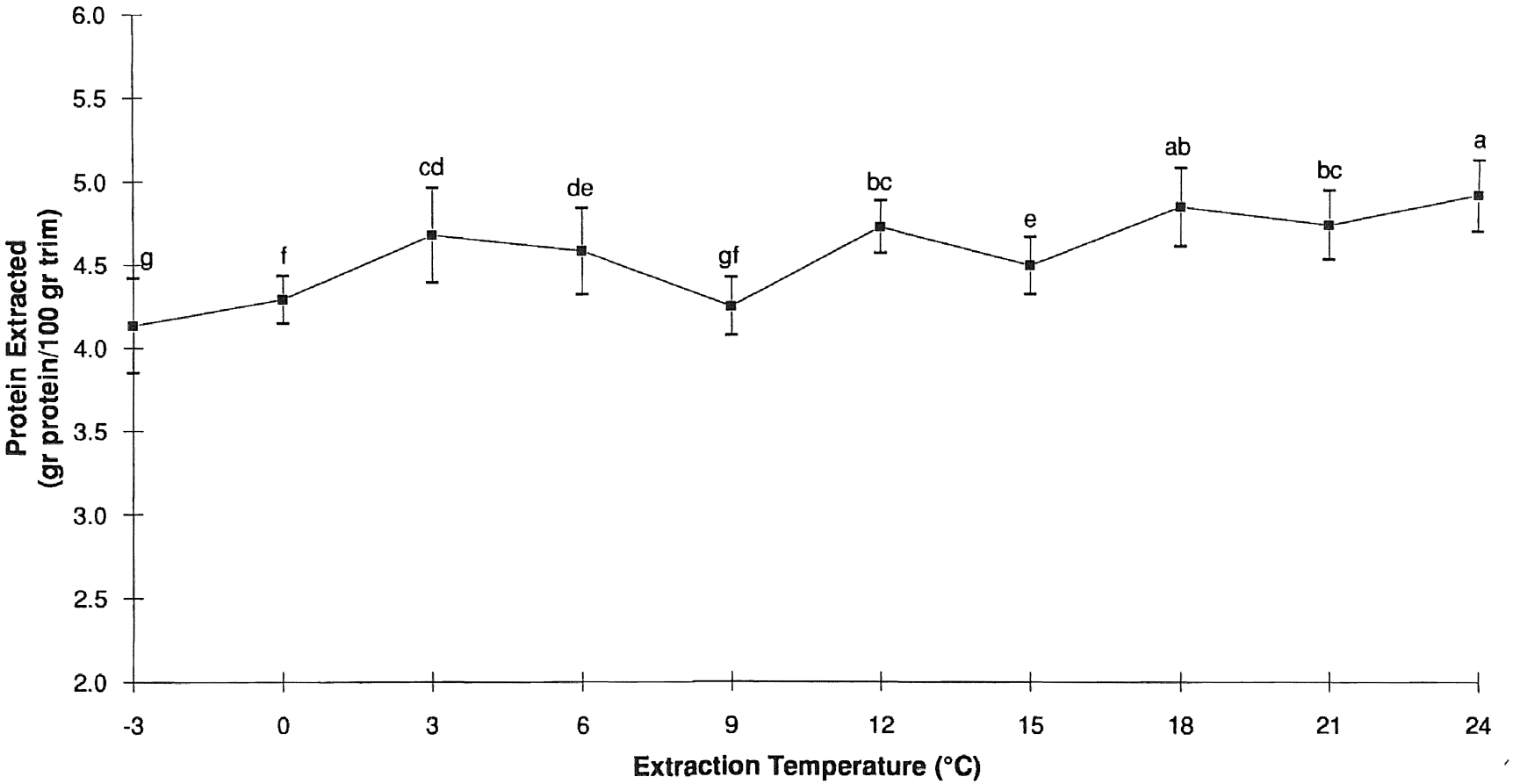

Figure 4. Effect of Temperature on the Protein Extracted from Cow Trimming. Means lacking a common superscript letter differ (P < .05)

The protein extraction-temperature profile for heart trimming (Figure 5) was different from either bull, blade or cow trimming in that a broad intermediate level of protein extraction occurred at 12-18° C. Maximas appeared at 3° and 21° C with both having the highest quantity of extracted protein. The lowest amount protein extracted occurred at extraction temperatures of -3° and 9° C. The broad intermediate level of extracted protein from 12-18° C may be explained by the relatively high sarcoplasmic protein content of heart trimming.

The extraction of protein from head trimming using 2% NaCl resulted in the protein extraction-temperature profile displayed in Figure 6. Three maximas of extracted protein occurred at -3 to 0°, 12° and 18° C. Intermediate amounts of extracted protein were located in three regions at 6-9°, 15° and 21-24° C. One low extraction region existed at 3° C. The intermediate protein extraction regions occurred over temperatures that differed ($p<.05$) from the intermediate region of heart trimming (Figure 5).

The effect of temperature upon the extraction of salt-soluble protein from plate trimming is presented in Figure 7. Data revealed two extraction temperature regions. Three maximas were observed at 0-3°, 9° and 15-24° C with the 15-24° C region appearing relatively broad. The lowest amount of protein was extracted at temperatures of -3°, 6° and 12° C. These results are relatively

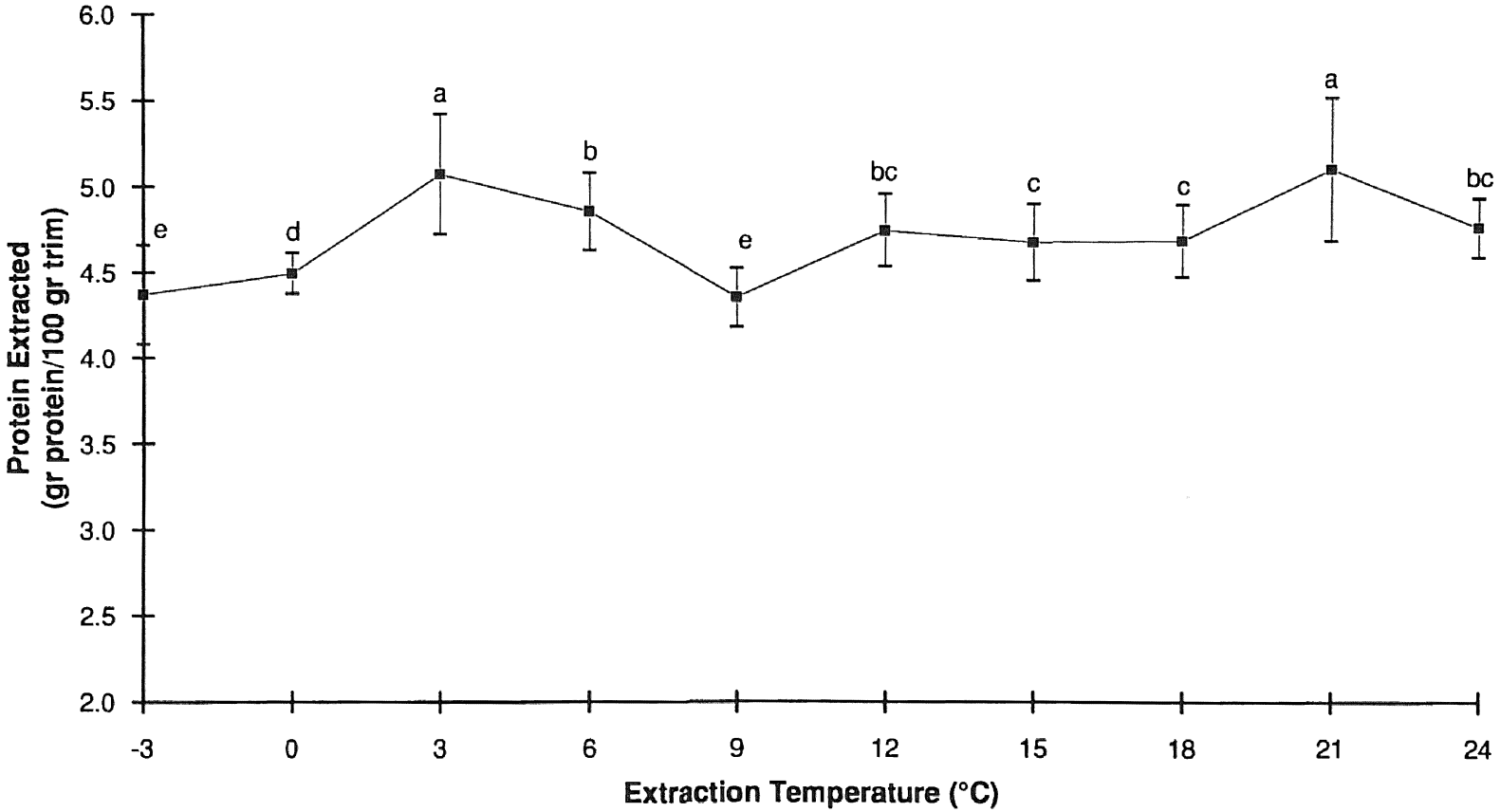

Figure 5. Effect of Temperature on the Protein Extracted from Heart Trimming. Means lacking a common superscript letter differ ($P < .05$)

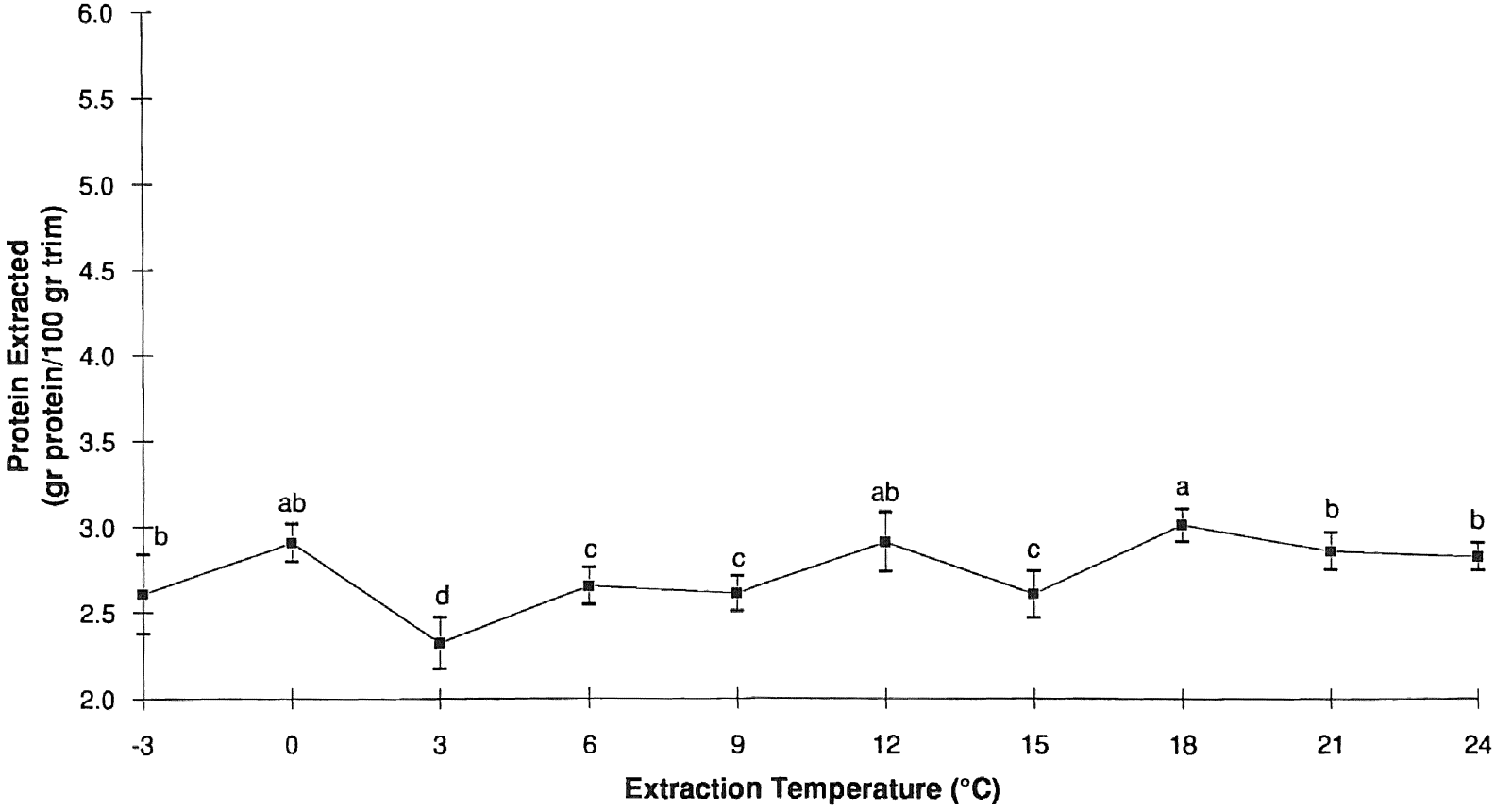

Figure 6. Effect of Temperature on the Protein Extracted from Head Trimming. Means lacking a common superscript letter differ (P < .05)

similar to the protein extraction-temperature profile obtained from head meat (Figure 6).

In general, blade trimming had the highest amount of extracted protein (5.37 g/100 gr trim obtained at 12° C) whereas the lowest amount of extracted protein occurred with head trimming (2.32 g/100 gr trim obtained at 3° C), a decrease of 57%. It seems difficult to explain this large variation besides the several maximas and minimas at each trimming extraction temperature. These data hypothesize that protein extraction is dependent on many factors such as species, age, sex, composition, pre- and post-mortem events, in addition to whether the meat was fresh or frozen. The lack of a standard method for determining salt extractable protein makes comparisons difficult between this work and others especially when a different salt concentration as well muscle trimming or species are used.

Protein extraction-temperature profiles exhibited several maximas and minimas ($P < 0.05$) with each meat trimming exhibiting a unique protein extraction-temperature relationship. The unique protein extraction-temperature profiles for each of the trimmings studied offers a new approach in formulating low-salt meat products that utilize the extracted proteins for bind, emulsions or other textural properties. Combining trimmings that have protein extraction-temperature

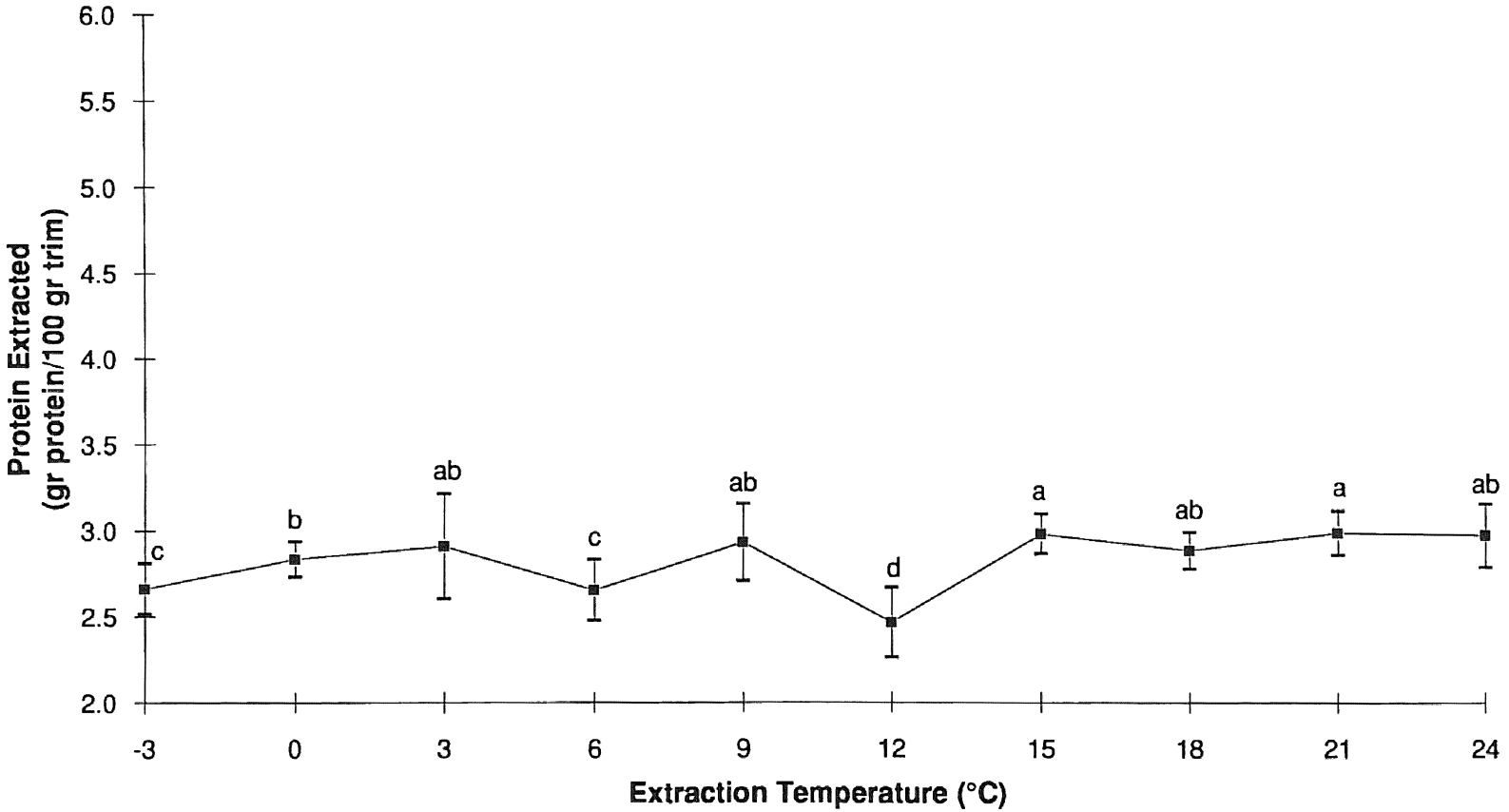

Figure 7. Effect of Temperature on the Protein Extracted from Plate Trimming. Means lacking a common superscript letter differ ($P < .05$)

profiles that take advantage of complimentary extraction maxima would appear to produce a meat block having high protein extractability over a broad processing temperature range. This would insure that protein extraction would be optimized over the temperature range encountered during processing. On the other hand, it may be desirable to minimize the extraction of protein to maintain the native textural property of the selected meat trimmings. In this case, combining trimming sources that share common minimas would minimize protein extraction as long as the processing temperature remained within the overlapping minima.

In conclusion, this research establishes that there are several common yet different optimal temperatures for the extraction of protein from different beef trimmings. Moreover, it may be useful for processors to utilize these differences in the manufacture of low fat meat products with their higher moisture contents. The results of this research may help meat processors to select the desirable combination of raw meat material. Much work, however, remains to identify optimal temperature protein extractability of the entire carcass and subsequent muscle groups.

LITERATURE CITED

Acton, J.C., Ziegler, C.R. and Burge Jr., D.L. 1983. Functionality of muscle constitutes in the processing of comminuted meat products. Crit. Rev. Food Sci. Nutrit. 18(2):99.

Acton, J.C. and Saffle, R.L, 1969. Preblended and prerigor meat in sausage emulsions. Food. Technol. 23:367.

Ahmed, P.O., Miller, M.F., Lyon, C.E., Vaughthers, H.M. and Reagon, J.O. 1990. Physical and sensory characteristics of low-fat fresh pork sausage processed with various level of added water. J. Food Sci. 55:625.

American Cancer Society. 1984. Nutrition and cancer, cause and prevention. An American Cancer Society special report. Canc. J. Clin. 34(2):121-126.

American Heart Association. 1986. "Dietary guidelines for healthy American adults. Arteriosclerosis 8, 218A." In Pearson, A.M., and Dutson, T.R. (Ed.), Meat and Health (p.3). Elsevier Applied Science, New York.

Anderson, M.L., King, F.J and Steinberg, M.A. 1963. Effect of linolic, linoleic and oleic on measuring protein extractability from cod skeletal muscle with the solubility test. J. Food. Sci. 28:286.

AOAC. 1984. Official Methods of Analysis, 14th Edition. Association of Official Analytical Chemists, Inc., Washington, D.C.

Asghar, A., Samejima, K. and Yasui., T. 1985. Functionality of muscle proteins in gelation mechanisms of structured meat products. Cri. Rev. Food Sci. Nutrit. 22(1):27.

Awad, A., Powrie, W.D. and Fennema, O. 1968. Chemical deterioration of frozen bovine muscle at -4°C. J. Food Sci. 33:227.

Bishcoff, J. 1990. Ingredients for a 90's diet. Meat and Poultry. 36(3):26.

Briggs, G.M., and Schweigert, B.S. 1990. "An over view of meat in the diet." In: "Advance in meat research." Vol. 6. Pearson, A.M. and Dutson, T.R. (Ed.), P. 2. Elsevier Applied Science., London and New York, NY.

Claus, J.R., Hunt, M.C., Kastmer, C.L. and Kropf, D.H. 1990. Low-fat, high-added water bologna: Effects of massaging, preblending, and time of addition of water and fat on physical and sensory characteristics. J. Food Sci. 55(2):338.

Claus, J.R., Hunt, M.C. and Kastner, C.L. 1989. Effects of substituting added water for fat on the textural, sensory, and processing characteristics of bologna. J. Muscle Food 1:1.

Decker, C.,D., Conley, C.C. and Richert, S.H. 1986. Use of isolated soy protein in the development of frankfurters with reduced levels of fat, calories, and cholesterol. Proc of 32nd European Mtg, Meat Research Workers P. 333.

Federal Register. 1988. 53(50):8425.

Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D. and Merkel, R.A. 1975. In Schweigert, B.S. (Ed.), Principles of Meat Science (p. 175), W.H. Freemans and Co., New York.

Gadea de lopez, G.D. 1990. Effect of temperature on the protein solubility of beef shoulder colds. Master Thesis. Oklahoma State University, Stillwater.

Gadea de lopez, G.D., and Hand, L.W., 1992. The effect of temperature on the protein solubility of beef shoulder clods. Okla. Ag. Ext. Stat. Rep. MP-135:67.

Gillett, T.A., Meiburg, D.E., Browm, C.L. and Simon, S. 1977. Parameters affecting meat protein extraction and interpretation of model system data for meat emulsion. J. Food Sci. 42(6):1606.

Gordon, A.M. and Yates, L.D. 1992. "Regulatory mechamism of contraction in Skeleton muscle. In: Sugi, Hauro (Ed.), Comparative & Envoronmental Physiology Vol 12. (p. 18), Springer-Verlag, New York.

Gornall, A.G., Bardawill, C. J. and David, M. M. 1949. Determination of serum proteins by means of the biuret reagent. J. Biol. Chem. 177:751.

Gummins, P, Perry, Sv. 1974. Chemical and immunochemical characteristics of the tropomyosins from striated and smooth muscle. Biochem. J. (141): 43.

Greaser, M.L. and Gergely, J. 1973. Purification and propeties of the components from troponin. J. Biol Chem. 246: 422.

Hand, L.W., Calkins, C.R. and Mandigo, R.W. 1985. A technique to measure the water uptake properties of meat. J. Anim. Sci. 61:290.

Hand, L., Hollingsworth, C.A., Calkins, C.R. and Mandigo, R.W. 1987. Effect of preblending, reduced fat and salt leves on frankfurter characteristics. J. Food Sci. 52(5):1149.

Hand, L.W. 1981. Effect of chloride salts on properties of frankfurters, hams and restructured pork roasts and of certain non-meat protein products on properties of pork fat emulsions and mortadella sausage. Master thesis. Texas A&M University.

Helander, E. 1957. On quantitative muscle protein determination. Acta Physiol. Scand. 41:9.

Hermansson, A.M., Harbitz, O. and Langton, M. 1986. Formation of water types of gels from bovine myosin. J. Sci. Food Agric. 37:69.

Hoogenkamp, W.H . 1991. Vegetable Protein Technology Value in Meat and Poultry Products, (p.13), Protein Technology International, Inc.

Huffman, D.L, Egbert, R.W., Chem, C. and Dylewski, D.P. 1991. Technology for low-fat ground beef. Proc. Recip. Meat Conf. 44:73.

Jauregui, C.A., Regenstein, R.M. and Baker, R.C. 1981. A simple centrifugal method for measuring expressible moisture, a water-binding property of muscle foods. J. Food Sci. 46:1271.

Keeton, J.T. 1991. Fat substitutes and fat modification in processing. Proc. Recip. Meat Conf. 44:79.

King, A.J. and Earl, L.A. 1988. Extraction of protein from slurries of unfrozen and frozen/thawed dark, ground turkey meat and skin with selected potassium and sodium salts. J. Food Sci. 53(5):1290.

Kinsella, J.E. 1976. Functional properties of protein in foods: a survey. Crict. Rev. Food Sci. Nutrit. 7:219.

Kinsella, J.E. 1982. "Relationships between structure and functional properties of food proteins."In Fox, P.F. and Condon, J.J. (Ed.), Food Proteins, (Ch. 3, p. 51), Applied Science Publisher, England.

Kinsella, J.E. and Fox, P.F. 1986. Water absorption by proteins: milk and whey proteins. Crit. Rev. Food Sci. Nutrit. 24(2):91.

Lawrie, R.A. 1979. In Meat Science, 3Ed., (p. 77), Pergmon, London.

Lin, K.C., Keeton, J.T., Gilchrist, C.L. and Cross, H.L. 1988. Comparisons of carboxymethyl cellulose with differing molecular features in low-fat frankfurters. J. Food Sci. 53:1592.

Mac Bride, M.A. 1986. Effect of materials equipment, and processing on the physical properties and microstructure of meat emulsion. PhD Thesis. Cornell University. Ithaca.

Marquez, E.J., Ahmed, E.M., West, R.L. and Johnson, D.D. 1989. Emulsion stability and sensory quality of beef frankfurters produced at different fat or peanut oil level. J. Food Sci. 54(4):867.

Miller, A.J., Ackerman, S.A. and Palumbo, S.A. 1980. Effect of frozen storage on functionality of meat for processing. J. Food Sci. 45:1466.

Miller, W.O., Saffle, R.L. and Zirkle, S.B. 1968. Factors which influence the water-holding of various types of meat. Food Technol. 22:1139.

Morr, C.V., German, B., Kinsella, J.E., Regenstein, J.M., Van Buren, J.P., Kilara, A., Lewis, B.A. and Mandigo, M.E. 1985. A Collaborative study to develop a standardized food protein solubility procedure. J. Food Sci. 50: 1715.

National Research Council. 1988. In Designing Foods: Animal Option in the Market Place, National Academy Press, Washington D.C.

Park, J., Rhee, K.S., Keeton, J.T. and Rhee, K.C. 1989. Properties of low-fat frankfurters containing

monosaturated and omega-3 polyunsaturated oils. J. Food Sci. 54(3):500.

Park, J., Rhee, K.S. and Zipin, Y.A. 1989. low-fat frankfurters with elevated levels of water and oleic acid. J. Food Sci. 55:871-874.

Pearson, A.M., and Young, R.B. 1989. In Muscle and Meat Biochemistry (p. 12), Academic Press, Inc, San Diego, California.

Regenstein, J.M. 1984. Protein-water interaction in muscle food. Proc. Recip. Meat Conf. 37:44.

Regenstein, J. M. and Regenstein, C. E. 1984. In Food Protein Chemistry, Acadiemic Press, Inc., Orlando, Fl.

Rongey, E.H. and Bratzler, L.J. 1966. The effect of various binders and meats on the palatability and processing characteristics of bologna. Food Technol. 20:1228.

Saffle, R.L. and Gelbreath, J.W. 1964. Quantitative determination of salt-soluble protein in various types of meat. Food Technol. 18(12):1943.

Samejima, K., Ishioroshi, M. and Yasui, T. 1981. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. J. Food Sci. 46:1412.

Sayre., R.N. and Briskey, E.J. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28:675.

Schut, J. 1976. In Friberg, S. (Ed.), Meat Emulsion, Chpt. 8 (p. 385), Marcell

Seifter, S. and Gallop, P.M. 1966. "The structure protein." In Pearson, A.M. and Young, R.B. (Ed.), Muscle and Meat Biochemistry (p. 12), Academic Press, Inc, California.

Shackelford, M.F., Millar,.M.F., Haydem, K.D. and Reagen, J.O. 1990. Effect of feeding elevated level of monosaturated fats to growing-finishing swine on acceptability of low-fat sausage. J. Food Sci. 55:1497.

Smith, D.M. 1988. Meat proteins in comminuted meat products. Food Technol. 43:116.

Smith,G.C., Carpenter, Z.L. and King, G.T. 1969. Considerations for beef tenderness evaluations. J. Food Sci. 34:612.

Sofos, J.N. and Allen, C.E. 1977. Effect of lean meat source and level of fat ans soy protein on the properties of wiener-type products. J. Food Sci. 42(4):875.

St. John, L.C., Buyck, M.J., Keeton, J.T., Leu, R. and Goetsch, U.J. 1986. Sensory and physical attributes of frankfurters with reduced fat and elevated monounsaturated fats. J. Food Sci. 51:1144

Tarrant, P.V. 1982. "Muscle protein in meat technology." In: Fox, P.F. and Condon, J.J. (Ed.), Food Protein, (p. 261) , Applied Science Publisher, England.

Townsend, W.E., Ackerman, S.A., Witnauer, L.P., Plam, W.E. and Swift, C.E. 1977. Effects of types and levels of fat and rates and temperature of comminution on the processing and characteristics of trankfurters. J. Food Sci. 36:261.

Trautman, J.C. 1967. Effect of temperature and pH on the soluble protein of ham. J. Food Sci. 31:409.

Wanger, J.R. and Anon, M.C. 1986. Effect of frozen storage on protein denaturation in bovine muscle. II. Influence on solubility, viscosity, and electrophoretic behavior of myofibrillar proteins. Food Technol. 21:547.

Whiting, R.C. 1984. Addition of phosphates, proteins, and gums to reduced-salt frankfurter batters. J. Food Sci. 49:1355

Wilding, P., Lillford, P.J. and Regenstein, J.M. 1984. Functional Properties of Proteins in foods. J. Chem. Tech. Biotechnol. 34B:182.

Wiley, E.L., Reagan, J.A., Carpenter, J.A. and Campion, D.R. 1997. Connective tissue profiles of various raw sausage materials. J. Food Sci. 44:918.

Yates, L.D. and Greaser, M.L. 1983. Quantitive determination of myosin and actin in rabbit skeletal muscle fibers. J. Mol. Biol. 168:123.

APPENDIXES

APPENDIX A

EXTRACTION OF TOTAL SOLUBLE PROTEIN

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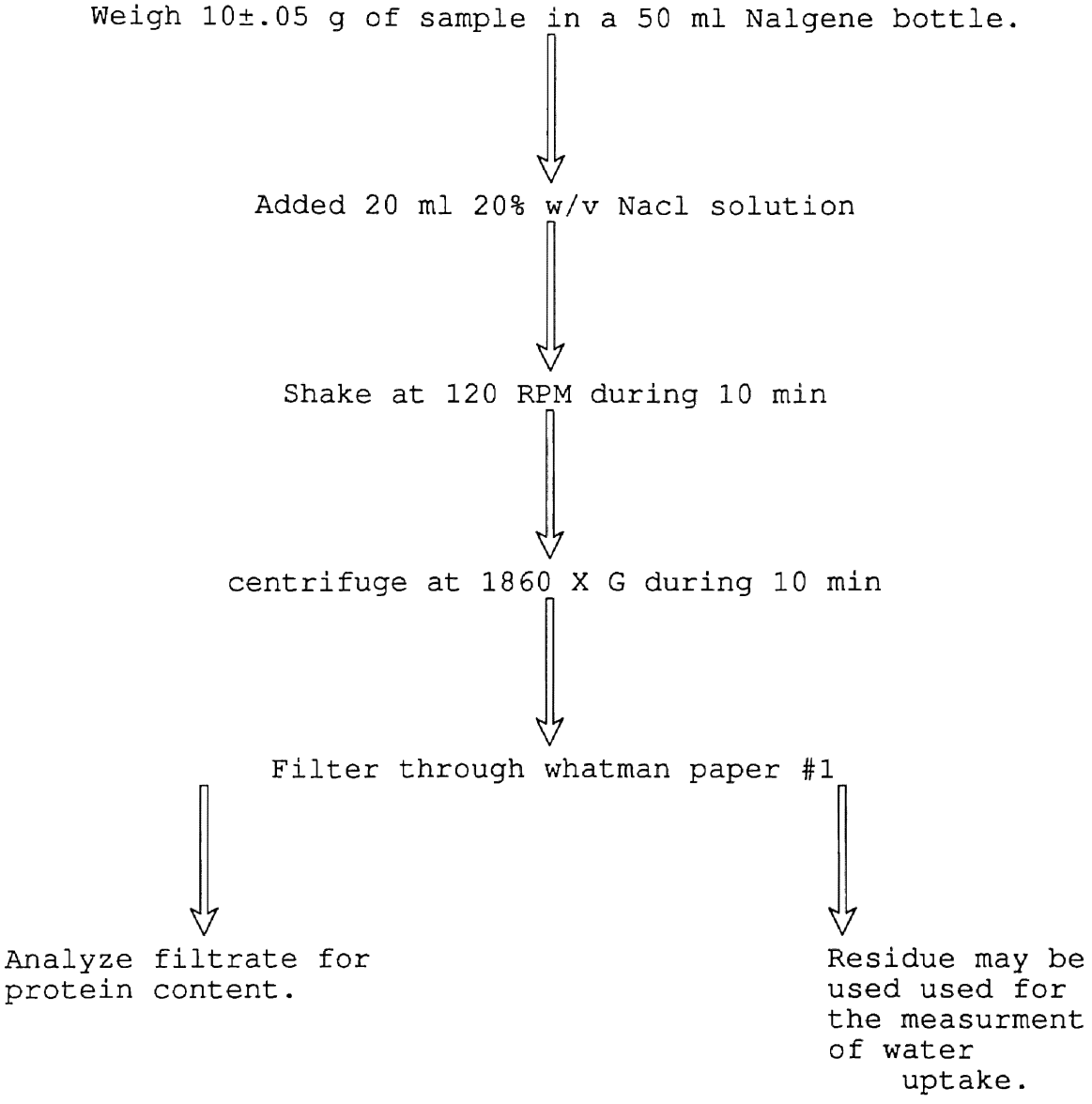

The grams of protein extracted from 100 gr of meat trim was calculated as follows. Variables measured are the sample mass, M_s (approximately 10 gr.), volume of the extracting solution, V_e (20.0 mL), and extracted protein in 0.2 mL, P_b (mg). Based on these variables, the protein extracted from 100 gr of trim was calculated by:

$$\frac{\text{gr Protein Extracted}}{\text{100 gr Trim}} = \frac{P_b \times V_e}{M_s} \times \frac{1}{0.2\ \text{mL}} \times \frac{1\ \text{gr}}{10^3\ \text{mg}} \times 100$$

where:

$\frac{1}{0.2\ \text{mL}}$ accounts for the volume of the extraction solution sampled for determining protein;

$\frac{1\ \text{gr}}{10^3\ \text{mg}}$ converts mg of measured protein into gr of protein extracted; and

100 accounts for 100 gr trim in the desired expression.

APPENDIX B

BIURET METHOD OF DETERMINATION

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1. Biuret Regents.

   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 1L.

2. Preparation of Standards.

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

To make standards, combine according to the following table.

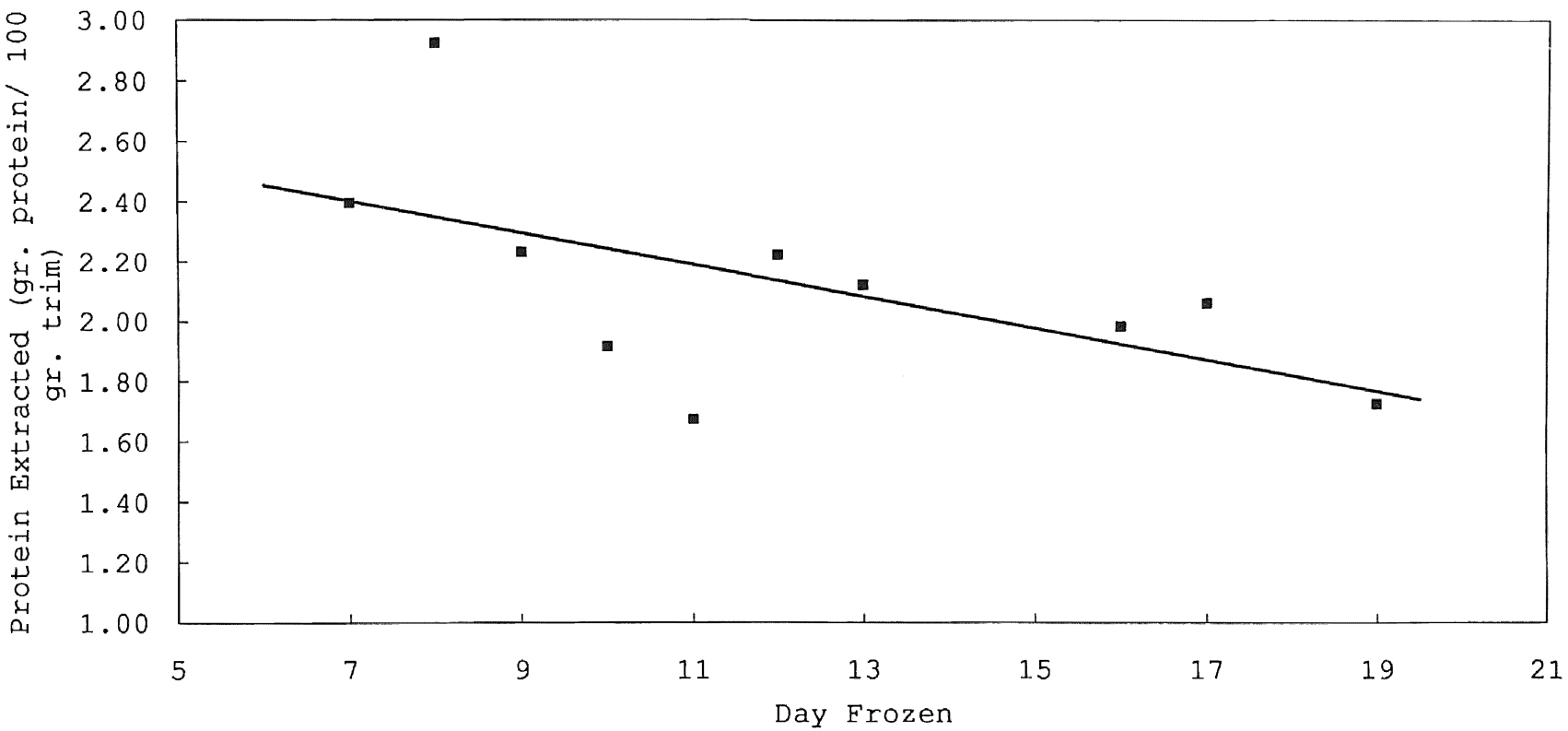
BSA(ml)	Biuret reagent (ml)	water (ml)
0.0	4.0	1.0
0.1	4.0	0.9
0.2	4.0	0.8
03	4.0	0.7
0.4	4.0	0.6
0.5	4.0	0.5

3. Sample preparation.

1-Add 4ml of Biuret reagent to the tube.
2-Add (0.2 ml protein extraction for total protein , 0.4 for sarcoplasmic or myofibrillar determinations).
3-Make volume to 5 ml with water , vortex and incubate during 30 min.
4-Read concentration at (540 nm).

APPENDIX C

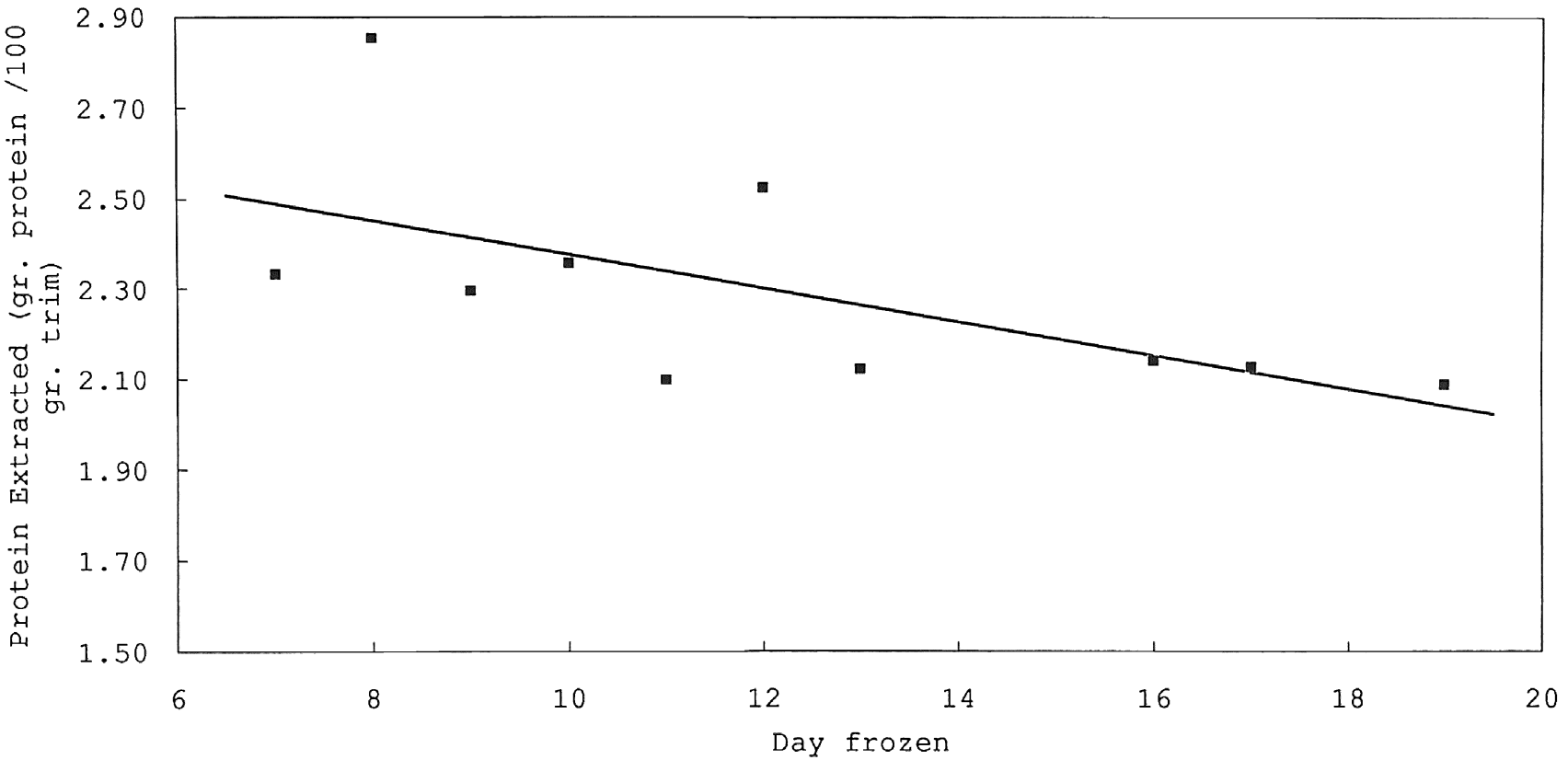
EFFECT OF TEMPERATURE ON THE TOTAL SOLUBLE PROTEIN OF BLADE TRIMMINGS



Effect of Frozen Storage of Blade Trimming on the Total Soluble Protein.

APPENDIX D

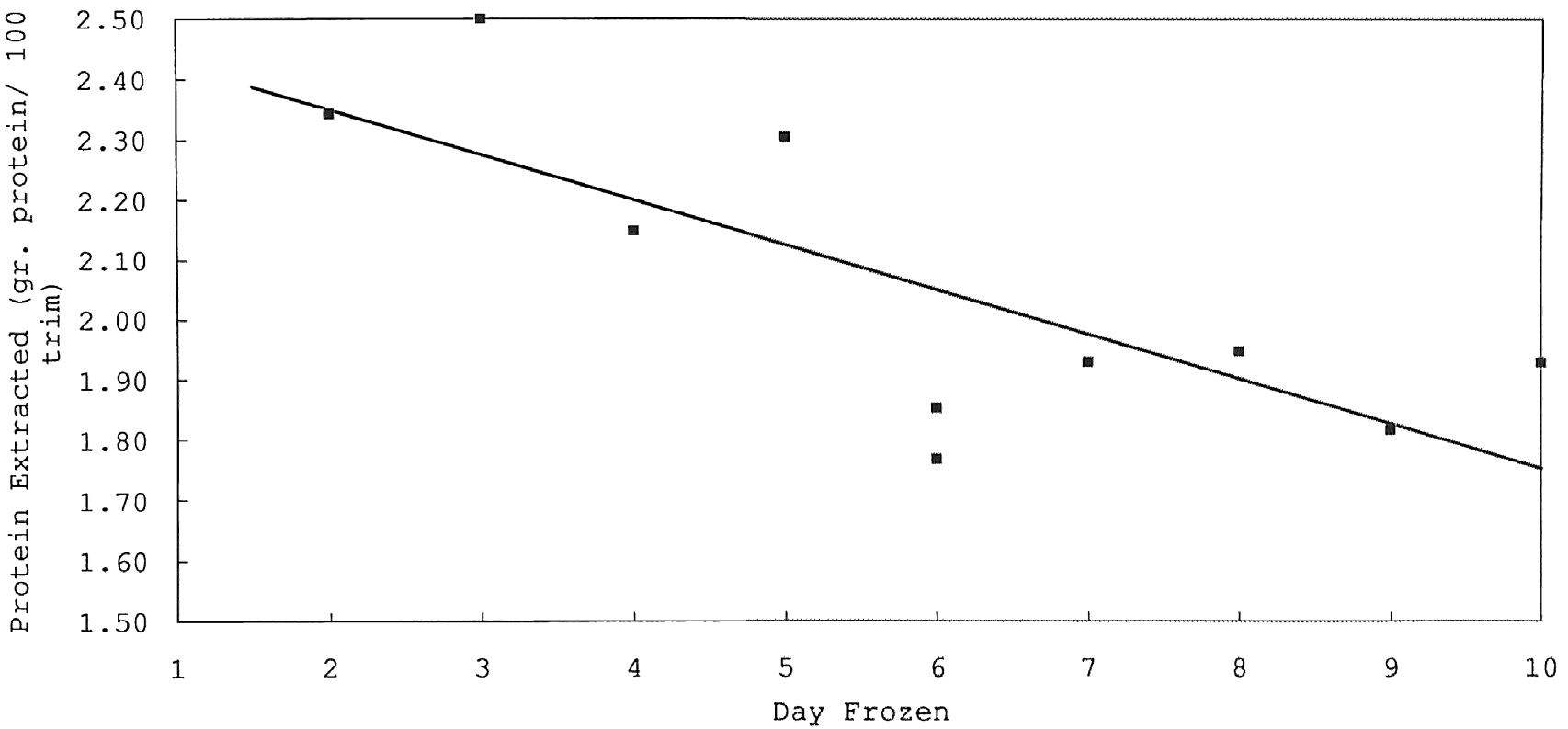
EFFECT OF TEMPERATURE ON THE TOTAL SOLUBLE PROTEIN OF COW TRIMMINGS



Effect of Frozen Storage of Cow Trimming on the Total Soluble Protein.

APPENDIX E

EFFECT OF TEMPERATURE ON THE TOTAL SOLUBLE PROTEIN OF HEART TRIMMINGS



Effect of Frozen Storage of Heart Trimming on the Total Soluble Protein.

APPENDIX F

EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF TRIM FORM SIX DIFFERNTS CARCASS SOUCES

EFFECT OF TEMPERATURE ON THE SOLUBILITY OF TOTAL SALT SOLUBLE PROTEIN OF SIX DIFFERENT BEEF TRIMMINGS.

	Sources of beef trimmings					
Temp(°C)	Bull	Blade	Cow	Heart	Head	Plate
-3	3.86^{f}	5.11^{a}	4.13^{g}	4.37^{e}	2.61^{c}	2.66^{c}
0	4.59^{bcd}	4.69^{f}	4.29^{f}	4.50^{d}	2.91^{ab}	2.83^{b}
3	4.53^{cd}	4.35^{g}	4.68^{cd}	5.07^{a}	2.32^{d}	2.91^{ab}
6	4.43^{de}	4.94^{e}	4.58^{de}	4.86^{b}	2.65^{c}	2.65^{c}
9	4.22^{e}	5.26^{ab}	4.25^{fg}	4.36^{e}	2.61^{c}	2.93^{ab}
12	4.76^{ab}	5.37^{a}	4.73^{bc}	4.75^{bc}	2.91^{ab}	2.46^{d}
15	4.43^{de}	4.89^{e}	4.50^{e}	4.68^{c}	2.61^{c}	2.98^{a}
18	4.8^{a}	5.03^{de}	4.85^{ab}	4.69^{c}	3.01^{a}	2.88^{ab}
21	4.77^{ab}	5.18^{bc}	4.74^{bc}	5.11^{a}	2.86^{b}	2.98^{a}
24	4.73^{abc}	5.31^{ab}	4.92^{a}	4.77^{bc}	2.83^{b}	2.97^{ab}

a-g Means with different letter superscript in the same column are significantly different as determined by LSD Test ($P<0.05$).

VITA

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Master of Science

Thesis: THE EFFECT OF TEMPERATURE ON THE PROTEIN SOLUBILITY OF BEEF TRIM FROM SIX DIFFERENT CARCASS SOURCES

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