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MATHEMATICAL MODELLING OF THERMAL GELATION OF MYOFIBRILLAR BEEF PROTEINS AND THEIR INTERACTION WITH SELECTED HYDROCOLLOIDS.

BY

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ABSTRACT

MATHEMATICAL MODELLING OF THERMAL GELATION OF MYOFIBRILLAR BEEF PROTEINS AND THEIR INTERACTION WITH SELECTED HYDROCOLLOIDS.

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This study was designed to provide the experimental base of a proposed mathematical model for predicting thermally-induced gelation of myofibrillar beef proteins and their interaction with selected hydrocolloids. Four commercially available vegetable gums were evaluated. These hydrocolloids were carrageenan, guar, locust bean and xanthan gums. The mathematical model was designed to predict the gelation of myofibrillar beef proteins as a function of the time-temperature history of the process and the protein concentration on a dry basis. The thermally induced gelation was measured as the Instron back-extrusion apparent viscosity. A model myofibrillar protein system was utilized.

The model system was a solution of extracted myofibrillar beef proteins. Protein solutions were heated at 54, 64, 70, 80 and 85 ^{o}C. The time-temperature history of the process, the back-extrusion apparent viscosity and the model variables were calculated by a combination of commercial software and computer programs developed specifically for this experiment.

It was found that under the conditions of this study, three terms of the model formerly reported as constants (A', a and α) were actually functions of the protein concentration. These mathematical relationships were developed and integrated into the basic model. The modified mathematical model was found to predict (R^2 from 0.88 to 0.97) the heat-induced gelation and the change in water holding capacity of myofibrillar beef proteins as a function of the time-temperature history of the process and the protein concentration (dry basis).

It was hypothesized that vegetable gums contribute to the framework of the thermally-induced protein gels. Correlation between experimental and predicted gelation values of gels from carrageenan, guar and locust bean gums with myofibrillar beef proteins (R^2 from 0.85 to 0.91)

support this premise. As predicted by the mathematical model these protein- hydrocolloid gels had a higher gel strength and water holding capacity than the control. Under the conditions of this experiment xanthan gum was found to inhibit gelation. However this protein-gum solution had the best water holding capacity value among all experimental units.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The thermal gelation of myofibrillar beef proteins is an important phenomenon which takes place in all heat processed meats products. These series of chemical reactions are one of the major factors affecting the final sensory attributes of meat products.

Men learned to process meat products by this mechanism in ancient times. Egyptian hieroglyphics found in pyramids are the first recorded use of manufactured meat products. The great Greek philosopher and historian Homer describes in "The Iliad" how the Greek soldiers, during the siege of Troy, produced manufactured meat products by mixing meat pieces with spices and salt and stuffing them in goat stomachs before cooking.

In the centuries that followed, men learned to modify the end-products of this thermally-induced gelation of meat proteins by trial and error. However, these changes were not accompanied by an understanding and a control of the chemical reactions responsible for this gelation. It was not until this century that knowledge about gelation reactions began to appear in technical literature, with most of the work concentrated in the last decade. It is now known that

protein-protein interaction is the functional event which is related to the structural integrity of meat products through orderly heat-induced protein aggregations, that myofibrillar proteins play a major functional role, and that the complete myosin molecule is necessary for attaining appreciable continuity and strength in the protein matrix. All factors that affect myofibrillar proteins will affect the final texture and the quality of end products of the thermally induced reactions. Some factors that are known to affect this reaction are pH, type and concentration of salts, ionic strength, concentration and type of protein and time and temperature of cooking. The characteristic viscosity of a thermally produced protein gel is considered a measure of the extent of gelation, and therefore, a function of the same factors that affect protein-protein interactions.

During commercial meat processing operations several factors remain constant e.g., pH, concentration and type of salts. However protein concentration and the time temperature history of the process is unique to each batch, and therefore it was considered that a mathematical model was needed to predict the final product of this thermally induced protein gelation as a function of the protein concentration and the time temperature history of the process.

If such a model could account for the overall time temperature history of the process and the change in protein concentration, it could greatly reduce research time and costs, and therefore facilitate and decrease expense of food product development.

A new type of meat product that has been proposed for development is a low fat product. However fats in meat products have several functional properties, e.g., they are a reservoir for flavor compounds and they contribute to the structure and texture of the final product. Therefore, reducing the fat content may alter the product quality.

To maintain the beneficial effect of fats in meat products several compounds may be necessary. Among these are the vegetable polysaccharides gums which seem to have several advantages. Polysaccharides gums are a group of chemical compounds that are extensively used in the food industry to regulate viscosity, as texture modifiers, and to form gels.

The objectives of this study were :

1. To develop a mathematical model that predicts the thermally induced gelation of myofibrillar beef proteins as a function of the protein concentration and the time temperature history of the process.

2. To apply the model developed to the thermal gelation of selected combinations of vegetable gums and myofibrillar beef proteins and verify its validity.

LITERATURE REVIEW

Gelation of proteins is an important phenomenon which takes place in all fabricated meat products during thermal processing. This tacky-sol transformation of meat proteins to a gelled state results in formation of the ultimate three-dimensional interlinked protein network. This phenomenon involves both intramolecular (conformational) and intermolecular changes in proteins. The protein network physically (due to capillarity) and chemically (due to noncovalent bonding) stabilizes water and physically or structurally restrains dispersed fat (in comminuted meats) from rendering (Schmidt et al. 1981; Acton et al. 1983; Gossett et al. 1984). This review will focus on (1) the main factors that affect gelation (meat proteins and chemistry and mechanism of protein-protein interaction), (2) the basis of a proposed mathematical model for predicting and (3) general characteristics of selected vegetable gums.

Properties of Meat Proteins

The composition of lean meat can be approximated in a broad sense as 75% water, 20% protein, 3% fat and 2% other substances. The 20% protein, calculated from 6.25 times percent nitrogen, includes 1 to 1.5% non-protein

nitrogeneous substances, such as amino acids, nucleotides, creatine and traces of other nitrogeneous compounds (Schut, 1976; Forrest et al. 1975; Schmidt, 1987).

Muscle proteins have been traditionally classified into various categories on the basis of distribution, organization, solubility and function in the living muscle. Roughly they can be divided into three groups, i.e., the sarcoplasmic proteins soluble in salt solutions of low ionic strength (< 0.1), the myofibrillar or structural proteins, which are soluble in concentrated salt solutions (ionic strength of 0.5 to 0.6) and the connective tissue proteins, which are insoluble in both, at least at low temperatures (Szent-Gyorgyi, 1951; Perry, 1956).

Sarcoplasmic Proteins

The soluble proteins of the sarcoplasm located within the sarcolemma are referred to as sarcoplasmic proteins. Among them some albumins and the so called myogens, to which belong most of the enzymes of glycolitic pathway, are the real water-soluble proteins (Schut, 1976). The other fractions of sarcoplasmic proteins are soluble in low salt concentration (ionic strength <=0.1). The recent trend is to partition the sarcoplasmic proteins into four fractions by sucrose gradient techniques involving ultracentrifugation. They include a nuclear fraction, a mitochondrial fraction, a microsomal fraction and a cytoplasmic fraction (Asghar and

Pearson, 1980; Bodwell and McClain, 1971). About 100 different proteins are known to be present in the sarcoplasmic fraction (Scopes, 1970) which constitutes about 30 to 35% of the total muscle protein or about 5% of the weight of muscle. Despite their diversity sarcoplasmic proteins have some common physicochemical characteristics. They are globular or rod shaped in conformation, have low viscosity, have isoelectric point between pH 6.0 and 7.0, and have molecular weights in the range 30,000 to 100,000 daltons (Bendall, 1964).

Myofibrillar Proteins

The salt-soluble proteins which compose the myofibrils within the muscle fibers are collectivelly defined as the myofibrillar proteins. They constitute about 55 to 60% of the total muscle protein or 10% of the weight of the vertebrate skeletal muscle (Lawrie, 1974). The major proteins in this category are the myosin-actin-actomyosin group, the tropomyosin-troponin complex, the minor myofibrillar components and the cytoskeletal proteins. Based on their physiological functions in muscle, myofibrillar proteins can be classified into two subgroups: (1) the contractile proteins and (2) the regulatory proteins.

The myofibrillar proteins, myosin and actin, which are directly involved in the contraction-relaxation cycle of live muscle are termed "contractile". Myosin and actin are

the major components of thick and thin myofilaments, respectively. Some of the salient characteristics of contractile proteins were published by Bandman (1987).

Myosin is the major constituent of the thick myofilaments of the sarcomeres. It accounts for approximately 35% of muscle protein and is the most abundant of all proteins found in muscle (Hanson and Lowy, 1964). It is a thread-like molecule with a high length-to-diameter ratio (40:1) having a molecular weight of 470,000 to 500,000 daltons (Frederiksen and Holtzer, 1968).

Actin is the major constituent of the thin myofilament and accounts for 22% of the myofibrillar protein (Portter, 1974; Yates and Greaser, 1983). It exists in two forms: globular (G-form) and fibrous (F-form) depending on environmental conditions (Steiner et al. 1952). At physiological concentrations of salt, globular G-actin polymerizes to form filaments (F-actin)which can interact with myosin filaments to produce mechanical energy for biological movements (Bandman, 1987; Miyanishi and Tonomura, 1981; Pollard et al. 1981). When actin is extracted with water from muscle tissue it is obtained in the globular form, having a molecular weight of from 44,000 to 49,000 daltons (Sender, 1971; Hay et al. 1973). Since the actin filaments have no defined length F-actin does not have a determined molecular weight.

In muscle, myosin and F-actin are present in a more or less complexed form called actomyosin. Actomyosin is the structural component which is responsible for contraction and relaxation in muscle of living animals (Granicher and Portzehl, 1964).

Tropomyosin-Troponin Complex

These myofibrillar proteins, which are not directly involved in cross-bridge formation but play a role indirectly in the contraction-relaxion cycle, are called regulatory proteins (Maruyama and Ebashi, 1970). Tropomyosin and troponin together account for 9.5% and 12.05% of the muscle proteins and have molecular weights of 36,000 and 70,000 daltons, respectively (Forrest et al. 1975; Porzio and Pearson, 1977). Both of these proteins are located in the groove of the actin filament and play an important role in the control of muscular contraction, in particular as a regulating system under the influence of calcium ions (Murray and Weber, 1974).

During the last few years a number of new proteins have been discovered in skeletal muscle. Quantitatively, most of them are insignificant, but are believed to be involved with the regulation of the filamentous structure of myofibrils, and hence they have been classified as minor regulatory proteins. However, a precise function for many of

them is not yet clear. According to Asghar et al. (1985) these proteins are distributed in different parts of the ultrastructure of filaments such as the M-line (e.g., M-protein or myomesin), A-band (e.g., C-, F-, H-, I- and X-protein), Z-disk (e.g., α-actinin, Z-nin, and Eu-actinin), and I-band (e.g., β-actinin and γ-actinin).

Cytoskeletal Proteins

Several researches have reported the existence of longitudinal filaments other than those of actomyosin. Those filaments look as if they are connecting the thin filaments on either side of the Z-line. Some researchers denoted them as S-filament (Maruyama, 1980). Other filaments were also seen as if they were connecting the edges of the thick filaments to the Z-line, and Sjostrand (1962) designated them as gap filaments. Locker and Leet (1975) also supported the existence of gap filament in the skeletal muscle, while other workers used the term T-filaments in their description (Maruyama, 1980). According to Wolosewick and Porter (1979) a three-dimensional filamentous lattice connects most of cytoplasmic structures. As the average diameter of these filaments is about 10 nm, which is between that of actin (6 nm) and myosin (15 nm) filaments, they are referred to as intermediate or 10 nm filaments (Bornstein and Sage, 1980; Gard et al. 1979; Lazarides, 1982; Price and Sanger, 1979; Price and Sanger, 1980; Steiner et al. 1952; Wang and Ramirez-Mitchell, 1983). Chemically, intermediate filaments

differ from contractile and regulatory proteins in many respects, and morphologically they resemble collagen fibers, which in contrast, exist extracellularly. Since the intermediate filaments are believed to strengthen the architecture of myofibrillar system in muscle, they have also been named cytoskeletal or backbone proteins (Granger and Lazarides, 1978; Obinata et al. 1981). These proteins represent longitudinal intrafibrillar and transverse interfibrillar bridges (Gracia-Nunzi and Franzini-Armstrong, 1980). The proteins which can tentatively be included in this group are titin (or connectin), nebulin, desmin (or skeletin), vimentin and symentin. Some important properties of cytoskeletal proteins are published by Asghar et al. (1985).

Connective Tissue Proteins

The interstitial space of muscle cells (syncytia) contains three proteins, namely, collagen, reticulin and elastin, which are fibrillar in nature. Collectively they are called connective tissue which, in fact, also contains some globular mucoprotein and non-protein components such as lipids and different mucopolysaccharides (hyaluronic acid, chondroitin sulfate A, B and C, keratosulfate, heparitin sulfate and heparin in the form of galactosamine or glucosamine). However, the extracellular proteins around

individual muscle fiber consist mainly of fine reticular and collagenous fibers which constitute the endomysium layer (Bendall, 1964).

Collagen consists of a triple helix that contains a higher hydroxyproline content than any other meat protein. Collagen fibers shorten to about one-third their original length when heated to 70 ^{o}C. At a temperature of about 80 ^{o}C or higher collagen is converted into gelatin. Reticulin, resembling collagen in many respects, does not produce gelatin on heating.

Elastin is a rather unique protein because it contains the uncommon amino acid residues desmosine and isodesmosine. These amino acids are involved in the crosslinking of the polypeptide chains and give elastin its characteristic elastic properties. Unlike collagen, elastin is not decomposed by heat, contains very little hydroxyproline, has very little swelling ability and is extremely resistant to acid and alkali (Bendall, 1964).

Role of Myofibrillar Proteins in Gelation

The presence of salt-extractable myofibrillar proteins has been shown to be necessary for satisfactory binding in both emulsion and restructured meat products. Using model emulsion systems, Acton and Saffle (1969), Miller *et al.* (1980) and Randall and Voisey (1977) showed that increasing

the proportion of salt-extractable myofibrillar proteins produced a concurrent increase in binding quality. A similar effect was observed by Acton (1972) and Siegel et al. (1978) with sectioned and formed products.

In addition to the research done on the binding of myofibrillar proteins as a group, work has been carried out to determine the role of the individual myofibrillar proteins in binding. Much of the initial work in this area was carried out by Fukazawa et al. (1961a,b), Samejima et al. (1969) and Nakayama and Sato (1971a,b) using the individual isolated myofibrillar proteins in model gelation systems. It was generally concluded that myosin and actomyosin were the proteins that produced the greatest gel strengths and, therefore, were the most important in binding. In addition they found that in most cases actomyosin was a more effective binding agent than myosin.

In contrast to these results, MacFarlane et al. (1977), Ford et al. (1978) and Turner et al. (1979) found that myosin was superior to actomyosin in binding meat pieces together in both a model binding system and in a reformed beef product. Although this difference seemed hard to reconcile, an explanation is found in the work of Yasui et al. (1980). Using a model gelation system they showed that the addition of myosin to actomyosin produced a gel that was much stronger than either myosin or actomyosin when

used separately. Hence, the results obtained by MacFarlane et al. (1977) is explained by the interaction of the added myosin with the actomyosin present in the surface of the meat to form a strong binding matrix and the inability of actomyosin to do similarly.

Gelation of Proteins

A discussion of gelation necessitates defining some commonly used terms associated with this phenomenon namely: denaturation, aggregation, coagulation and gelation.

Denaturation has been defined as: (1) a process (or sequence of processes) in which the spatial arrangement of the polypeptide chains within the molecule is changed from that typical of the native protein to a more disordered arrangement (Kauzmann, 1959), (2) as a process in which a protein or polypeptide is transformed from an ordered to a disordered state without rupture of covalents bonds (Scheraga, 1963), or (3) any process, except chemical modification, not involving rupture of peptide bonds which causes a change in three-dimensional structure of a protein from its native in-vivo form (Haschemeyer and Haschemeyer, 1973). These definitions suggest that denaturation is not an "all-or-nothing" phenomenon but rather a continuous process with various areas of the protein molecule changing at different rates (Paul and Palmer, 1972).

Tanford (1968) qualified Kauzmann's definition by requiring that there be no alteration in the protein's primary structure. Denaturation can, therefore, be restricted to the continuous process of native protein structural changes involving the secondary, tertiary, or quaternary structure in which alterations of hydrogen bonding, hydrophobic interactions, and ionic linkages occur during the transition to the denatured state (Anglemier and Montgomery, 1976).

Bond energies that contribute to native structure and maintenance of a protein's conformation were published by Acton and Dick (1984). These bonds can also be viewed as important in protein that is denatured. Once a new structure is formed, the same types of bonding can contribute to the stability of the new structure (Acton and Dick, 1984).

Denaturation involves protein-solvent interactions and leads to changes in physical properties, such as loss of solubility of the protein. Sometimes unfolding of the protein structure is considered part of denaturation (Gossett et al. 1984). Denaturation is usually irreversible if the methods are drastic, the molecular weight of the protein is large and aggregation occurs to prevent a return to the native state.

Aggregation is a general term which has been used to describe many types of protein-protein interactions, with formation of complexes of higher molecular weights (Hermansson, 1979). Aggregation is usually governed by a balance between attractive and repulsive forces. Attractive forces can involve hydrogen bonds, covalent bonds such as disulfide linkages, and hydrophobic association, whereas, repulsive forces can involve coloumbic forces which are affected by the net charge of the protein molecule or the ionic strength of the solution (Egelandsdal, 1980). Aggregation causes the meat protein matrix to shrink which limits the amount of water the matrix can bind and reduces the strength of the forces immobilizing the water. The end result of these changes is a decrease in water holding capacity (Hermansson, 1982).

Coagulation is the random protein-protein interaction of denatured protein molecules, in which polymer-polymer interaction are favored over polymer-solvent reactions (Schmidt et al. 1981). The coagulum is often turbid, and the formation of the coagulum is usually thermally irreversible (Shimada and Matsushita, 1981). A coagulum may settle out of solution because randomness does not lead to an orderly structural assembly of the final aggregate.

Gelation is the orderly interaction of proteins, which may or may not be denatured and which leads to formation of a three dimensional well-ordered structural matrix (Hermansson, 1978). Polymer-polymer and polymer-solvent interactions, as well as attractive and repulsive forces are balanced such that a well-ordered matrix can be formed (Schmidt et al. 1981). Since denaturation is involved in this definition of gelation it is evident that native protein structure is altered when the gelled protein matrix is formed.

Mechanism of Protein Gelation

The classic explanation of protein gelation is the two-step process proposed by Ferry (1948):

Native protein --> denatured protein --> aggregated protein

The first step is considered a denaturation process and the second step an aggregation process. Comparison of the rate of the denaturation step vs that of the aggregation step helps determine gel characteristics. For example, Ferry (1948) suggested that for a given rate of denaturation the rate of aggregation will be slow if the attractive forces between the denatured proteins chains are small. The resulting gel will be a finer network of proteins chains, will be less opaque and will exhibit less syneresis than one with a faster rate of aggregation. A coarser network of

protein chains yields an opaque gel with large interstices capable of holding solvent which is easily expressed from the matrix.

Hermansson (1978) elaborated on Ferry's mechanism stating that contrary to coagulation, where aggregation of the protein molecules is random, gelation involves the formation of a continuous network exhibiting a certain degree of order. Furthermore, when aggregation is suppressed prior to denaturation the resulting network can be expected to exhibit a higher degree of elasticity than if random aggregation precedes denaturation. The slower the second step relative to the first, the more orderly the denatured chains will tend to orient themselves and therefore a more finer gel network will be produced.

Ferry (1948) estimated that the number connecting points between protein molecules was very small, and that for gelation as few as 5-6 loci per chain were sufficient to form a rigid network. The mechanism of gel formation differs among proteins, predominantly in the type of interactions which stabilize the gel (Schmidt et al. 1981). The type and strength of these connections influence the response of the gel to stresses such as mechanical agitation or temperature change (Paul, 1972). Anglemier and Montgomery (1976) stated that long proteinaceous fibers form a three-dimensional

network primarily through the establishment of interprotein hydrogen bonds, and probably not through salt bridges which would be highly solvated in aqueous gels.

Schmidt et al. (1981) suggested that if aggregation occurs simultaneously with denaturation an opaque, less-elastic gel results. During the storage of a gel syneresis or loss of fluid may occur as a result of the formation of additional interprotein bonds which decrease the number of loci available for binding water and reduce the amount of intermolecular space available to immobilize water through capillary forces.

Since the kinetics of the denaturation step relative to the aggregation step appear to be important in determining the type of gel produced, some kinetic terms that aid in describing the gelation process will be reviewed. The first is the reaction rate constant k (min^{-1}), which is obtained from the first order relationship (Lund, 1975):

$$-\frac{dC}{dt} = kC \qquad (1)$$

where C is concentration and -dC/dt is the rate at which concentration decreases. Rearranging Equation (1) we obtain:

$$-\frac{dC}{C} = k\,dt \qquad (2)$$

Integrating between limits C_1 at time = 0 and C at time t Equation 2 gives:

$$\text{Ln } C = \text{Ln } C_1 - kt \qquad (3)$$

A plot of Ln C vs t gives a line of slope -k.

The rate constant is usually temperature dependent and can best be described by the Arrhenius equation:

$$k = S\, e^{-Ea/RT} \qquad (4)$$

where S = frequency factor (min^{-1});
Ea = activation energy (cal/mole);
R = gas constant (1.987 cal/mole °K);
T = absolute temperature (°K).

A plot of Ln k vs 1/T gives a straight line of slope -Ea/R.

Mechanism of Gelation of Myofibrillar Proteins

For muscle proteins during processing, thermal energy is the single most important driving force in protein transition from the native state to the denatured state. For myosin and the actomyosin complex it is a continuous process of native protein structural changes involving secondary, tertiary and/or quaternary structure. Hydrogen bonding, hydrophobic interactions and electrostatic linkages are altered during transition to the denatured state (Anglemier and Montgomery, 1976). While electrostatic and hydrogen

bondings become weaker upon heating the potential for hydrophobic bonding increases and with conformational changes there is greater tendency for more interchain hydrophobic interaction to occur (Acton and Dick, 1984).

Heat induced formation of a three-dimensional protein matrix by myosin and actomyosin, termed gelation, can be represented by two stages of reactions. Each stage involves distinct segments of the myosin molecules. More critically, the stages occur in separate temperature regions during heating. One stage of aggregation occurs between 30 and 50 ^{o}C and the second stage occurs at temperatures > 50 ^{o}C. Thus, in the protein- protein interactions of myosin, each stage by temperature region can be represented independently using Ferry's two-step sequence of reactions. It is obvious that in applying this to processed meats, heating to a final internal temperature of between 65 and 71 ^{o}C involves both stages as both temperature regions are traversed (Acton and Dick, 1984).

The first stage involves aggregation of the globular head regions of the molecule. It is an irreversible reaction assuming heating will be continuous with continuous temperature elevation on the system. Through studies with the S-1 fraction, HMM (heavy meromyosin) segment and myosin, the aggregation is thought to be dependent on oxidation of -SH groups which are predominantly found in the globular

head region (Samejima et al. 1981; Ishioroshi et al. 1979). While -SH group reduction (moles -SH/mole segment) progressively increases from 20 to 70 °C, considerable reduction of -SH content occurs in the early temperature range of 20 to 50 °C for myosin or segments containing a globular head position (Acton and Dick, 1984).

Studies from Ishioroshi et al. (1980) support the role of sulfhydryl group involvement in head-to-head aggregation as one factor of the protein interactions. For sulfhydryl group oxidation to occur it is necessary for head-to-head aggregation to have been preceded by a rapid conformational change in the head region, particularly if covalent disulfide bonds are formed. Foegeding et al. (1983) reported that myosin gels heated to 70 °C were stabilized by non-covalent and disulfide bonds. In addition, they reported that gels heated to 50 °C were less difficult to solubilize (by guanidine hydrochloride and urea) than gels formed at 70 °C.

Liu et al. (1982) concluded on the basis of ease of solubilization (by 2% SDS) of actomyosin gels heated to 48 °C, that hydrophobic interactions were the predominant force in actomyosin aggregation below 50 °C. Solubilization of myosin gels heated to 50 °C by guanidine hydrochloride

and urea, as reported by Foegeding et al. (1983) also implies that hydrophobic and hydrogen bonding are more important than sulfhydryl group reduction.

The two-step sequence of conformational change in the head region and head-to-head aggregation satisfies Ferry's mechanism in the first stage between 30 and 50 °C (Acton and Dick, 1984).

The second stage is associated with structural change of the helical rod segment of myosin which culminates in network formation through cross linking of these segments.

While the globular head interaction predominates in the first stage there is also apparent early disruption of the α-helix at the hinge region in moving from the coiled-coil (α-helix) to a random coil type structure in the same lower temperature region. Further helical disruption of the tail portions requires a higher energy input, thus these helical alterations predominate in the second stage at temperatures > 50 °C.

The coiled-coil to random coil conformational change in the tail region is extremely important to aggregation occurring in the second stage of events. The LMM (light meromyosin) (Ishioroshi et al. 1982) and the myosin rod

(Samejima et al. 1981) ultraviolet absorption difference spectra at 285 nm, where the aromatic side chains of the protein absorb, confirmed that absorption increases as both segments are heated from 20 to 65 °C. In addition several workers have reported an increase in binding and enhancement of fluorescent intensity for actomyosin (Niwa, 1975) and myosin (Lim and Botts, 1967) upon heating in the presence of ANS (8-anilino-1-naphtalene sulfonate). ANS is a fluorescence probe capable of binding with hydrophobic regions of proteins when the conformational structure allows reaction with nonpolar residues. The binding of ANS is initiated at temperatures beginning in the 35-45 °C range. The fluorescent intensity increases with further temperature increase. The exposure of hydrophobic residues facilitates hydrophobic interactions, and thus, increases the potential for tail-to-tail cross-linking in establishing the gel framework. (Acton and Dick, 1984).

In the gelation type protein-protein interaction, it is evident that there is a second reaction to aggregation driven by thermal energy input where the product of the first step becomes the reactor for aggregation in the second step. This sequence of reactions or steps in the formation of three dimensional protein matrices has been proposed by Acton et al. (1983).

Effect of Temperature on Gel Strength

Yasui et al. (1980) investigated the effect of temperature on rigidity of rabbit myosin and actomyosin gels. They found that the gel strength started to increase at 40 °C and reached a maximum at 60 °C. A similar result was obtained by Grabowska and Sikorski (1976) using fish myofibrils, with the difference being that the increase in gel strength started at 30 °C and continued up to a temperature of 80 °C. These results were confirmed in principle by the work of Quinn et al. (1980) who showed, using differential scanning calorimetry, that denaturation of meat (beef) proteins begins at about 50 °C and continues with increasing temperature up to 90 °C. This work and that of Wright et al. (1977) shows that the temperature range of denaturation of the different protein components is a characteristic of the species of animal from which the protein came, the pH and the ionic strength. This information explains the varying results obtained by different workers in this area, as each group used different combinations of animal species, pH and ionic strength.

Acton (1972) investigated the effect of temperature of cooking on the binding ability of poultry loaves. The results obtained on binding ability were basically the same as those obtained by workers measuring gel strength of isolated proteins. In essence, the binding strength started

to increase at 40 °C and reached a maximum at 80 °C, after which it decreased slightly with temperature up to 100 °C.

The effect of temperature on the binding ability of crude myosin (beef) was investigated by Siegel and Schmidt (1979). Their results showed that binding strength started to increase at 55 °C, then increased linearly with temperature to 80 °C but did not show the same decrease in binding ability after 80 °C as reported by Acton (1972). The difference in response of binding ability to temperature obtained by the two different groups of workers may have an explanation similar to that given for the variation in gels of purified proteins, which was the difference in species of animal, pH and ionic strength used by the two groups.

From all the gelation and meat binding studies, the temperature region above 50 °C appears most critical. Gels do not reach appreciable strength until the myosin tail portion has undergone helix-coil transformation and subsequent cross-linking. The myosin head region is important since, from ultrastructure studies, it appears to form the initial super-junctions upon which the super-thick filament network interlinks (Siegel and Schmidt, 1979; Samejima et al. 1981). Similar studies of gelation of natural actomyosin (Deng et al. 1976; Acton et al. 1981; Liu et al. 1982; Ziegler and Acton, 1984) have shown that the ultrastructure of actomyosin gels is one of thinner

filamentous strands with larger pore size distribution and a different cross-linked appearance when compared to myosin gels (Yasui et al. 1982). These ultrastructure studies showing morphological differences between myosin and actomyosin gels imply that in processed meats, differences in textural attributes between prerigor and postrigor raw materials may emerge in the finished product (Asghar et al. 1985).

From the research that has been done on the effect of temperature on gel-strength and binding ability of meat proteins, it can be concluded that there is an interaction between the temperature of heating and the presence and concentration of different salts. The exact interaction has not been clearly elucidated, but the implications is that the temperature at which maximum binding occurs is dependent on the presence of specific salts and hence the ionic strength and pH (Quinn et al. 1980).

Theoretical Basis of the Mathematical Model

To evaluate the effects of heat treatment on the thermal gelation of myofibrillar beef proteins a universal method is needed by which all heating processes can be quantified and compared to a common reference point. This method would facilitate comparison between controlled

constant temperature processes and variable temperature-time treatments and incorporate effects of variable heating rates, variable protein concentration and added gums.

A mathematical model was developed by Morgan (1987) and is based on fundamentals of protein kinetics and polymer rheology. The model combines theories of how temperature and protein concentration at constant shear rate affect viscosity of non-Newtonian fluids and protein polymerization (gelation) reaction kinetics and assumes that apparent viscosity is a relative measure of gel strength.

Concentration Effects

A logarithmic relationship of moisture to dough viscosity was proposed by Harper et al. (1971). Later the same group used a semiempirical logarithmic mixing rule to describe the effect of moisture content on viscosity of corn flour dough and proposed a formula that predicts concentration effects on viscosity of suspensions or solutions.

$$Nc = Nco\,e^{B(\text{concentration})} \qquad (5)$$

where C is concentration of total dry matter, B is the parameter which quantifies relative effects of concentration, Nc is the viscosity index at a particular

concentration and Nc_0 is the viscosity of the solvent. This model assumes that C remains constant and that the trapped water has a lubricant effect.

Rheological and Kinetics Background

A pseudo first-order reaction is used as starting point to model the heat activated chemical changes responsible for thermal gelation of myofibrillar beef proteins. The reaction model is based on the assumption that rheological and kinetic theory presently used in studying plastic polymers might be used as starting point for modelling gelation of protein solutions. Considerable theory has been developed for predicting polymerization phenomena of plastics and similar materials. Mathematical relationships have been developed using molecular and physical entanglement theories to predict rheological properties of polymers (Ferry, 1970). Sha and Darby (1976) used molecular weight data to successfully predict apparent viscosity of polyethylene melts which had weight-average molecular weights ranging from 57,700 to 139,000 daltons.

Several problems are encountered when attempting to draw analogies between plastic polymers and protein reactions. Polymers generally undergo various reversible melting and irreversible polymerization reactions during thermoplastic extrusion. However, proteins undergo irreversible denaturation with network entanglement and

possible cross-bonding. Generally, first or second order reactions are assumed with reactive plastic polymer and monomer species, whereas the thermal gelation of myofibrillar beef proteins involves several higher order reactions. However, perhaps the end effect of this heat-mediated gelation of myofibrillar proteins could be described via a first order reaction analogy (Blum, 1960; Penny, 1967; Deng et al. 1976; Ziegler and Acton, 1984), and then a simple reaction could be used to approximate the average overall viscosity effect due to thermal denaturation of the major myofibrillar proteins.

When a solution of myofibrillar beef proteins is heated, the proteins undergo irreversible denaturation with network entanglement and cross-linking that involve several higher order reactions which occur simultaneously or in cascade. These denaturation reactions will affect their size, shape and molecular weight. Since apparent viscosity is related to all of them, it seems logical to assume that molecular changes caused by the gelation reaction will significantly affect the material's apparent viscosity.

The pseudo first-order kinetic model used in this study to model temperature-time history effects of myofibrillar protein denaturation (gelation) on apparent viscosity

assumes that concentration of the reactive polymer species will remain constant and predicts disappearance of the monomer species.

The pseudo first-order reaction is described by the formula:

$$Mc(t) = Mi e^{-kt} \quad (6)$$

where Mc is concentration of the monomer at time t, Mi is the initial monomer concentration, and K is the first order reaction rate constant. The molecular weight of a polymer can be approximated by:

$$MWp = MWm\ DP \quad (7)$$

where MWp is the polymer molecular weight, MWm is the monomer molecular weight, and DP the degree of polymerization (Williams, 1971). Williams, (1971) also approximated for pseudo first order polymerization the number-average degree of polymerization (DP) as

$$DP = \left(\frac{Mi - Mc}{Pc} \right) \quad (8)$$

where Pc is the reactive polymer species concentration and Mi and Mc as describe in Equation 6.

Ferry (1970) reported a power law relationship for correlating the zero shear rate limiting Newtonian viscosity (η) of polymers with their molecular weight:

$$\eta = k_2(MWp)^{\alpha} \qquad (9)$$

where k_2 is a viscosity coefficient and α a dimensionless constant. Theoretically, α is derived to be 1.0 for low molecular weight polymers and 3.5 for high molecular weight polymers. Sha and Derby (1976) reported that observed values of α found in the literature range from 3.4 to 8.0 for high molecular weight polymers.

Therefore, it was assumed that effects of changing molecular weight during protein polymerization is described (at constant shear rate) by:

$$\eta_{(MWp)} = k_2(MWp)^{\alpha} \qquad (10)$$

where k_2 is assumed to be dependent on t and Mc according to Equation 6 with a material-constant factor included in the expression.

Equations 6, 7 and 8 were combined with Equation 10 yielding η, as a function of reaction time (t), for constant temperature (T).

$$\eta(t) = k_2\left(\frac{MWm\ Mc}{Pc}\right)^{\alpha}\left(1-e^{-k_1 t}\right)^{\alpha} \qquad (11)$$

Equation 11 describes the increase in protein gel viscosity due to heat-induced denaturation and assumes that total dry matter remains constat and shear rate is very be low (near zero). This increase is a function of time for all temperatures (T) greater than the threshold temperature (T_d).

The pseudo first-order reaction used in developing Equation 11 assumes that temperature is constant and greater than the reaction threshold temperature. The coefficient k_1 is defined as the polymerization rate constant. Kinetic theory implies that k_1 is related to temperature by:

$$k_1 = k_4 e^{-Ed/RT} \qquad (12)$$

where k_1 is a specific reaction constant and Ed is the activation energy of protein denaturation. According to Eyring and Stearn (1939) k_1 is related to absolute temperature by:

$$k_1 = \frac{T\ k_t\ k_b}{h} \qquad (13)$$

where k_t is a transmission coefficient, k_b is Boltzman's constant and h is Plank's constant.

Application of Equation 12 and 13 to Equation 11 requires that temperature remain constant with time. However, temperature in most experimental conditions, as well as in almost all commercial processes, will increase from its initial temperature to process temperature in a variable time. Therefore, each process will result in a distinctive and variable temperature-time history within meat products. To meet this need an integral temperature-time history (TTH) developed by Morgan et al. (1979) was incorporated into Equation 11. This function is defined as:

$$TTH = \int_0^{\infty} T(t)\, e^{-\Delta Ea/RT} dt \qquad (14)$$

where Ea = activation energy, R = universal gas constant and T(t) is the temperature-time profile above some minimum threshold temperature (Td).

Morgan (1979) developed the TTH function based upon work by Eyring and Stearn (1939). TTH is defined as zero for all T(t) below the Td. Its assumed that the gelation reaction is not initiated until the temperature is at or exceeds Td and its effect is a multiplicative increase in viscosity due to the myofibrillar protein thermal activated gelation.

Assuming constant moisture, Equations 11 and 14 could be combined to give an expression for the incremental change in apparent viscosity due to the thermal gelation of myofibrillar beef proteins.

$$\eta(T-t) = \eta_0(1+A(1-e^{-aTTH})^{\alpha}) \qquad (15)$$

where η represents the viscosity of the myofibrillar protein in solution before heating, "A" is a parameter which relates the ratio of maximum heat-induced gel viscosity to initial viscosity, "a" is related to the reaction rate constant for protein denaturation and cross-linking, α is a function of the shear rate and a relative measure of molecular entanglement during shear and TTH is defined by Equation 14. Equation 15 is the basic model utilized in this study for predicting the combined effects of temperature, protein concentration, time-temperature history and selected hydrocolloids gums (carrageenan, guar, locust bean and xanthan) on apparent viscosity of myofibrillar beef proteins solutions, where protein gelation was heat induced.

Similar models have been used to model TTH effects of protein denaturation on apparent viscosity index (Morgan et al. 1988; Harper et al. 1978; Smith et al. 1988). Harper et al. (1978) successfully applied this approach to heat setting of bovine plasma proteins suspensions, whereas Smith et al. (1988) found good correlation when they applied

such a simple reaction model to approximate the gel strength in the thermal gelation of chicken myofibrillar protein suspensions and drew the analogy between kinetic losses of protein tertiary structure and pseudo first-order polymerization. This analogy assumes that increases in viscosity due to protein gelation is analogous to the viscosity increase brought about by increased polymer molecular weight during a classical polymerization process. This evidence supported the idea that such an approach was adequate for modelling the thermally-activated gelation of myofibrillar beef proteins.

Characteristics of Hydrocolloids

Macromolecular hydrocolloids or gums, as some are more commonly known, are used by the food industry as texture modifying agents in many different types of products. The term gum refers to a wide variety of compounds including polysaccharides of plant and microbial origin, animal proteins such as gelatin and some chemical derivatives of cellulose (Andres, 1975). Many gums have the ability to form gels at low concentration, physically binding water into a three dimensional structure. The water held by these gels exhibits physical properties similar to those of free or bulk water and is not easily removed from the structure when physically stressed.

Hydrocolloids have several properties that are valuable to meat technologists and are used for several different functions, such as stabilizers and structure forming (gelling) agents. These functional properties are related in part to the ability to imbibe and retain large amounts of water, to interact with proteins and to bind fat (Wallingford and Labuza, 1983).

Some of the gums that have been reported in meat products are: xanthan, guar, locust bean and carrageenan. Guar gum is a very effective water binder in comminuted meat products, canned meats and pet foods. The anionic xanthan gum and locust bean gum have been shown to prevent fat separation in canned meat, whereas, carrageenan stabilizes the texture of frankfurter emulsions against acid deterioration (Pedersen, 1980; Abd El-Baki et al. 1981; Fox, 1983).

Xanthan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate with Xanthomonas campestris, and is purified by recovery with isopropyl alcohol, dried and milled. The linear portion of this colloid is composed of repeating units of D-glucose and is chemically identical to cellulose. The side chains which accounts for xanthan's water solubility are made up of D-mannose and D-glucuronic acid subunits and also contain approximately 3% by weight

pyruvate (McNeely and Kang, 1973). It is a white to cream colored powder that is readily soluble in hot and cold water and is prepared as a mixture of potassium and sodium salts. Because of its chemical structure it is able to form highly viscous and stable solutions at low concentrations at room temperature. These solutions exhibit pseudoplastic characteristics as a result of the rigid cellulosic backbone that is stabilized by the side chains (Morris et al. 1977). The principal properties exhibited by solutions of xanthan gum which are important to the meat industry are: a high degree of pseudoplasticity, a high tolerance to salts, very high stability towards extremes of temperature, pH, ionic strength and shearing force, an ability to suspend particulate matter, the synergy with galactomannan gums and the resistance to enzymatic degradation.

Guar gum is a seed gum composed of linear chains of D-mannose with numerous short side units composed of D-galactose. It has a molecular weight of approximately 220,000 daltons and forms colloidal dispersions in cold water (Goldstein and Alter, 1973). Guar gum is not highly branched, with the side chains consisting of single galactose subunits. Therefore, guar dispersions, which have fairly high viscosities in comparison to more highly branched molecules of equal molecular weights, should absorb less water but still give a relatively high water holding capacity.

Locust bean gum consists of a linear chain of D-mannose as does guar gum. However, it differs in the level of substitution of D-galactose on the side chains, with one substitution every fourth or fifth molecule of mannose compared to every second molecule for guar gum (Rol, 1973). It has a molecular weight of the same order as guar gum, approximately 310,000. Locust bean gum has a fairly low water holding capacity (WHC) at 515g water per 100g dry gum solids. The dispersability of locust bean gum at room temperature appears to be the key to this gum's low WHC. Crystalline regions within the gum's structure fail to solubilize at room temperature, only breaking up as the solution is heated. Cold water dispersions of locust bean gum have a significantly lower viscosity (about ten times) than hot water dispersions (Rol, 1973; Andres, 1975).

Carrageenan is not a well-defined substance, but rather a designation (a family name) for a group of salts of sulphated galactans. They have been defined as that group of galactan polysaccharides extracted from red algae (Rhodophyceae) of the Gigartineceae, Solieracea, Hypneacae and Phyllophoracea families, and that have an ester content of 20% or more and are alternately α 1-3, β 1-4 glycosidically linked. Various types or fractions of carrageenan are defined according to idealized structures and designated by greek letters lambda, kappa, iota, etc.

The different carrageenan fractions (kappa, iota, lambda, etc.) occur in varying ratios in various red seaweeds. By selection of seaweeds it is, therefore, possible to obtain carrageenans which are predominantly of one type. Blends with controlled intermediate properties are produced by blending extracts or seaweeds before the extraction step.

Galactose is the most common repeating monomer. The solubility of carrageenan depends on the hydrophilic sulfate half-ester groups present and the galactopyranosyl unit, and therefore, a range of solubility is found for the various types of carrageenans (Stoloff, 1973).

Carrageenan is reported to have a molecular weight of about 300,000 daltons (Marine Colloids, Inc.) which is much lower than that of xanthan gum. Carrageenans are capable of forming viscous solutions at low concentrations in cold water with the viscosity dependent on temperature, pH, concentration, type of carrageenan molecules and solutes present.

METHODS AND MATERIALS

Experimental Design

This study was conducted in two parts, the first of which was a model system experiment designed as the experimental base for developing a mathematical model for the thermal gelation of myofibrillar beef proteins. The second part of this study was designed to test if the model developed in part I could be applied to the thermal gelation of myofibrillar beef proteins combined with selected hydrocolloids.

Experiment I

This experiment was designed to be the experimental base for testing a proposed mathematical model developed by Morgan et al. (1987) and to determine if it could be applied to a model meat system of myofibrillar beef proteins. The model proposed was:

$$\eta(T-t) = \eta_0(1+A(1-e^{-a\Pi H})^{\alpha}) \qquad (15)$$

where individual model components were described previously.

This model describes the gelation of myofibrillar beef

proteins (measured as the back-extrusion apparent viscosity) as a function of the time-temperature history of the process and the protein concentration of the samples. The experiment was carried out as described below.

Meat Sample Preparation. Beef was excised from the bottom round (biceps femoris) of six young bulls slaughtered at the meat laboratory facility (MSU) and allowed to age three days. The exterior and seam fatty tissue and the epimysial and perimysial connective tissue deposits were physically removed prior to grinding the muscles. After passing the tissue twice through a chilled grinder with plate orifices of 4.8 mm diameter, the muscle mince was divided into lots of approximately 600 g, vacuum packaged and stored at -30 ^{o}C, until required for the experiment.

Isolation of Myofibrillar Proteins. Myofibrillar beef proteins were extracted in a 2 ^{o}C cold room following a procedure describe by Eisele and Brekke (1981) and Smith et al. (1988) with some modifications. The frozen ground meat samples were allowed to thaw overnight in a 2 ^{o}C cold room. A meat sample (about 600 g) was then blended for 30 sec in a Waring blender at maximum speed with 4 volumes (2400 ml) of 0.1M sodium chloride and 0.05M sodium phosphate buffer, pH = 7.1. The suspension was stirred for 60 min at 1200 rpm using an electronically speed-controlled stirrer (Heller GT-21) equipped with a LM Jiffy Mixer stirrer shaft

(Thomas Scientific Apparatus, cat. 8634-S20) to avoid air incorporation into the protein suspension. The solution was transferred to 250 ml centrifuge plastic bottles and centrifuged at 9000 x G for 15 min at 0 °C in an automatic refrigerated centrifuge (Sorval RC-2B). Any connective tissue which accumulated on the propeller was discarded. The supernatant containing fat and sarcoplasmic protein was discarded and the pellet resuspended in 2400 ml of fresh buffer. The extraction procedure of stirring, centrifuging, and resuspension was repeated 3 times.

The final pellet was analyzed for nitrogen content and adjusted to a selected protein concentration (1, 2, 3, 4, 5 or 6%) and to pH 6.5. Buffer salts concentration in the water phase were 0.5% (w/w) sodium chloride and 0.5% (w/w) sodium phosphate.

Heat Treatment. Protein suspensions were transferred to 16 x 100 mm disposable culture tubes (approximately 10 g per tube) and thermally processed in a water bath (model FG-103, Eberbach, Corp., Ann Arbor, MI.) at six different temperatures (54, 64, 70, 75, 80, and 85 °C). Samples were taken at variable intervals ranging from 30 to 13,000 sec. Heat-treated protein solutions were immediately transferred to an ice-water bath, permitted to cool for 30 minutes and stored overnight at 2 °C.

During the thermal process the temperature at center of each tube was monitored with a thermocouple thermometer (model 450-TT, Omega Engineering, Inc. Stamford, CT.) inserted in the center of the tube. Temperature was recorded every 30 sec until the center of the tube reached the temperature of water bath.

Gel Strength. Before measuring the gel strength, cooked protein samples were transferred to a water bath at 20 ^{o}C and allowed to equilibrate for 2 h. Gel strength was evaluated as the back extrusion apparent viscosity using an Instron Universal Testing Machine (Model 4202, Canton, OH) equipped with a 50 N load cell and coupled with a microcomputer (Hewlett Packard 86B). The computer ran a program specifically developed for this experiment (program Rodrigo, appendix A). This program established Instron operating variables: speed 20 mm/min, travel distance 30 mm, load calibration cell 50 N. Distance (mm) and Force (N) were read by the program every 300 milliseconds as a 7.33 mm diameter plunger (flat tip) penetrated the gel at constant speed. Distance-force data were used by the computer to calculate the back-extrusion apparent viscosity and the apparent elasticity using the procedure described by Hickson et al. (1982).

The peak plunger force was calculated as the equivalent force of a linear force deformation triangle which resulted

in an area under the force deformation curve equivalent to the observed curve. The deformation base of the triangle was the same as that of the observed curve. The equation for computing equivalent peak force (Fp) was:

$$Fp = \frac{2\ AREA}{Lp} \qquad (16)$$

where area = area under the force deformation curve;
Lp = plunger travel distance..

The viscosity index (η) was computed using Equation 17 (Hickson, et al. 1982).

$$\eta = \left(\frac{1}{2\pi V_p}\right)\left(\frac{F_p}{L_p}\right)(1-K^2)Ln\left(\frac{1}{K}\right)\left(1+\frac{\alpha}{Ln\ K}\right) \qquad (17)$$

where Vp = velocity of plunger (mm/min);
K = ratio of plunger diameter to tube diameter;
α = $(1-K^2)/(1+K^2)$;
Lp = plunger travel distance (mm);
Fp = plunger force as describe before (N).

Evaluation of the Thermal Process. The time-temperature history (TTH) of the gels was calculated with a microcomputer (Hewlett Packard 86B) using a program specifically developed for this purpose (program Mariana, Appendix C). Basically the program used the experimental time-temperature data collected during the thermal gelation of the myofibrillar proteins and calculated the Fourier

number (Fo) and the dimensionless temperature ratio θ using Equations 18 and 19.

$$Fo = \frac{k\ t}{Cp\ r^2\ \rho} \qquad (18)$$

$$\theta = \frac{(Ti - T_\infty)}{(Tc - T_\infty)} \qquad (19)$$

where t = time in seconds;
r = internal radius of test tube;
Cp = caloric capacity calculated as 1.675 + 0.025 Moisture;
ρ = density as weight/volume (1069 Kg/m^3);
T_i = initial temperature of sample (normally 20 °C);
Tc = temperature at the center of test tube (°C);
T_∞ = temperature of the water bath (°C);
K = thermal resistant constant from Equation 20.

Thermal resistance values were not found in the literature for this type of gel. Therefore a formula which gave "K" as a function of temperature was developed. For this purpose, data published for veal, lean beef, and pure water were used (Heldman, 1985). Because the value of "K" changed with moisture and temperature, an average "K" for 90% moisture was calculated (i.e. the moisture expected in this gel). Then using values for 40 and 80 °C, the following linear relationship was created:

$$k = 0.274 + 1.4146\ E(-4)\ T \qquad (20)$$

where T= temperature in °C.

With the above data the Biot number was calculated for each one of the experimental time-temperature points using an approximation to the general equation. This approximation was found to be good for a range of Fo values greater than 0.15. The approximation was calculated using a simplification of the general formula for transient heat transfer in an infinite cylinder (Equation 21).

$$\theta = \sum_{n=1}^{\infty} A_n J_0\{\mu_n \tfrac{r}{R}\} e^{-\mu_n^2 F_0} \qquad (21)$$

where $A_n = \dfrac{2\ Bi}{[\ J_0(\mu)\ (\ \mu^2+Bi^2\ )\ ]}$

θ = as defined in Equation 19;
Jo = Bessel function;
μ = root 1 of Bessel function;
r = radius of point for temperature 1;
R = radius of tube;
Fo = as defined in Equation 18;
Bi = Biot number.

For the special case of the center of the tube (r/R = 1) and considering only the first root of μ Equation 21 could be written as:

$$\theta = A_1 J_0\{\mu_1\} e^{-\mu_1^2 F_0} \qquad (22)$$

or

$$Ln(\theta) = Ln(A_1)+Ln(J_0\{\mu_1\})-\mu_1^2 F_0 \qquad (23)$$

Using values reported in the literature (Luikov, 1968), it was found that for Fo > 0.15 Biot number was related to μ and A_1 (R^2 = 0.99 and 0.989 respectively) by the equations:

$$Ln(B_i) = 0.08359\mu^{4.35842959} \qquad (24)$$

and

$$Ln(B_i) = 0.060637\ A_1^{8.078} \qquad (25)$$

Combining Equations 24 and 25, it is found that for this special case A_1 is related to μ by:

$$Ln(A_1) = 0.03974+0.5395\ Ln(\mu) \qquad (26)$$

When n= 1, the argument of the Bessel function approximates (0) and then Jo{μ}= 1 and therefore Ln(Jo{μ}) = 0. When this value and Equation 26 are substituted in Equation 23, it gives:

$$Ln(\theta)+\mu^2 Fo-0.03974-0.5395\ Ln(\mu)=0 \qquad (27)$$

A computer subroutine (program Mariana) using the secant method was utilized to find μ values that satisfied Equation 27 and then utilized these values to calculate a Biot number for each experimental time-temperature point as:

$$B_i = e^{0.08359\mu^{4.35842959}} \qquad (28)$$

Followed by the average Biot for a user-selected range.

After defining the Bi number from experimental data, the time-temperature profile was calculated based on a transient heat transfer model for infinite cylinders reported by Parker et al. (1970). This model predicts temperature-time data for ten different radial positions within a given test-tube cross-section based on the initial, center and bath temperatures for each treatment. The cross-section was divided into ten concentric rings defined by the preselected radial position.

The computer program utilized the general formula for an infinite cylinder again (Equation 21), but in this case r/R was a variable and μ was calculated to its sixth root. Then the general equation could be represented as:

$$\theta = \sum_{n=1}^{6} A_n J_0\left\{\mu_n \frac{r}{R}\right\} e^{-\mu_n^2 F_0} \qquad (29)$$

where μ roots where calculated using Equation 30.

$$\mu_n J_0(\mu_n) - B_i J_0(\mu_n) = 0 \qquad (30)$$

The solution of the general equation gave a value of Θ as function of r/R and time (as Fo). Using the calculated value of Θ, temperature Tc for any point r/R at any time (Fo_1) could be calculated by rearranging of Equation 19, as shown in Equation 31.

$$T_c = \theta(T_i - T_\infty) + T_\infty \qquad (31)$$

This generated an 11 x 60 time-temperature matrix which was used to calculate the effect of the temperature at each selected r/R point as an A' factor. The A' factor was defined as:

$$A' = T e^{-Ea/RT} \qquad (32)$$

where Ea : Activation energy;
T : Absolute temperature (^{o}K);
R : Ideal gas constant.

The TTH value was the accumulative effect of the average A' of two points times the time interval between these two points (30 sec) as represented by Equation 33.

$$TTH = \sum_{i=1}^{\infty} \left(\frac{A_i - (A_i - 1)}{2} \right) \qquad (33)$$

These procedures calculated the TTH for each ring which then were averaged over rings to obtain a final TTH average.

Determination of Activation Energy. The activation energy of this pseudo first order reaction was calculated in two steps. The first step estimated a range of values for the activation energy. To do that, high, medium and low values of apparent viscosity were selected in such a way that values for time of cooking at different temperatures were obtained for the same apparent viscosity (protein concentration = 4.09%). A plot of natural logarithm of time versus the inverse of the absolute temperature of cooking yielded a slope equal to -Ea/R where R = constant of ideal gases (1.987 cal/^{o}K-mol). These plots gave a range of values for activation energy between 16,000 and 42,000 cal/mol.

The second step calculated the final Ea value by an optimizing computer routine. TTH values for Ea = 16,000 and 42,000 cal/mol were plotted versus apparent viscosity and the linear range selected. Selected values for the linear region (all temperatures) were utilized as starting points. The computer routine basically did the following: upon an

input of Ea value, calculated TTH values for each temperature, plotted them versus η and calculated the regression line. Then, Ea was adjusted and TTH values recalculated until a maximum regression coefficient for the regression line was obtained. A microcomputer (Zenith 148) with the computer package Framework II (version 1.0) linked to the graph-statistical package Plotit (version 1.1) were used for these calculations.

A first estimated value for the material constants A and α were calculated using the computer program Mariana. The final values of these constants were calculated using a non-linear regression analysis (Marquardt, 1963). These calculations were performed by a microcomputer (Zenith 148) with the computer package Framework II and the graph statistical package Plotit.

Experiment II

This experiment was designed to test the hypothesis that the mathematical model developed in experiment I could be applied to the thermal gelation of myofibrillar beef proteins combined with selected hydrocolloids. The gums utilized were Xanthan gum (Miles Laboratories, Inc.), Guar gum (Colony Import & Export Corp.), Locust bean gum (TIC Gums, Inc.) and Carrageenan gums (Gelcarin XP 4039, FMC Corp.).

The model meat system used was basically the same as the one used in experiment I. The main differences were that protein concentration was selected to be 2.5% and temperature of cooking was 70 °C. Concentration of gums was 0.5% and they were added mixed with the salts (sodium chloride and phosphate salts).

Chemical and statistical analysis, as well as mathematical calculations, were identical to those in experiment I, except that the Ea value was assumed to be equal to that calculated for the myofibrillar system.

Methods Of Analysis

Determination of Water Holding Capacity

Water Holding Capacity (WHC) was determined on the ruptured gel/protein suspensions after gel strength testing. The protein/gel in 16 x 100 ml culture tubes was centrifuged at 2000 x G for 2 h at 4 C in an automatic refrigerated centrifuge (Sorval RC-3). The weight of the water released was used to calculate the WHC as:

$$WHC = \frac{W_g - W_l}{W_g} 100 \qquad (34)$$

where W_g = weight of the gel;
W_l = weight of the water lost.

Buffer Preparation

Fresh buffer was prepared the day prior to the experiment. The buffer batch was prepared as follows: 33.2 g of monosodium phosphate monohydrate, 81.9 g of disodium phosphate, 87.6 g of sodium chloride and 2797.3 g of glass bidistilled deionized water were mixed with an magnetic stirrer until all the salts dissolved (approximated 20 min.). The concentrated salt solution was tranferred to a 20 l plastic bin and 12 l of water added with agitation. The pH of the solution was checked and found to be between 7.1 and 7.2. The buffer solution was allowed to cool overnight in a cool room at 2 ^{o}C before use.

Protein Determination

Nitrogen was determined by the Micro-Kjeldhal procedure (A.O.A.C., 1985, 23.009) using a Buchii automated nitrogen analyzer 322/342 equipped with an Epson HX-20 minicomputer. Protein samples weights were between 0.2 and 0.3 g and the protein content determined as 6.25 times percent nitrogen. Concentrations of chemicals were hydrocloric acid 0.05N, boric acid 4% w/w and sodium hydroxide 30% w/w. Water was glass bidistilled and deionized.

Statistical Analysis

Linear regression analysis was performed by the least square method using the integrated graph-statistical program

Plotit (Eisensmith, 1985). Nonlinear regression analysis was performed with the algorithm for least squares estimation of non-linear parameters developed by Marquard (1963) using the integrated graph-statistical program Plotit (Eisensmith, 1981).

Computer Programs Utilized

This experiment required intensive use of computer time to expedite the mathematical development of the TTH model for myofibrillar beef proteins. The computer software utilized were of two types: (a) availabe commercial programs and (b) programs especially developed for this experiment. The commercial programas utilized were: Framework II (Ashton Tate, 1985), Plotit (Einsensmith, 1985) and TK!Solver (Software Arts, Inc. 1983).

Specifically developed for this experiment were three computer programs. They were named: program Rodrigo, program Mariana and program Delia. These programs were developed in Basic for a Hewlett Packard 86B computer.

Program Rodrigo

This program was developed to: (1) calibrate and control the working parameters of the texturometer INSTRON (speed, travel distance, calibration of load cell, calibration of plotter and calibration of load balance), (2) to read every 300 milliseconds the distance-force data

generated as a 7.33 mm diameter plunger (flat tip) penetrated the gel at predetermined and constant speed, (3) to calculate the back extrusion apparent viscosity from these data and (4) to save these values for future reference. Figure 1 is a flow diagram of this program.

Program Delia

This program was developed to read raw data generated and saved by the program Rodrigo. It calculated the back extrusion apparent viscosity and the shear force at the tube wall from the distance-force data generated and saved during the texture measurement. Figure 2 shows a flow diagram of this program.

Program Mariana

This program was developed mainly to estimate the time temperature history of the process. This program calculated the following individual parameters of the thermal process: the Fourier number, the Θ value, the Biot number, the time temperature matrix for ten concentric rings, the TTH values for calculated concentric rings and an all-over average TTH. This program was also designed to calculate a first estimate of some of the constants of the basic model (Equation 15) namely: the (a), the α and A' values.

The thermal process was calculated using the experimental time-temperature data collected during the

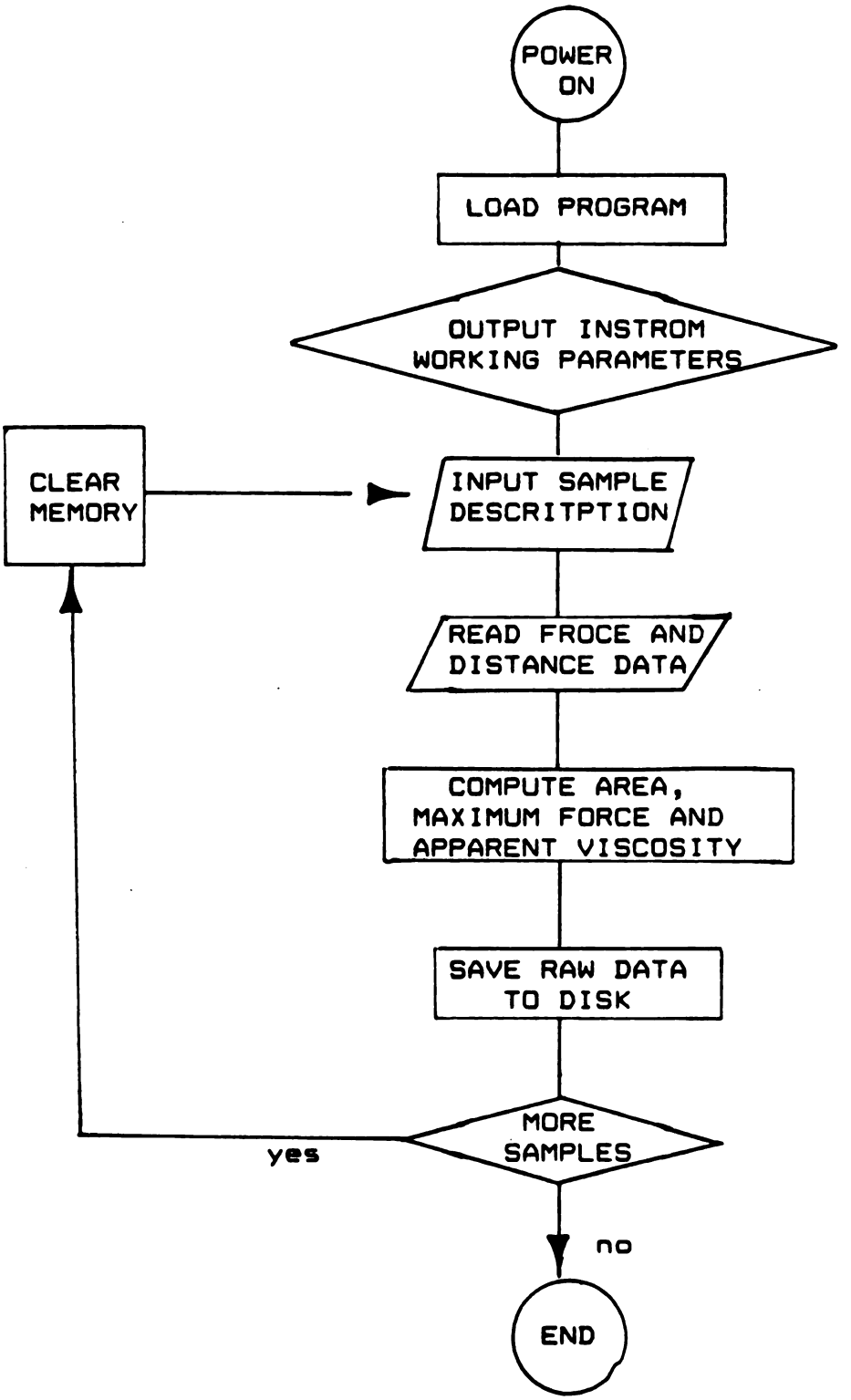


Figure 1. Flow diagram of computer program Rodrigo.

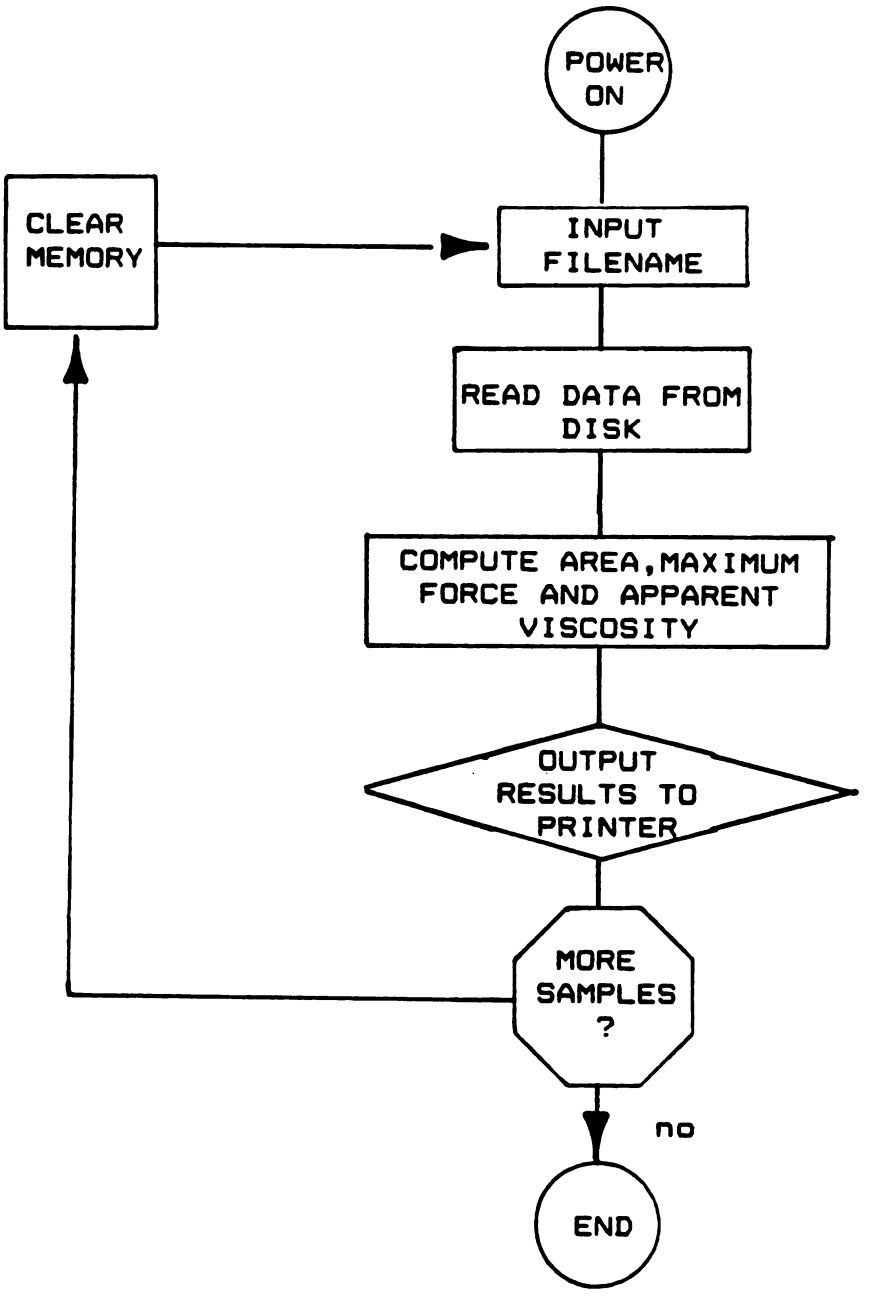


Figure 2. Flow diagram of computer program Delia.

thermal treatment of the protein gels. These time temperature values were utilized to calculate the Fourier number and the Θ values, which in turn were utilized to calculate the Biot number. The next step divided the cross section of the test tube (used for cooking the gel sample) into ten concentric rings with equal area and then calculated the time-temperature profile for each ring.

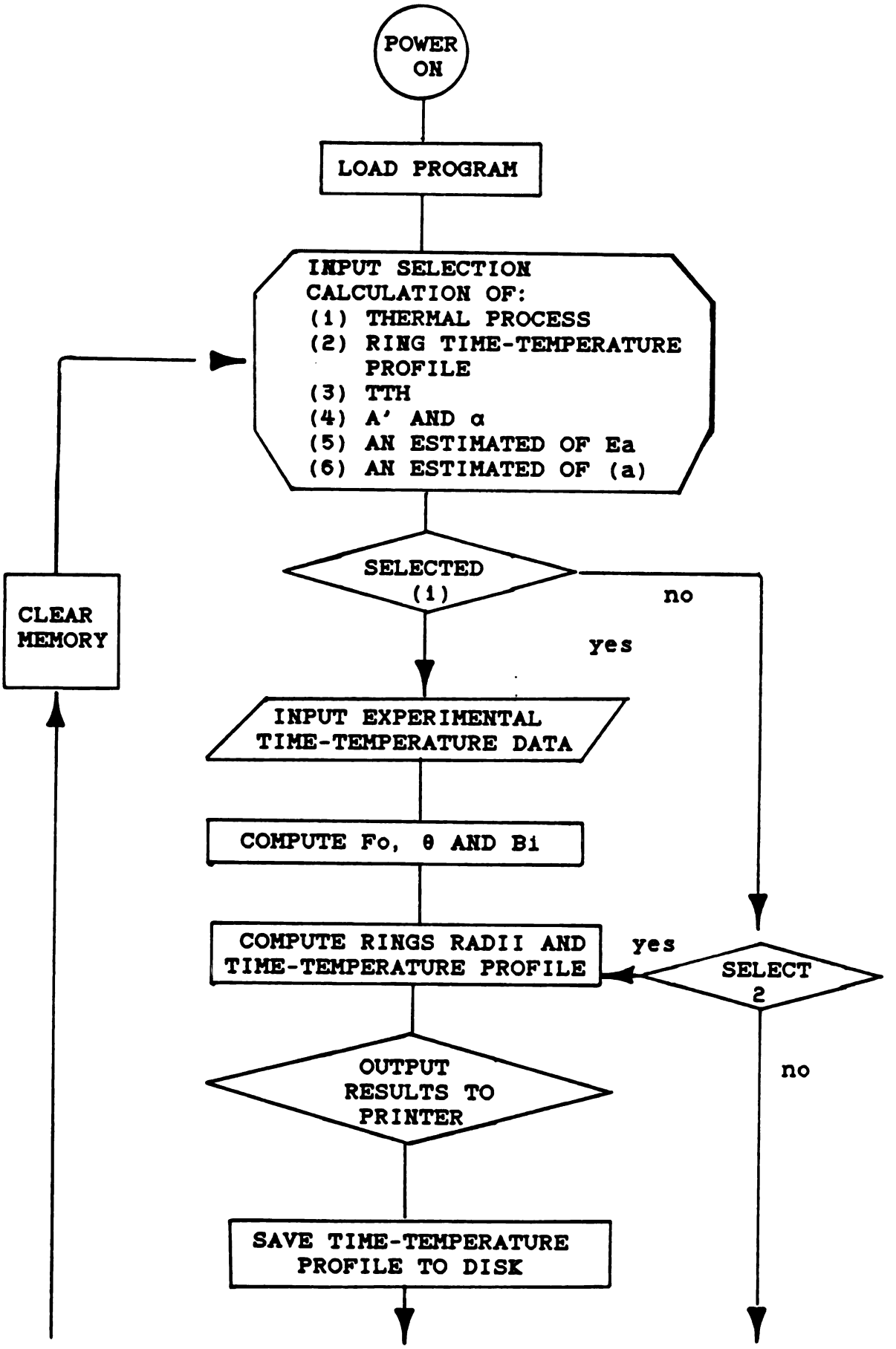
The next step calculated the TTH value for each ring, using time intervals of 30 sec. Final TTH values were obtained by averaging individual TTH values for each ring at every 30 sec. The program contains provision for a variable Ea.

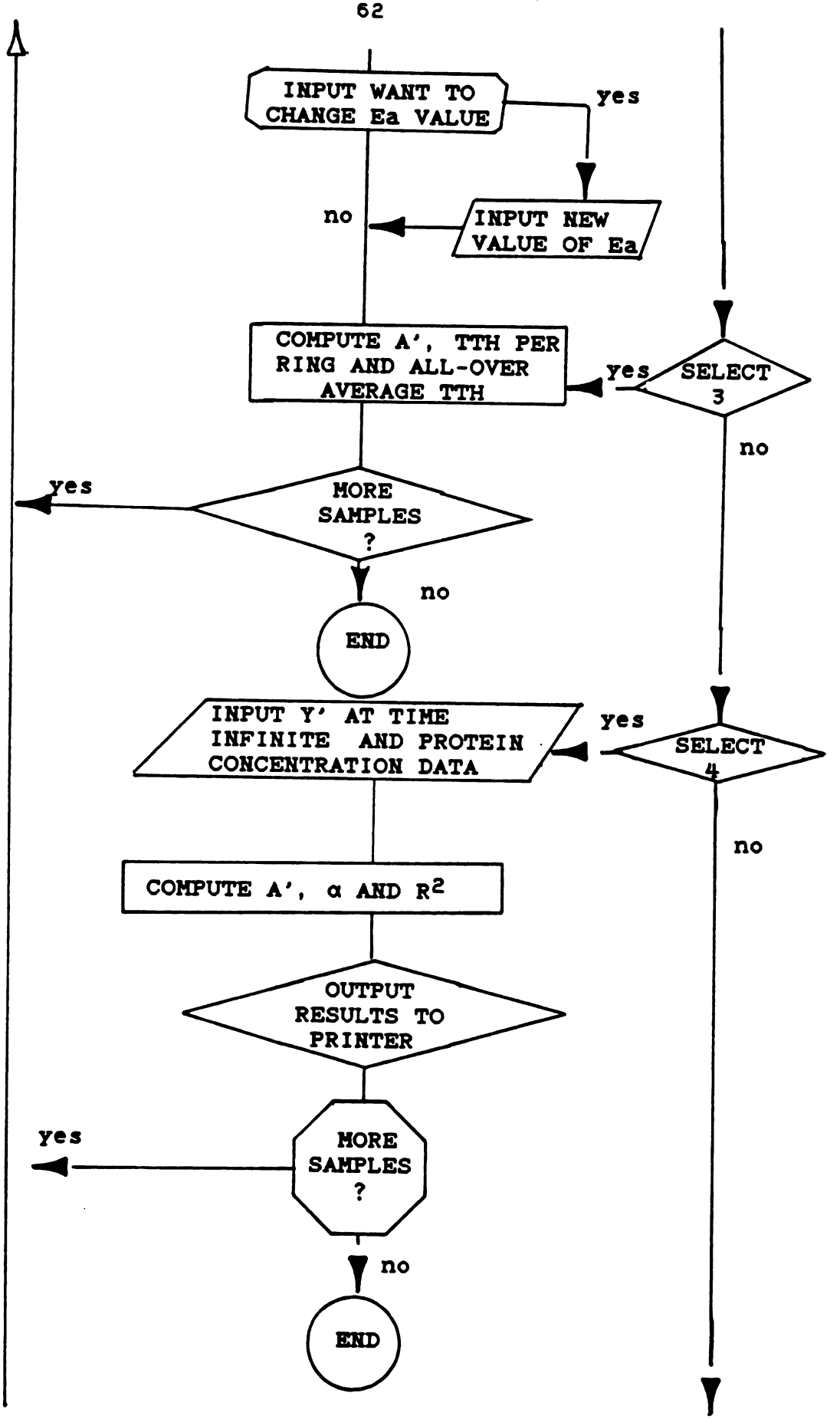
The second section of the program calculated values of α and A using a regression analysis of selected values of η and their corresponding protein concentration (C), whereas a preliminary value of (a) was obtained by iterating several values of Y' and their corresponding TTH values using Equation 35.

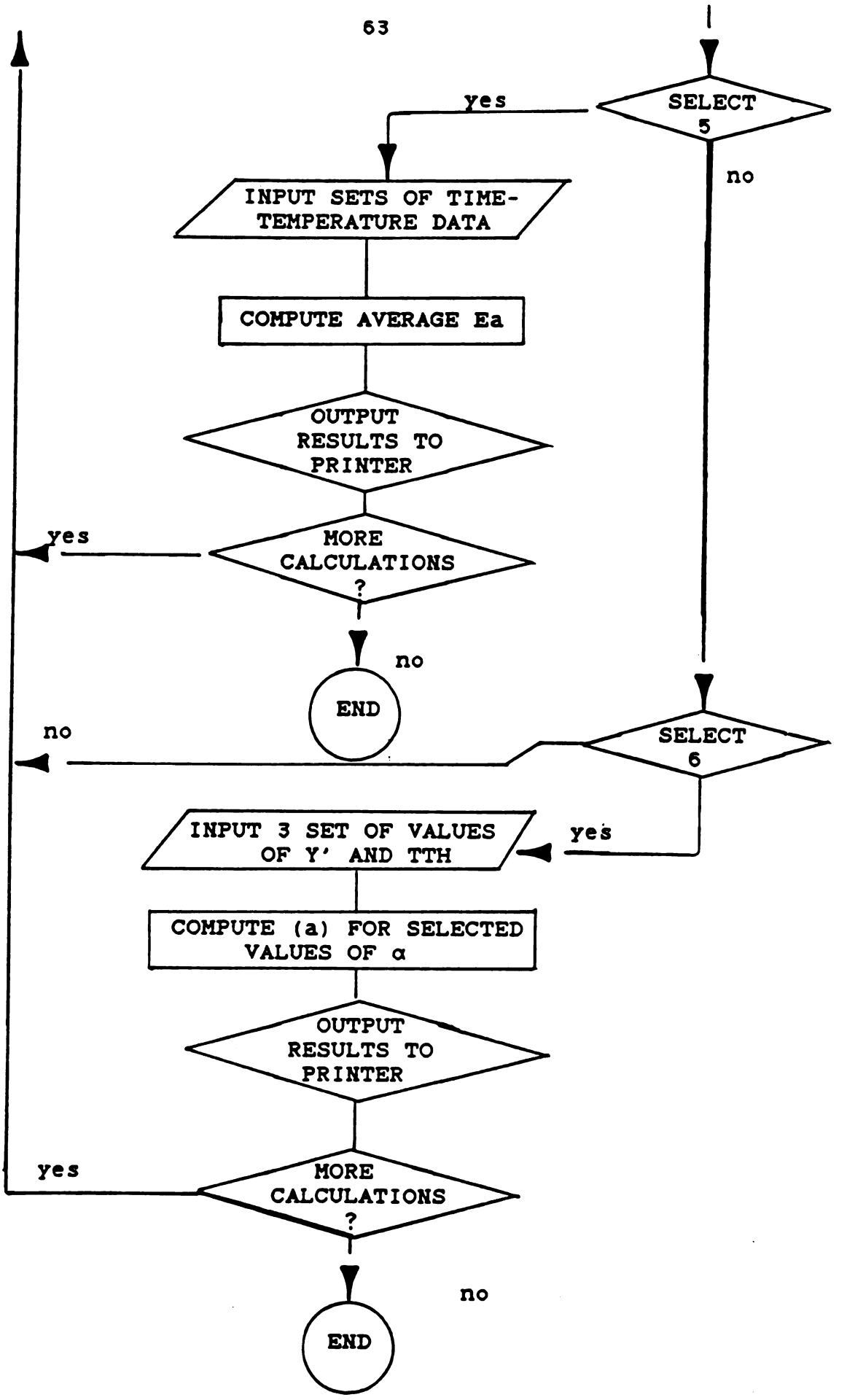
$$\frac{Y_1}{Y_2} - \frac{B(1-e^{-aTTH_1})^{\alpha}}{B(1-e^{-aTTH_2})^{\alpha}} = 0 \qquad (35)$$

Figure 3 shows a flow diagram of the steps followed by this program.

Figure 3. Flow diagram of computer program Mariana.


POWER ON
LOAD PROGRAM
INPUT SELECTION
CALCULATION OF:
(1) THERMAL PROCESS
(2) RING TIME-TEMPERATURE PROFILE
(3) TTH
(4) A' AND α
(5) AN ESTIMATED OF Ea
(6) AN ESTIMATED OF (a)
SELECTED (1)
no
yes
CLEAR MEMORY
INPUT EXPERIMENTAL TIME-TEMPERATURE DATA
COMPUTE Fo, θ AND Bi
COMPUTE RINGS RADII AND TIME-TEMPERATURE PROFILE
yes
SELECT 2
no
OUTPUT RESULTS TO PRINTER
SAVE TIME-TEMPERATURE PROFILE TO DISK


INPUT WANT TO CHANGE Ea VALUE
yes
no
INPUT NEW VALUE OF Ea
COMPUTE A', TTH PER RING AND ALL-OVER AVERAGE TTH
yes
SELECT 3
no
yes
MORE SAMPLES ?
no
END
INPUT Y' AT TIME INFINITE AND PROTEIN CONCENTRATION DATA
yes
SELECT 4
no
COMPUTE A', α AND R2
OUTPUT RESULTS TO PRINTER
yes
MORE SAMPLES ?
no
END


SELECT 5
yes
no
INPUT SETS OF TIME-TEMPERATURE DATA
COMPUTE AVERAGE Ea
OUTPUT RESULTS TO PRINTER
MORE CALCULATIONS ?
yes
no
END
SELECT 6
no
yes
INPUT 3 SET OF VALUES OF Y' AND TTH
COMPUTE (a) FOR SELECTED VALUES OF α
OUTPUT RESULTS TO PRINTER
MORE CALCULATIONS ?
yes
no
END

RESULTS AND DISCUSSION

Determination of the Activation Energy Value

The value of the Activation energy (Ea) of this pseudo-first order reaction was calculated in two steps. The first step estimated a range of values where the Ea value was expected to be, whereas, the second step focused in that range to calculate the final value. This first step was based on kinetic theory, which predicts the reaction rate constant for a given Ea, temperature and time period (Equation 12). For a selected time condition Equation 12 could be written as:

$$k_i = k_\infty e^{-Ea/RT} \qquad (36)$$

If the natural logarithm of both terms is obtained and this is followed by a rearrangement of the terms, then Equation 36 could be written as:

$$Ln(t_i) = Ln\left(\frac{k_i}{k_\infty}\right) + \frac{Ea}{R}\frac{1}{T} \qquad (37)$$

Equation 37 implies a linear relationship between Ln time (sec) and the inverse of the temperature (^oK) of cooking. The slope of the line is the Ea (cal/mol) divided by the constant of the ideal gases (R=1.987 cal/mol^oK).

A basic assumption of these experiments was that the heat-induced back-extrusion apparent viscosity (Y') measured the extent of the thermal induced gelation reaction of the myofibrillar beef proteins. Therefore, by using the time to obtain a predetermined reaction extent (Y'= 1.0, 2.0, 2.5, 3.0 and 3.5) at different experimental temperatures it was theoretically possible to calculate the Ea of this reaction.

This first step was carried out as follows: A protein solution (4.2%) was thermally processed at 54, 64, 70, 80 and 84 ^{o}C and the Y' calculated for different time-temperature conditions (Table 1). The back-extrusion apparent viscosity (BEAV) induced by the heat treatment was found to be a function of time for the experimental range and follow the mathematical model described by Equation 38.

$$Y' = A\left(1-e^{-Bt}\right)^{C} \qquad (38)$$

where Y'= thermally induced BEAV;
A,B,C = constants related to the basic model (Equation 15);
t = time (sec).

Experimental Y' and related time values were utilized to obtain the constant (A, B and C) values for each temperature of cooking, using a non-linear regression algorithm (Marquard, 1963). The calculated constants A, B

Table 1. Thermally induced back-extrusion apparent viscosity (Y') for the time range of 30 to 350 sec and for five different temperatures.

TIME	TEMPERATURE °C				
seconds	54	64	70	80	84
30	.19	.06	.06	.11	.30
60	.30	.19	.32	.54	1.21
90	.39	.37	.75	1.15	2.15
120	.47	.56	1.25	1.81	2.83
150	.55	.77	1.75	2.40	3.28
180	.62	.99	2.20	2.91	3.54
210	.69	1.20	2.58	3.31	3.70
240	.75	1.42	2.90	3.63	3.79
270	.81	1.62	3.15	3.86	3.84
300	.87	1.81	3.35	4.04	3.87
330	.92	2.00	3.50	4.17	3.88
360	.98	2.18	3.62	4.27	3.89
390	1.03	2.34	3.71	4.34	3.90
420	1.08	2.49	3.78	4.39	3.90
450	1.13	2.64	3.84	4.43	3.90
480	1.17	2.77	3.88	4.46	3.90
510	1.22	2.89	3.91	4.48	3.90
540	1.26	3.01	3.93	4.49	3.90
570	1.31	3.11	3.95	4.50	3.90
600	1.35	3.21	3.96	4.51	3.90
630	1.39	3.30	3.97	4.52	3.90
660	1.43	3.38	3.98	4.52	3.90
690	1.47	3.45	3.98	4.52	3.90
720	1.51	3.52	3.98	4.53	3.90
750	1.55	3.58	3.99	4.53	3.90

and C values are shown in Table 2.

Calculated models were found to predict reasonably well the experimental data (CD = 0.95 to 0.99), and therefore were utilized to estimate the time required to achieve a preselected Y' (1.0, 2.0, 2.5, 3.0 and 3.5) for each temperature used. These values are shown in Table 3.

The first range of values for Ea was calculated by plotting the Ln of time (sec) and the inverse of absolute temperature (Figure 4). This method of calculating Ea value assumes that the temperature of cooking is constant throughout the thermal process and that the sample attains the temperature of the process instantly. However, experimental values showed that the equilibrium temperature required a relatively long time, which is explained by the relatively low heat transmission coefficient of this protein solution. Experimental samples arrived at equilibrium temperature (bath temperature) at about 10 min, which is the time range where most of the thermal induced changes in BEAV occurred. This explains why different time-temperature ranges produced different estimates of Ea, nevertheless these values indicated the range where the Ea values were to be found.

Values of Ea found by this method (Table 4) ranged from 14,147 to 33,989 cal. This range agrees with reports from

Table 2. Calculated constant values for modeled experimental data cooked to five different temperatures.

TEMPERATURE	CONSTANT*			
^{o}C	A	B	C	R^2
54	4.603	0.2987e-3	0.679	0.984
64	3.995	0.3236e-2	1.774	0.991
70	3.997	0.9591e-2	3.048	0.952
80	3.950	0.1525e-1	4.280	0.961
85	3.903	0.1940e-1	3.118	0.946

* A, B and C as defined by Equation 38;
R^2 = coefficient of determination.

Table 3. Time of cooking in seconds required to produce a selected thermal induced apparent viscosity for five different temperatures.

APPARENT VISCOSITY	TEMPERATURE °C				
	54	64	70	80	84
1.0	374	181	105	83	54
2.0	1123	329	167	129	85
2.5	1760	421	203	155	104
3.0	2500	538	260	188	130
3.5	3690	710	329	227	174


Ln Time (sec)

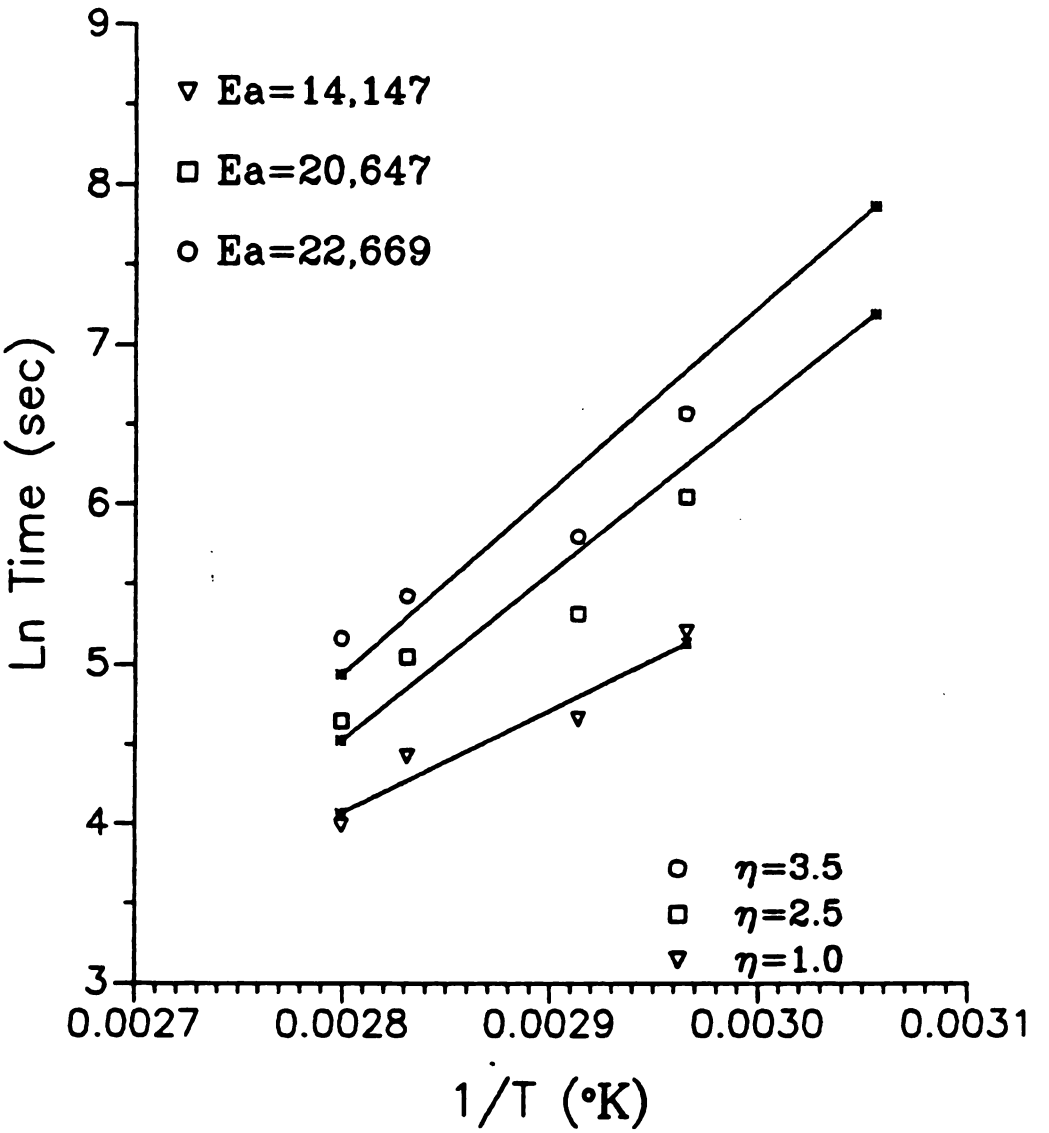


Figure 4. Estimation of activation energy range for five temperatures and three thermal-induced apparent viscosities (η).

Table 4. Estimated values for Ea (cal/mol) for different Y's and temperature ranges.

TEMPERATURE RANGE ^{o}C	HEAT-INDUCED APPARENT VISCOSITY 1.0	2.0	2.5	3.0	3.5
54 to 84	14,147	18,828	20,647	21,664	22,669
54 to 70	17,513	25,391	30,264	30,292	33,989
80 to 84	28,469	27,629	26,429	24,434	17,610

Ziegler and Acton (1984) who reported that the apparent heat of activation for the heat mediated interaction of actomyosin is in the range of 17.1 to 27.0 Kcal and with Smith et al. (1988) who found that the activation energy for the thermal gelation of myofibrillar poultry proteins is 20,000 cal.

The second step in calculating the Ea value was based on a second assumption of this experiment namely: The reaction extent (Y') is a function of the time-temperature history of the process (TTH), other factors constant. This is described by Equation 39.

$$Y' = F\left(\int_0^t T e^{-Ea/RT}\, dt\right) \qquad (39)$$

Equation 39 implies that Y' values are a function of the time-temperature history of the process, which was one of the basic assumptions of this experiment. Graphically this relationship will produce (when selected Ea is correct) one single curve when experimental Y' is plotted versus the time-temperature history of the process, independently of the temperature of the water bath. When Ea is incorrect, several curves (one for each temperature of process) will be produced.

The range of values for Ea calculated previously (14,147 to 33,989 cal) where used as starting values. The TTH value of the thermal process of the myofibrillar beef proteins was calculated using a transient heat transfer model for infinite cylinders (Parker et al. 1970). It predicted temperature-time data for ten different radial positions within a given test-tube cross-section based on the initial, center and bath temperatures for each treatment as described before. The cross-section was divided into ten concentric rings defined by the preselected radial position. TTH was computed for each ring and then used to compute a mass average TTH. Morgan et al. (1987) demonstrated that this method significantly reduces error in estimating kinetic parameters. It more accurately accounts for the variation in reaction rates within a sample due to temperature-time profiles. They also concluded that using the center temperature-time data, which is normally used in lethality studies, results in errors commonly exceeding 100%, while using mass average temperature-time data results in greater than 50% error, compared to the technique of using concentric rings to compute kinetic parameters.

During the first stage of the gelation reaction, Y' is a linear function of Ln (TTH). This property was used to determine the Ea value. Those values of Y' that were in the linear region for five different processing temperatures (54, 64, 70, 80 and 84 ^{o}C) were selected. These selected

experimental Y' values were plotted against their corresponding TTH values utilizing different values for Ea. A linear regression analysis was performed each time a new value of Ea was used until a maximum linear coefficient of determination was obtained (CD= 0.96) when Ea had a value of 29,500 cal/mol. The relationship between experimental Y' values and the values of the TTH model for the temperature range of 54 to 84 °C and for the Y' range of 0 to 3.5 is shown in Figure 5. The linear regression analysis is presented in Table 5. During this thermal analysis it was observed that the Y' values for the thermal process at 54 °C lagged somehow behind values for higher temperatures (Figure 6). This suggested that another reaction with higher Ea was exerting a significant influence at this temperature.

Even though the overall heat mediated aggregation reaction has been reported to follow first order reaction, the interaction of sarcoplasmic protein molecules apparently proceeds through two steps. Acton et al. (1981) reported two temperature reaction zones for the formation of natural actomyosin aggregates in dilute solutions (0.5 mg/ml) and for the formation of continuous structural aggregates in more concentrated solutions (7.5 mg/ml). The second stage was associated with structural changes of the helical rod segment of myosin which culminates in network formation

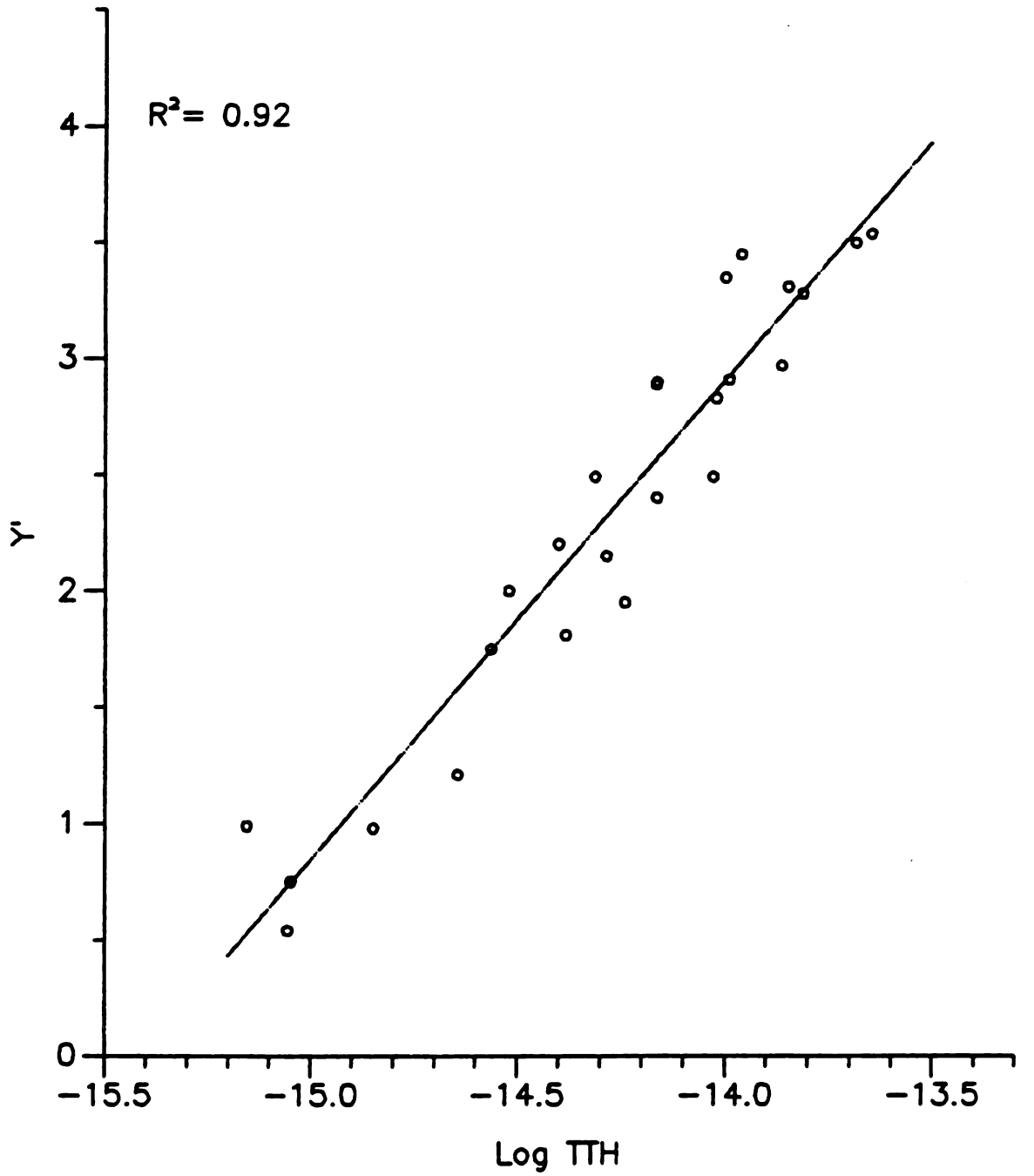


Figure 5. Correlation of Y' values obtained in the temperature range of 64 to 84 °C and their estimated TTH value.

Table 5. Statistical analysis of the linear regression of experimental Y' values and corresponding TTH values. Ea utilized was 29,500 cal/mol.

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Mean	1	137.5460	137.54600	
Regression	1	18.4604	18.46035	250.2
Residual	23	1.6972	0.07379	
Total	25	157.7036	Sig. of F Value:	.0000

REGRESSION STATISTICS

Regression Coefficient	Standard Error	Student's T Value	Sig	Confidence Limits Lower	Upper
B(0) 31.70035	1.8567	17.07	.00	30.43	32.97
B(1) 2.05713	0.1301	15.82	.00	1.97	2.15

Coefficient of: Determination .916 Correlation .95

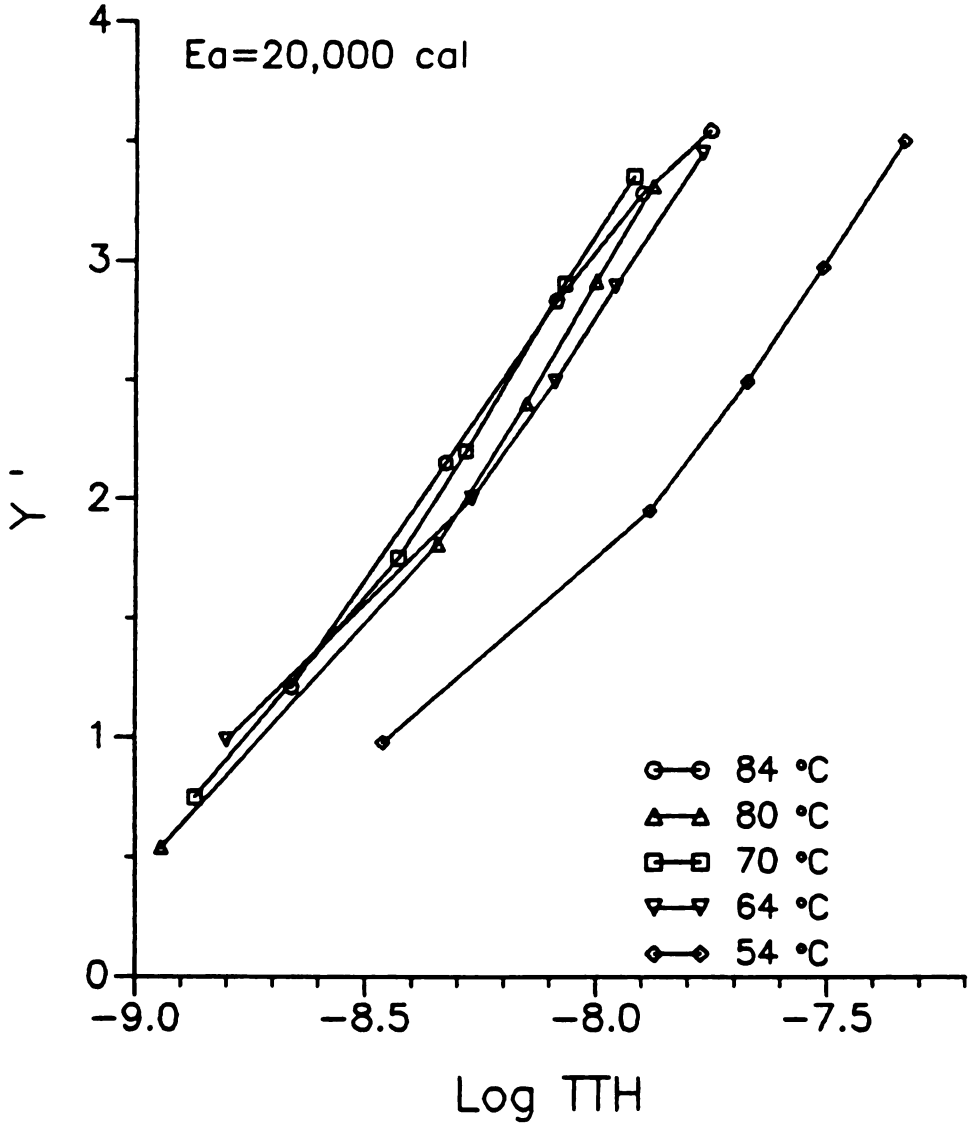


Figure 6. Relationship between experimental values of Y' and the Log of TTH.

through cross-linking of these segments. Montejano et al. (1984) reported that a uniform and rapid increase in rigidity of myofibrillar beef proteins started at 56 °C, indicating the formation of stable, stiff and elastic structured matrix. Other reports also indicate this type of behavior (Ziegler and Acton, 1984; Liu et al. 1982) and it is consistent with the proposed reaction mechanism for the formation of protein gels (Ferry, 1948).

$$xPn \xrightarrow{\text{step 1}} xPd \xrightarrow{\text{step 2}} (Pd)x$$

where x is the number of protein molecules P, with n denoting native state and d denatured state.

The helix to coil transformation starts at about 55 °C and is the starting point of polymerization and gelation. This will make the gelation process at 54 °C very slow and even though the overall reaction remains as a first order reaction, the predominant step (and hence the activation energy) that will predominate will be that of some other reactions which precede this step and start at lower temperatures (dissociation of F-actin and the conformational changes in the head of myosin).

The test of the model using temperature values from 64 to 84 °C notably increased the value of the coefficient of

determination of the regression line (CD= 0.98 versus 0.92) and assigned to Ea a value 20,000 cal. This new linear relationship of the experimental values of Y' and the values of TTH are shown in Figure 7, whereas the regression analysis is shown in Table 6.

Experimental data suggest that the thermal gelation of myofibrillar proteins does not occur at any significant rate until the temperature of the process is above 54 °C and probably is not significant before 60 °C. This agrees with Yasui et al. (1979) who reported that the gelation of myosin reaches a maximum at 60-70 °C and with Ziegler and Acton (1984) who concluded that the transition occurring at 55 °C is possibly the most crucial, since gels do not attain appreciable strength until this temperature is reached. This also agrees with commercial practice for meat products where the minimum temperature utilized to obtain a firm cooked product is about 65 °C. For these reasons, it was decided to consider only the temperature range of 64 to 84 °C, and therefore, to consider a final Ea value of 20,000 cal/mol.

Determination of (a)

The value (a) represents the product of the transmission coefficient (K_t) and the Boltzman's constant

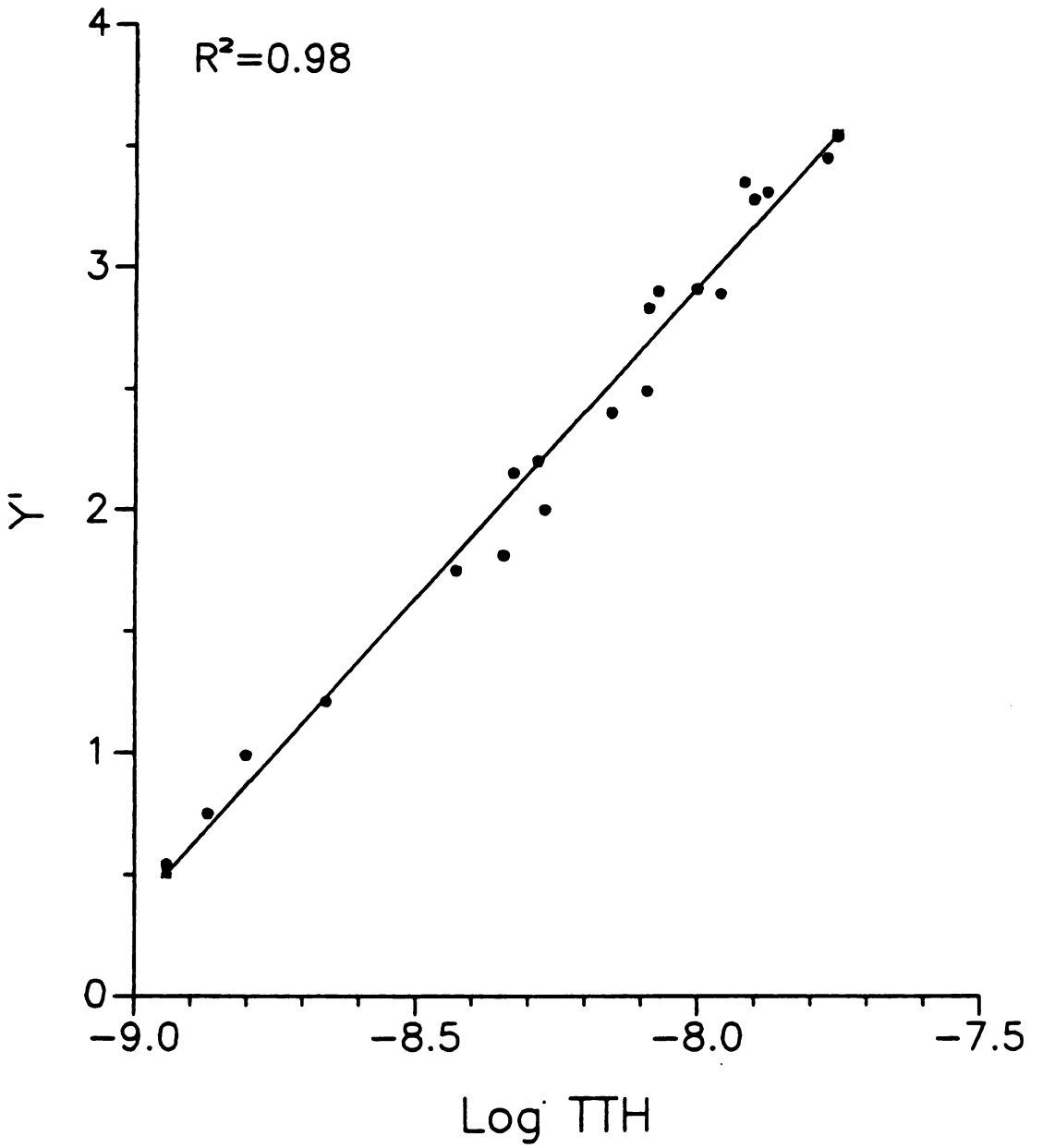


Figure 7. Correlation of Y' values obtained in the temperature range of 64 to 84 °C and their estimated TTH.

Table 6. Statistical analysis of the linear regression of experimental Y' values and corresponding TTH values. Ea utilized was 20,000 cal/mol.

A N A L Y S I S O F V A R I A N C E

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Mean	1	109.27810	109.278	
Regression	1	16.07738	16.0774	921.0
Residual	18	0.31420	0.0175	
Total	20	125.6697	Sig. of F Value:	.0000

R E G R E S S I O N S T A T I S T I C S

	Regression Coefficient	Standard Error	Student's T Value	Sig	Confidence Limits Lower	Upper
B(0)	23.34136	0.69271	33.70	.00	22.86	23.82
B(1)	2.55376	0.08415	30.35	.00	2.50	2.61

Coefficient of: Determination .981 Correlation .990

divided by Plank's constant or 2.83E+10 k_t. Because (a) is an implicit constant (can not be isolated) to calculate its value an iteration process was required.

If the basic model (Equation 15) is considered at two different reaction stages (stage 1 and 2) at a selected protein concentration, they could be expressed by Equations 40 and 41.

$$Y_1' = B(1-e^{-aTTH_1})^{\alpha} \tag{40}$$

$$Y_2' = B(1-e^{-aTTH_2})^{\alpha} \tag{41}$$

The product of the division of Equations 40 and 41 gave the following expression:

$$\frac{Y_1}{Y_2} = \frac{B(1-e^{-aTTH_1})^{\alpha}}{B(1-e^{-aTTH_2})^{\alpha}} \tag{42}$$

Equation 42 could also be written as:

$$\frac{Y_1}{Y_2} - \frac{B(1-e^{-aTTH_1})^{\alpha}}{B(1-e^{-aTTH_2})^{\alpha}} = M \tag{43}$$

where M= 0 when the iterated value of (a) is correct.

Equation 43 indicates a relationship between (a) and α, therefore, its solution for (a) requires the predetermination of α. The value of α for myofibrillar beef proteins was not found in the literature, but Morgan et al. (1987) reported that values of α for the gelation of proteins are in the range of 1 to 3. Preliminary experiments yielded tentative values of 0.52 and 0.80. Based on this, it was decided to calculate the value of (a) with these five different values of α (0.52, 0.8, 1.0, 2.0 and 3.0) and to obtain a graphic relationship between these two material constants.

The value of (a) for each α value, was calculated by an iteration computer subroutine which gave values to (a) until the equality of Equation 43 was met (tolerance 1E-14). For this purpose 20 values of Y' ranging in values from 0.54 to 3.54 and their corresponding TTH value were selected from samples processed at 64, 70, 80 and 84 °C. These Y'-TTH values were sorted and divided in two groups (ten lower and ten higher) before being utilized in Equation 43. The calculated average (a) value and (k_t) value for each preselected α is shown in Table 7.

The value of (a) was found to have a power relationship with α under the conditions of this experiment as shown in Figure 8 (CD= 0.99). This mathematical relationship is expressed by Equation 44.

Table 7. Calculation of (a) and k_t values using an average of 20 TTH values. Ea used was 20,000 cal/mol.

A LEVEL	(a)	Kt
0.53	1.87e+7	8.98e-4
0.8	1.02e+8	4.91e-3
1.0	1.63e+8	7.84e-3
2.0	3.50e+8	1.68e-2
3.0	4.60e+8	2.21e-2

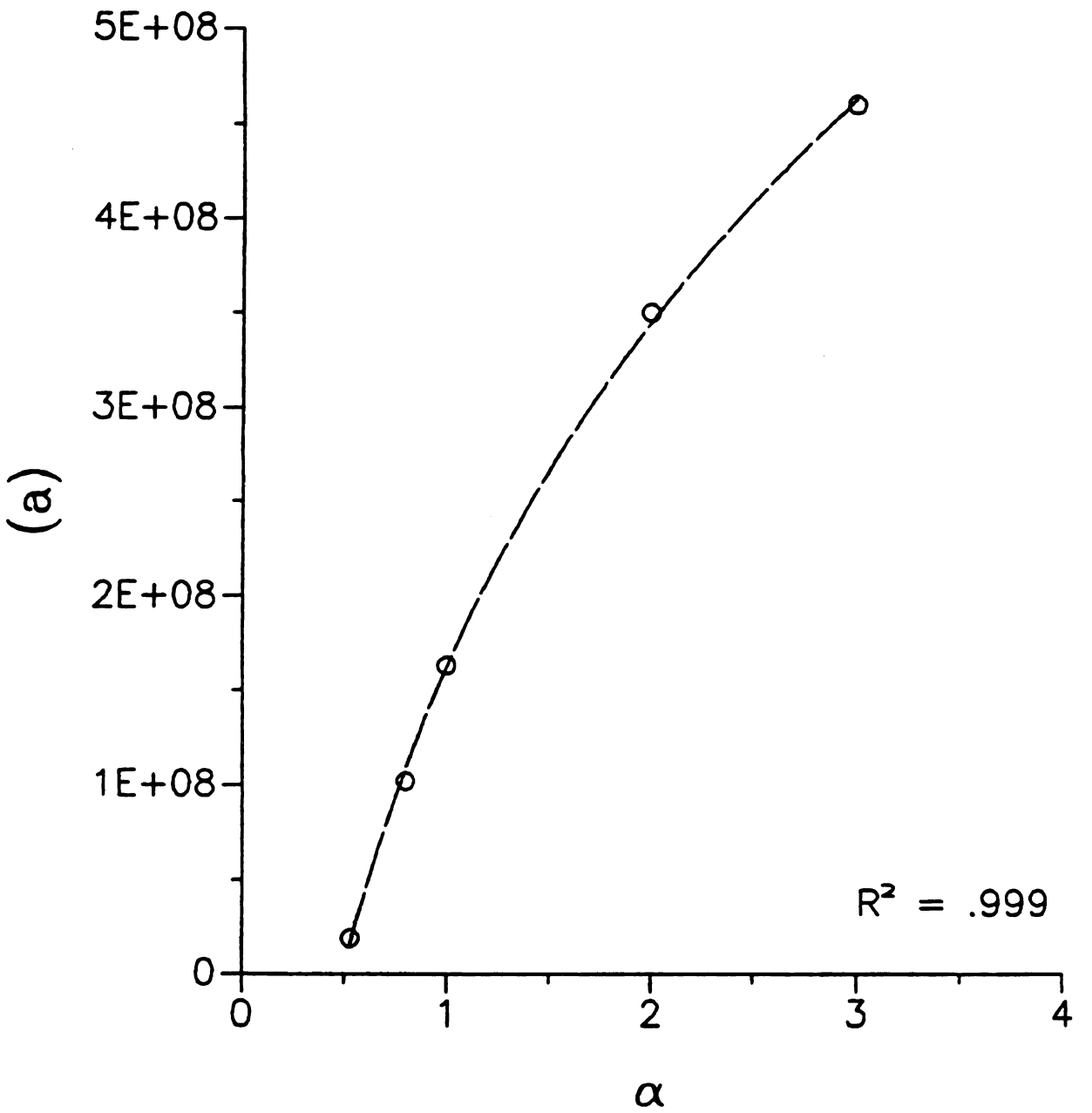


Figure 8. Correlation between α and (a).

$$a = -0.114578\ E(+10) + 0.13082\ (E+10)\ \alpha^{0.188084} \quad (44)$$

The value of α and therefore of (a) required by the mathematical model was calculated by assigning to α the predetermined values of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 5.0 and 8.0 followed by a calculation of their corresponding (a) value. A non-linear regression algorithm (Marquart, 1963) was utilized to fit the basic mathematical model (Equation 15) to experimental data using these sets of α and (a) values. The selected α-(a) combination was that which produced the best coefficient of determination.

Experimental values were obtained from five different experimental units processed at a similar temperature (70 ^{o}C) and with protein concentrations of 25.3, 31.9, 38.4, 41.5 and 46.2% (dry basis).

The plot of the R^2's determined for each set of values of α and (a) and for each protein concentration is shown in Figure 9. These results were not expected because they suggest that the values of α are a function of the protein concentration, when it was expected to be constant.

To investigate this possible correlation, the values of α which produced the best coefficient of determination

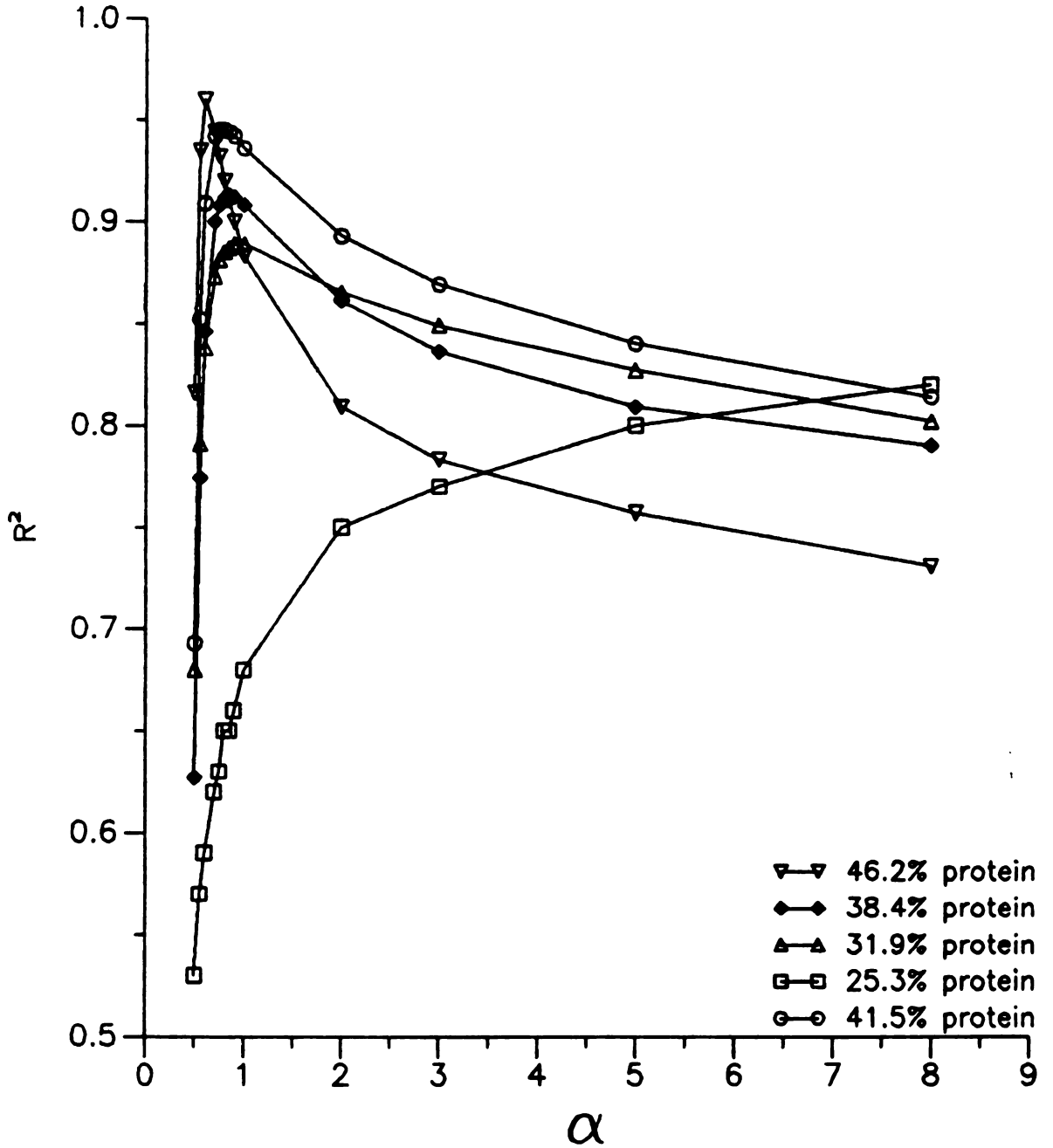


Figure 9. PLot of α versus R^2 for five protein concentrations. Protein concentration is in dry basis.

were plotted against their corresponding protein concentrations. It was found (Figure 10) that the values of α decrease exponentially as the value of protein concentration increases. This function is expressed by Equation 45.

$$\alpha = 0.734377 + 2074856\ e^{-0.4971416\,C} \qquad (45)$$

Where C is protein concentration (dry basis).

Determination of A'

The value of A' was estimated using the special case of the general model (Equation 15) for an infinite time of cooking. Under this conditions the value of the second term of the model tend to one and therefore the model could be simplified to:

$$Y'_{\infty} = A'\ C^{\alpha} \qquad (46)$$

where C is protein concentration on a dry basis and A' and α are material constants.

To calculate this parameter, data were obtained from five different experimental units thermally processed at similar time-temperatures conditions but with protein concentration on a dry basis varying between 25.3 and 44.6%.

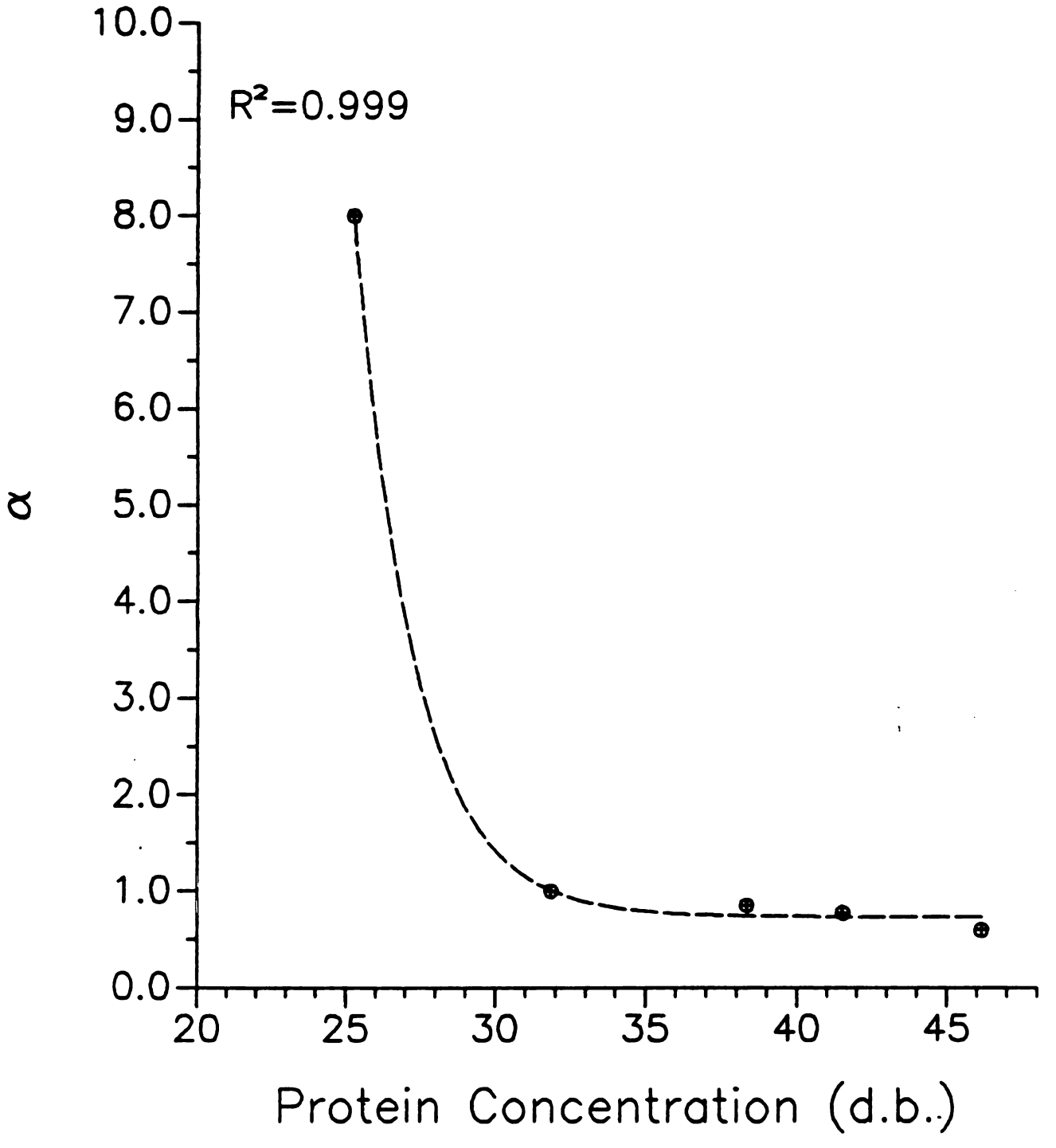


Figure 10. Correlation between α and protein concentration (d.b.).

The thermally induced BEAV was calculated as mentioned before. The experimental data was fitted to the basic model (Equation 15) and Y' at time infinite calculated using a non-linear regression algorithm (Marquard, 1963). A plot of the asymptotic values of Y' and their corresponding protein concentration (dry basis) is presented in Figure 11.

Equation 46 predicts the value of Y' at time infinite for any concentration of protein where gelation occurs and could be rearranged as:

$$A' = \frac{Y_\infty}{C^\alpha} \qquad (47)$$

Calculated values of A' were found to increase as protein concentration increased. A plot of these values (Figure 12) exhibited a A' values increasing exponentially with an increase in protein concentration. Their calculated mathematical relationship is described by Equation 48.

$$A' = 0.6154426 \left(1 - e^{-0.1049527\ C}\right)^{65.609} \qquad (48)$$

The dependency of A' on protein concentration as described in Equation 48 set the calculated TTH model for the thermal gelation of myofibrillar beef proteins as:

$$Y' = A'\ C_i^{\alpha} \left(\ 1 - e^{-a\ TTH}\ \right)^{\alpha} \qquad (49)$$

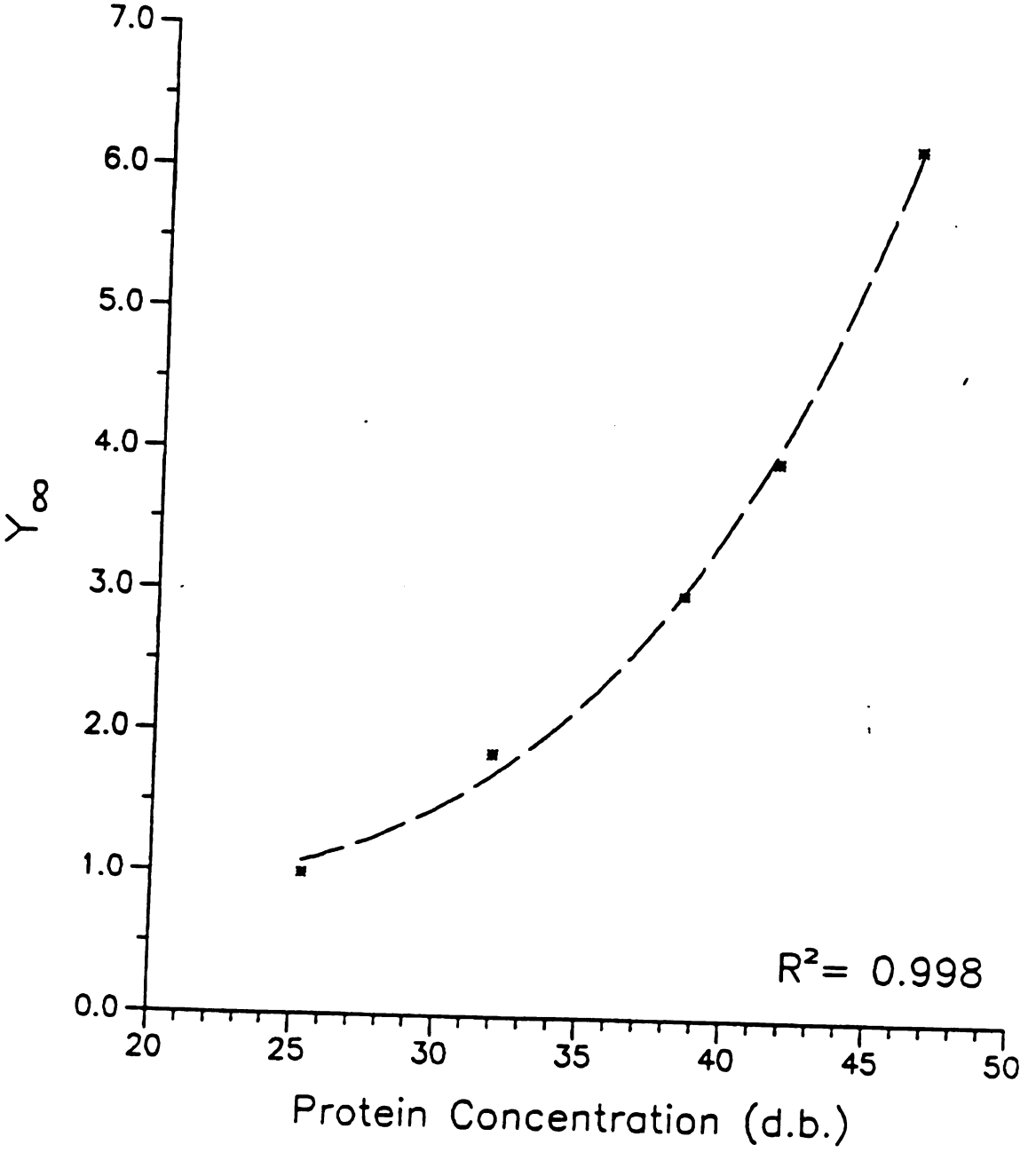


Figure 11. Correlation between protein concentration and Y'_{∞}

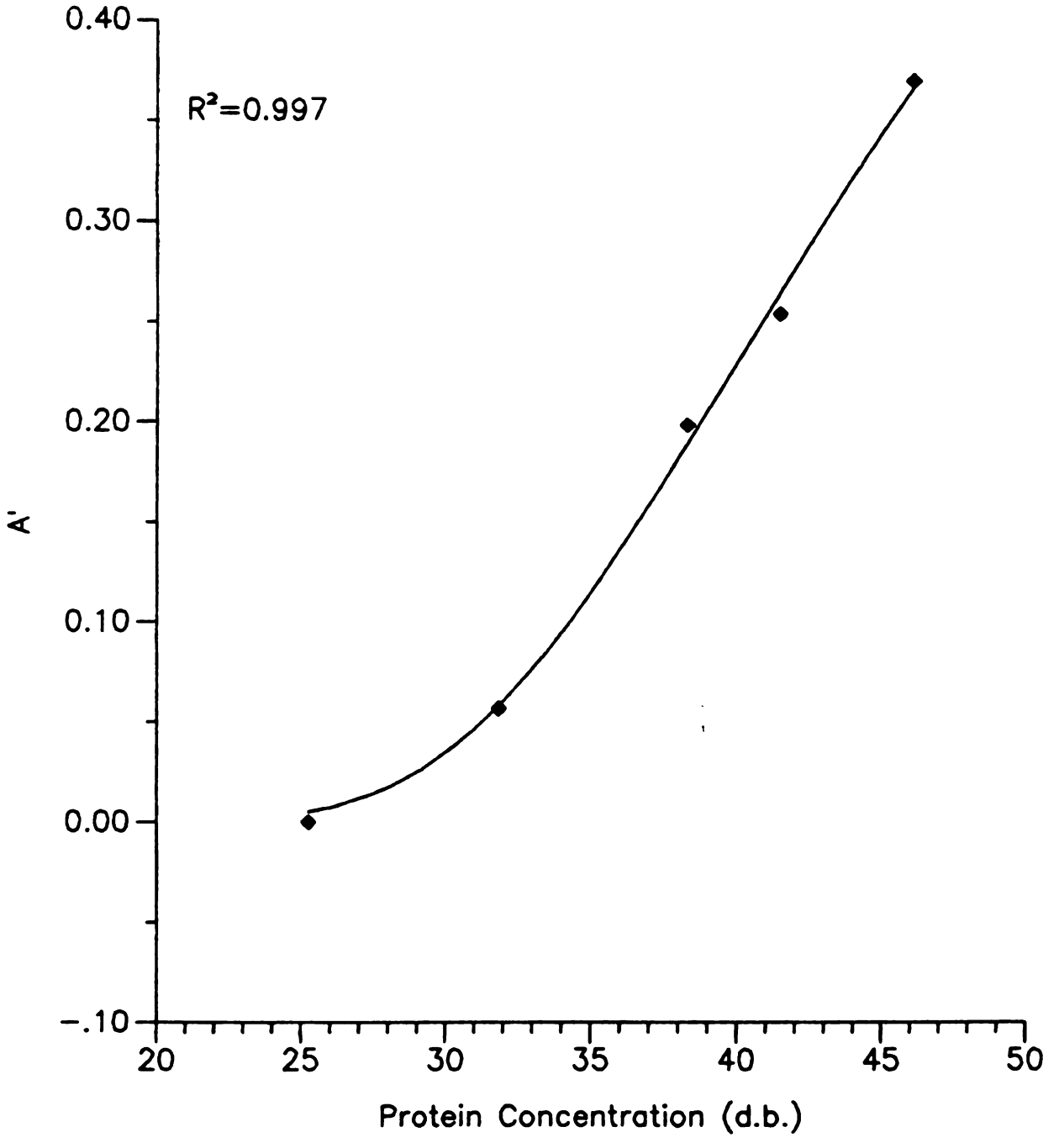


Figure 12. Correlation between protein concentration and A' (d.b.= dry basis).

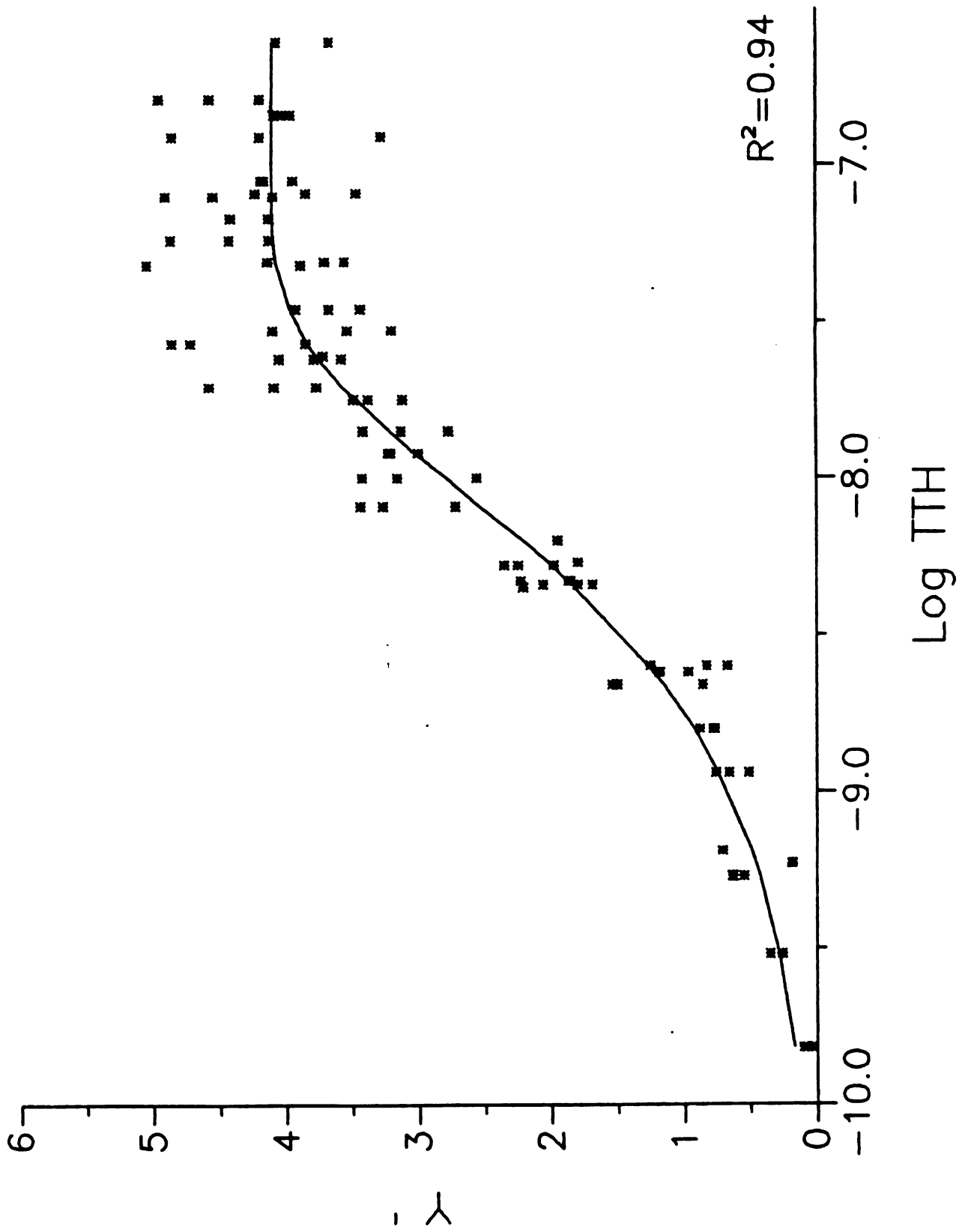
Where Y' = heat induced BEAV;
A' = as describe by Equation 48;
α = as describe by Equation 45;
a = as describe by Equation 44;
TTH = the time temperature history of the process;
C = protein concentration in dry basis.

Test and Verification of the Model

Test of the Model

The developed mathematical model (Equation 49) theoretically described the thermally induced back extrusion apparent viscosity of myofibrillar beef proteins, for samples thermally processed on the temperature range of 64 to 84°C and have a protein concentration on a dry basis between 25.6 and 44.6%. According to this model, if the protein concentration is kept constant, the model should predict the value of the thermally induced BEAV as a variable of the time-temperature history of the process. This was tested as follows: Experimental samples from five different experimental units with an approximate protein concentration of 42.5% (dry basis) were cooked at 64, 69, 71, 80 and 84 °C and their Y' calculated as described earlier. Experimental Y' values were plotted against values predicted by the TTH model. The model was found to describe reasonably well (CD=0.94) the experimental Y' values obtained from these experiments. Figure 13 shows experimental Y' values and values predicted by the model against the time-temperature history of the process (TTH).

Figure 13. Experimental Y' values for temperature range of 64 to 84 ^{o} versus values predicted by the TTH model.


R^2=0.94
Log TTH
-10.0
-9.0
-8.0
-7.0
0
1
2
3
4
5
6
- Y

A second form of testing the model was by using the protein concentration as a variable (at a constant temperature). Under these conditions the model should predict the value of Y' as a variable of the protein concentration, for any time of cooking. This was tested using three different experimental units with a protein concentrations on dry basis of 30.6, 38.4 and 46.2%. These samples were thermally processed at 69 ^{o}C. Experimental Y' values and predicted Y's were plotted against the time-temperature history of the process and found to correlate reasonable well (R^2= 0.89, 0.91 and 0.94 respectively). These correlations are shown in Figure 14.

From the information showed by Figures 13 and 14 it was concluded that under the conditions of this experiment, protein concentration on a dry basis in the range of 25.3 to 44.2% (dry basis) and a temperature in the range of 64 to 84 ^{o}C, the developed mathematical model (Equation 49) predicts the change in Y' (and presumedly the thermal gelation of myofibrillar beef proteins) as a function of the protein concentration and the time-temperature history of the process.

Verification of the Model

A basic assumption of the model developed was that the heat induced BEAV (Y') is a measure of the thermal gelation

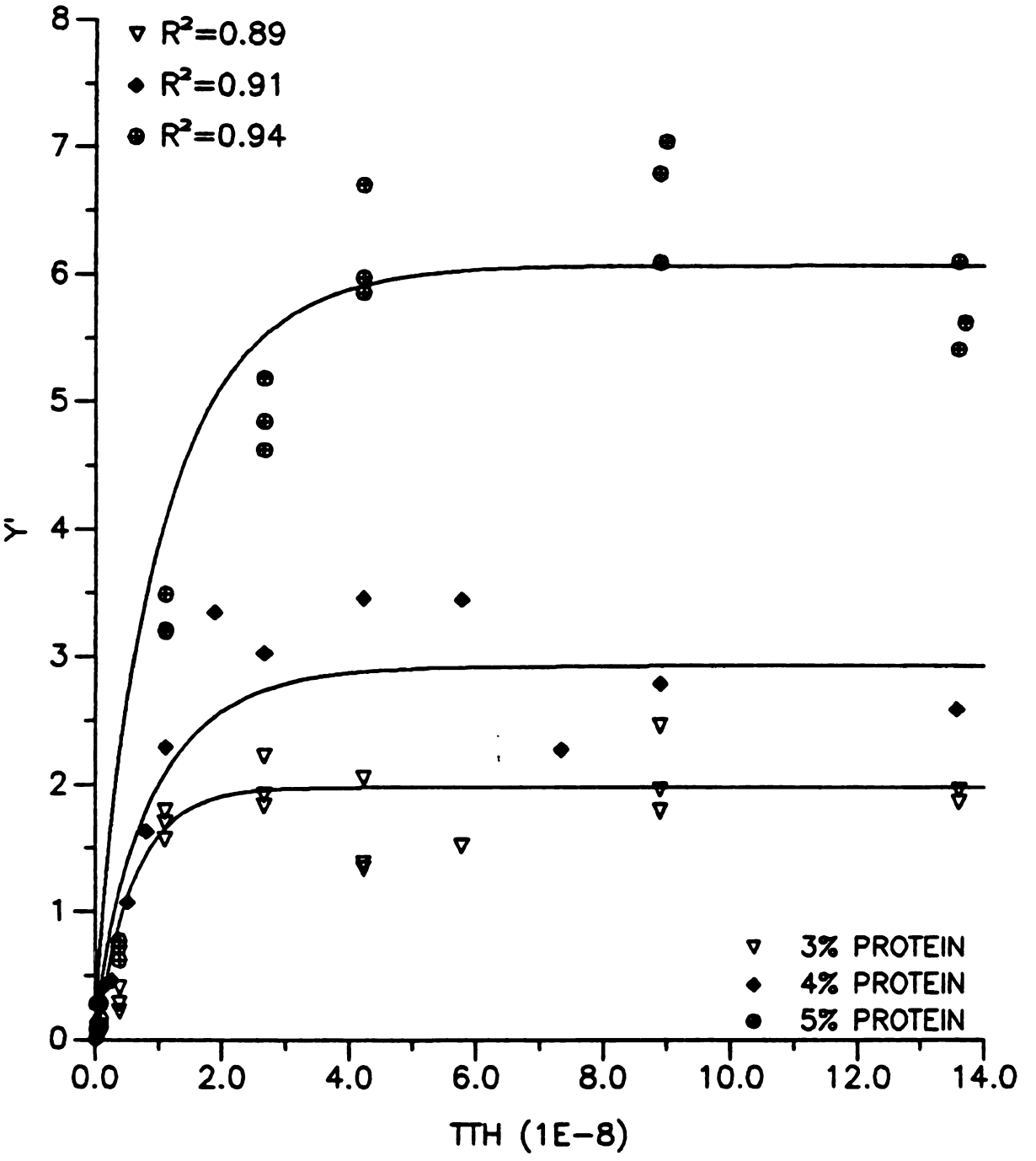


Figure 14. Experimental Y' values for three different protein concentrations versus values predicted by the TTH model.

of myofibrillar beef proteins. To verify this assumption a parallel method of measuring the gelation reaction was sought. The extent of the thermal gelation of proteins can be measured in several ways namely: loss of solubility, increase in turbidity, reduction of water holding capacity, etc. (Acton and Dick, 1984). Samejima et al. (1985) reported that the water holding capacity and gelation properties are the important factors that determine the quality of comminuted meat products. They also reported that these two properties are closely interrelated.

The water holding capacity is a measure of the water released by the gel during the cooking process. When the thermally induced protein matrix is formed, water is trapped inside of the protein network. As the thermal process continues, an increase in the points of interaction occurs with
a reduction of the interstitial space occupied by the trapped water. The net effect is a release of water which then indirectly measures the extent of the polymerization reaction.

The water released was determined on the ruptured gel/protein suspensions after gel strength testing. This technique warranted that the experimental units had the same chemical composition and experimental treatment as

those for thermally induced BEAV. The experimental samples were obtained from protein solutions thermally processed at 70 ^{o}C and with two different protein concentrations (30.6 and 38.4%, dry basis).

The trend of experimental values of water released over time of cooking (Figure 15) showed a tendency very similar to the inverse of the basic TTH - Y' model described by Equation 38. The next step was to test if the change in water released during the thermal process could be described by the model developed for Y'. Experimental data confirmed the general knowledge that water holding capacity is inversely related to protein concentration. Because Y' and water holding capacity were inversely related, the mathematical model (Equation 49) to be fitted to the water released had to be applied to the inverse of the protein concentration.

Results showed (Figure 16) that the experimental values of water released closely correlate with values predicted by the basic model. Coefficients of determination were 0.99 and 0.97 for protein concentration on a dry basis of 30.6 and 38.4% respectively.

The correlation between water released and the TTH model support the hypotesis that the thermally induced gelation of myofibrillar beef proteins is measured by the

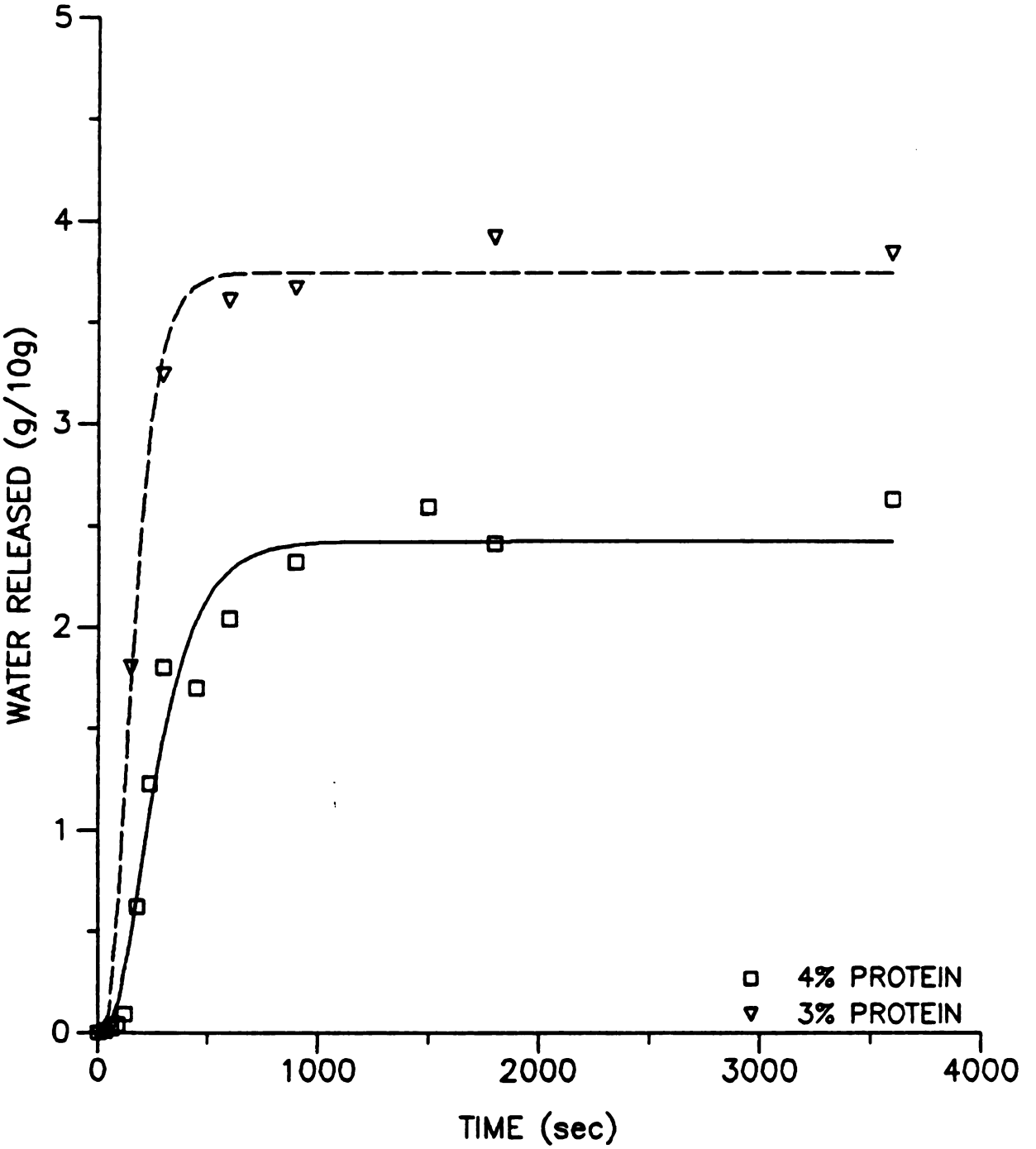


Figure 15. Relationship of water released and time of cooking for two protein concentrations.

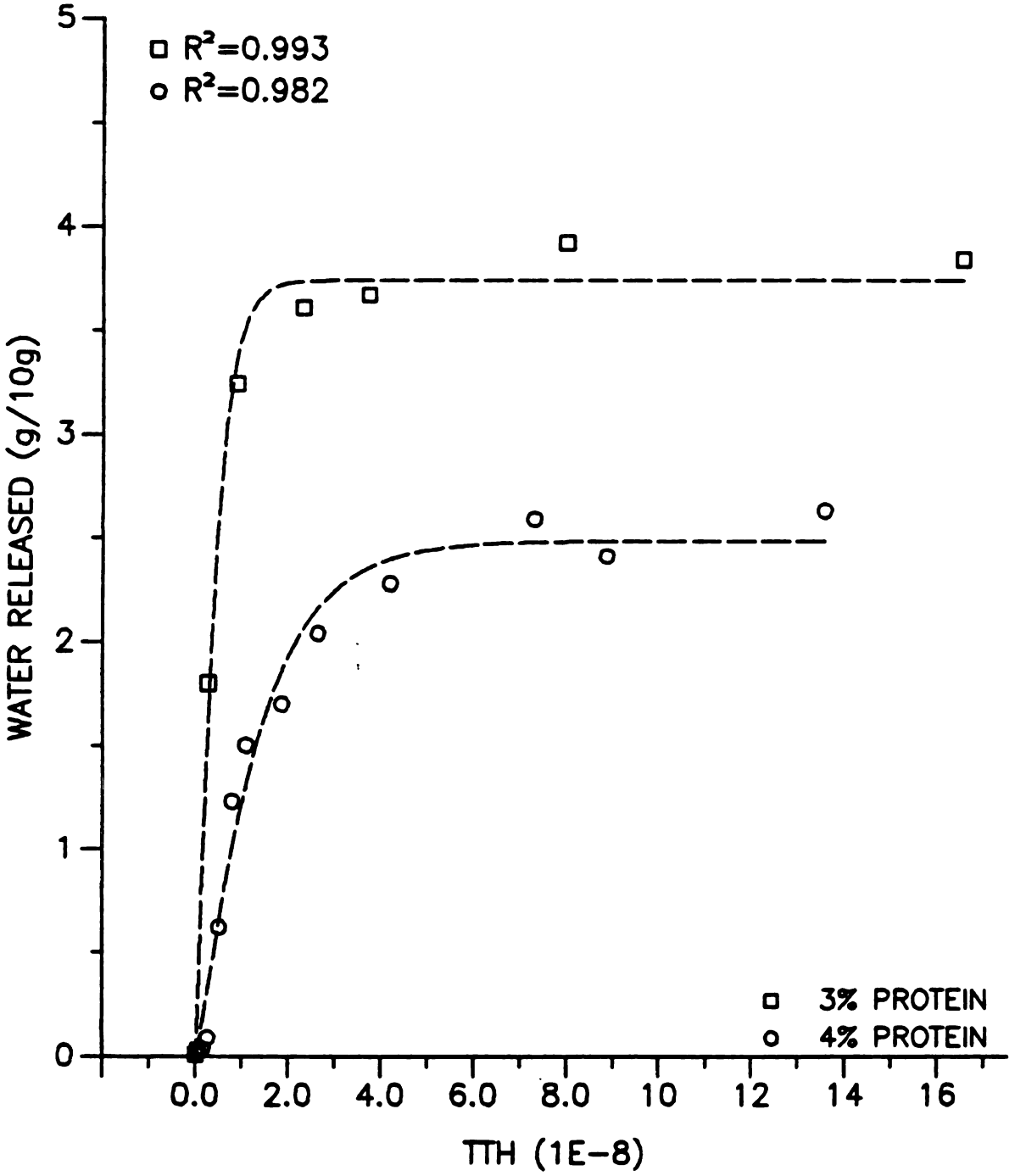


Figure 16. Experimental water released values versus values predicted by the TTH model.

Instron back-extrusion apparent viscosity. This correlation supports the hypothesis that the gelation process is a function of the protein concentration and the time-temperature history of the process. It also supports a basic premise of this experiment, i.e., The thermal gelation of myofibrillar beef proteins could be predicted by the TTH model described by Equation 49.

Effect of Vegetable Gums

It was theorized that the addition of vegetable gums to myofibrillar beef proteins solutions will have the effect of increasing the equivalent protein concentration, and therefore, alter the final three dimensional protein network of the thermally-produced gel. Vegetable gums are polysaccharides with different molecular weights, basic unit composition and level of side-branching, but in general they are more homogeneous and have more polar groups than myofibrillar proteins. These polar groups are responsible for the high water holding capacity exhibited by these compounds. They may also serve as linking points between gums and myofibrillar beef proteins, therefore, helping in the creation of the three dimensional network of the thermally-induced gel produced by myofibrillar beef proteins.

If vegetable gums interact with myofibrillar proteins they should increase the apparent viscosity induced by the thermal process. This will show up as an apparent increase in the protein concentration (C) and the degree of polymerization (A'), and produce a more orderly entanglement (reduction of α).

To test these assumptions the following experiment was carried out: An extracted myofibrillar beef protein solution with a protein concentration of 29.9% (dry basis) was divided into five lots. Each lot received 0.5% of a selected vegetable gum (carrageenan, guar, locust bean or xanthan gum) and was thermally processed at 70 ^{o}C. The TTH, Y' and water released values were then calculated as mentioned before.

Experimental Y' for all protein-gum combinations were plotted against their corresponding TTH values and the mathematical model developed for myofibrillar proteins (Equation 49) fitted to each of them. Figures 17 to 20 show plots of experimental Y' against TTH values for each gum as well as values predicted by the TTH model.

The Y' at time infinite for each protein-gum combination was calculated by fitting Equation 48 to the experimental data by means of a non-linear regression algorithm (Marquard, 1963). The equivalent protein

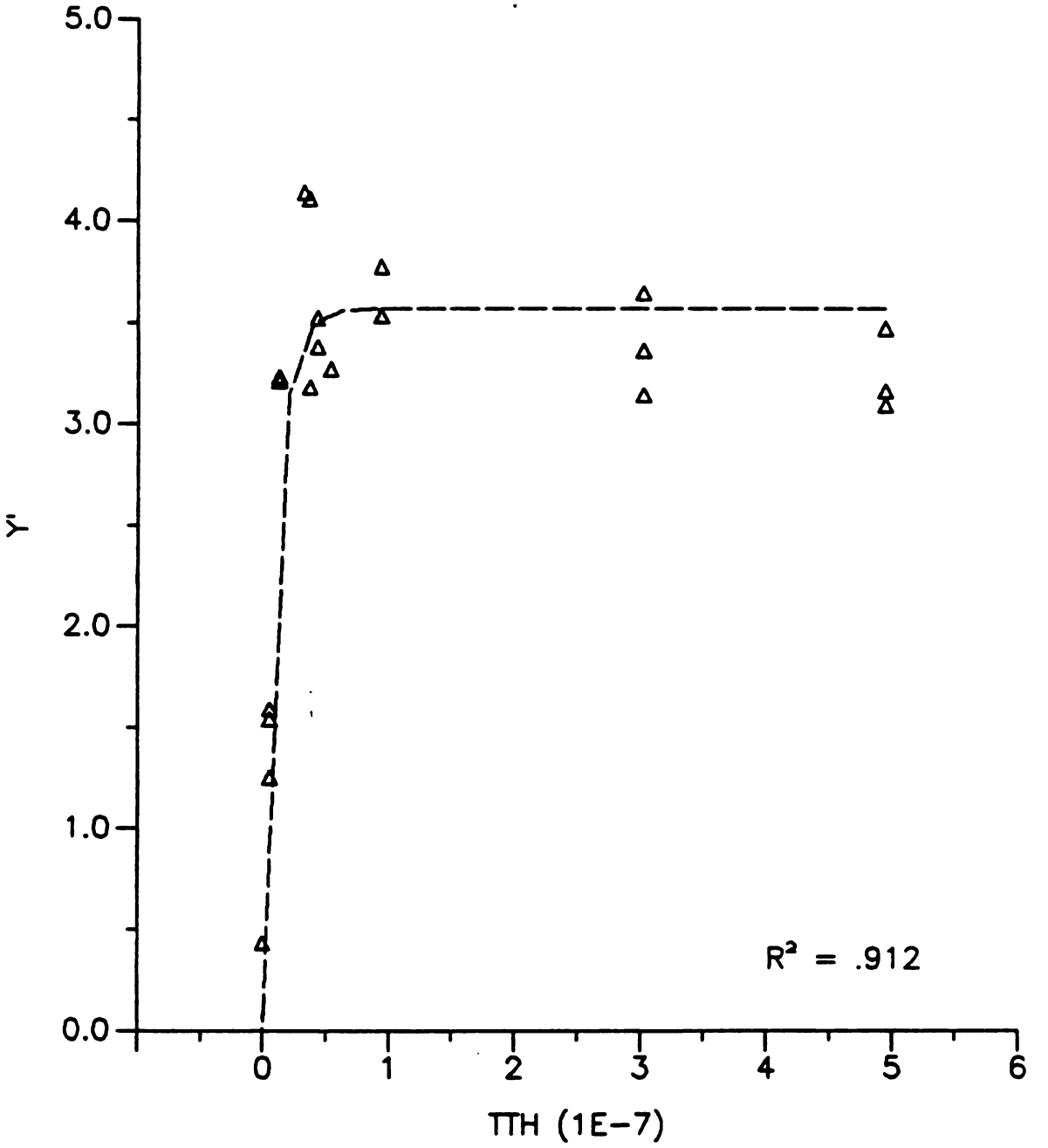


Figure 17. Experimental Y' values for carrageenan–protein solution and values predicted by the TTH model.

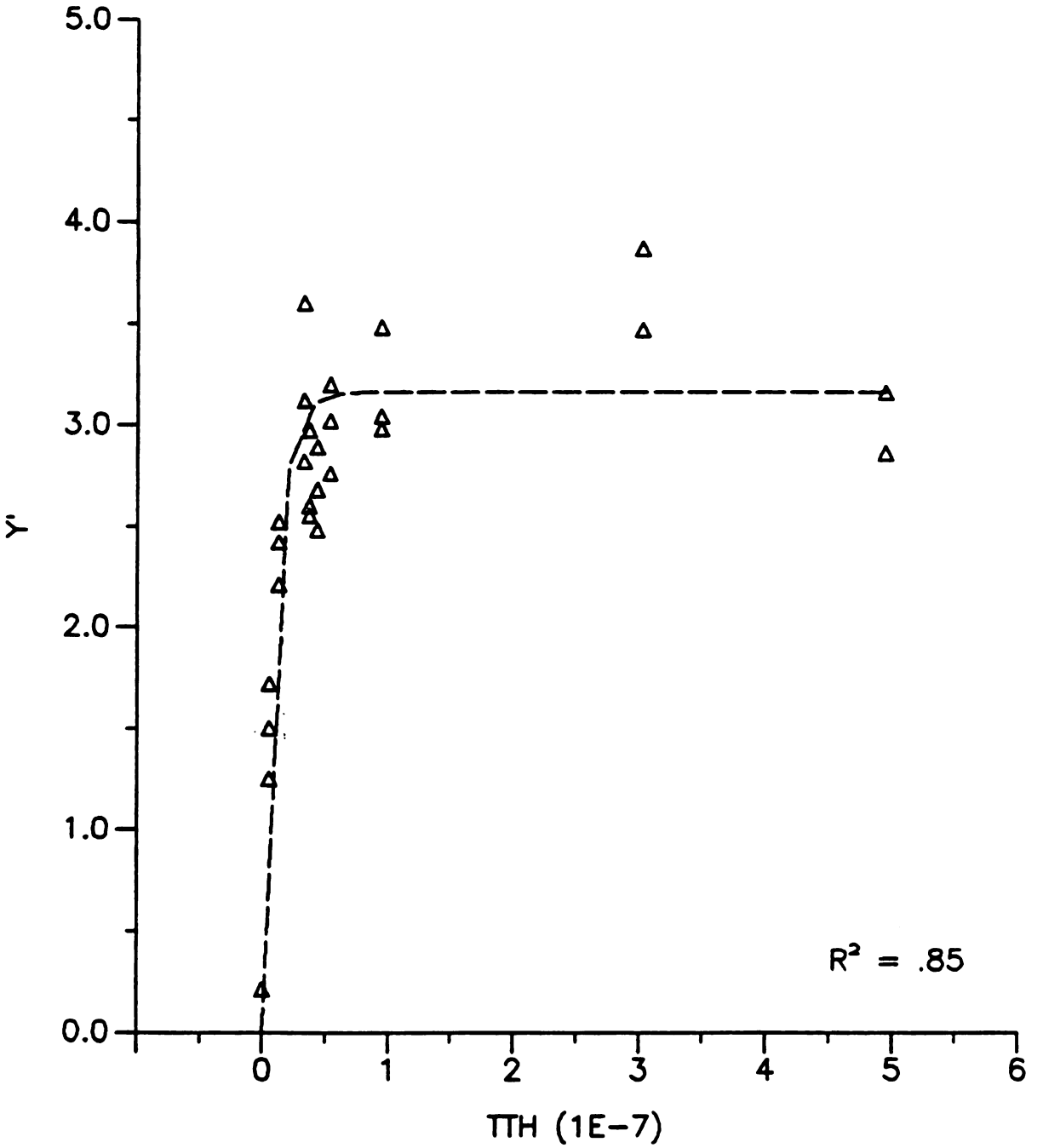


Figure 18. Experimental Y' values for guar-protein solution and values predicted by the TTH model.

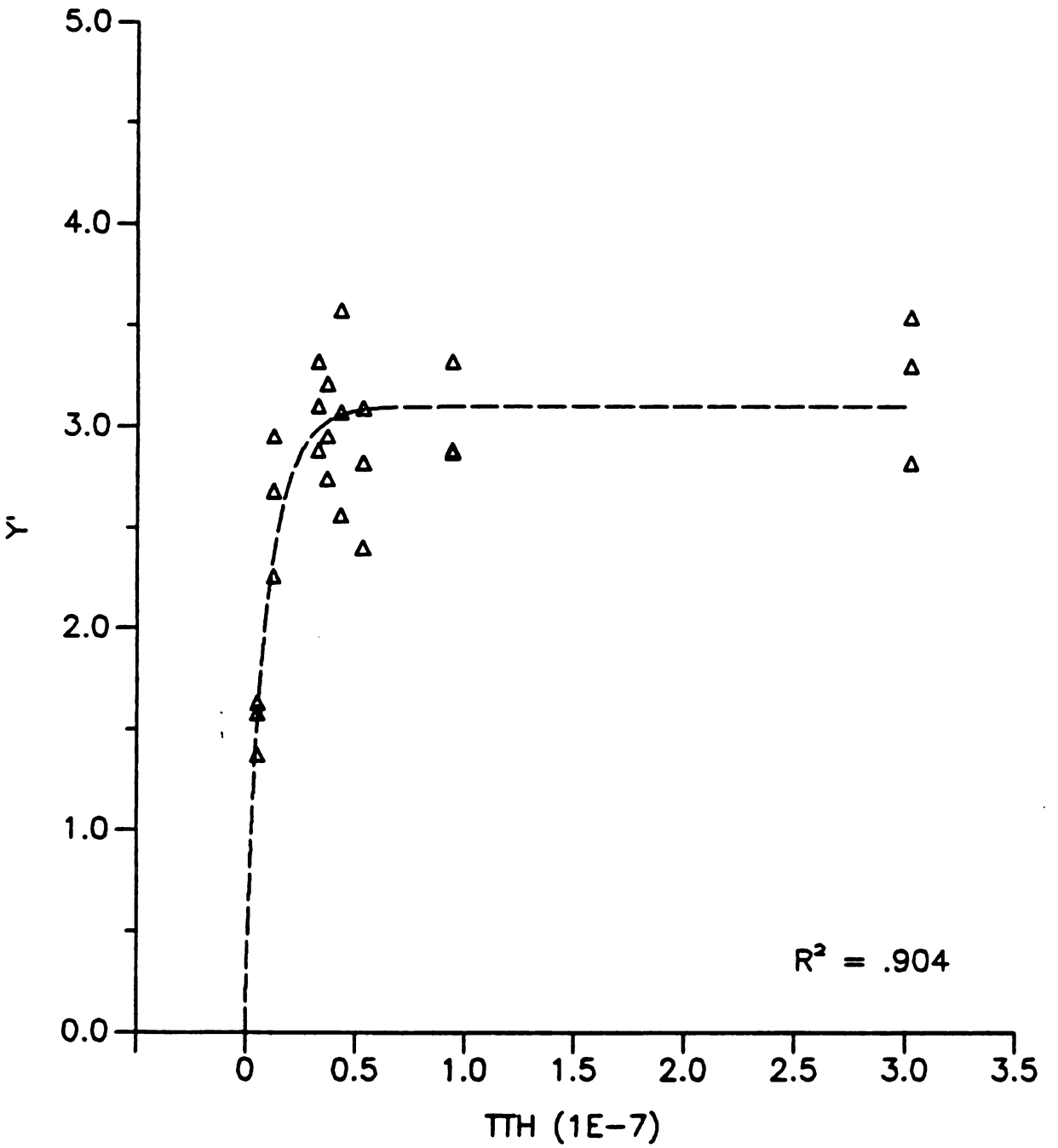


Figure 19. Experimental Y' values for locust bean-protein solution and values predicted by the TTH model.

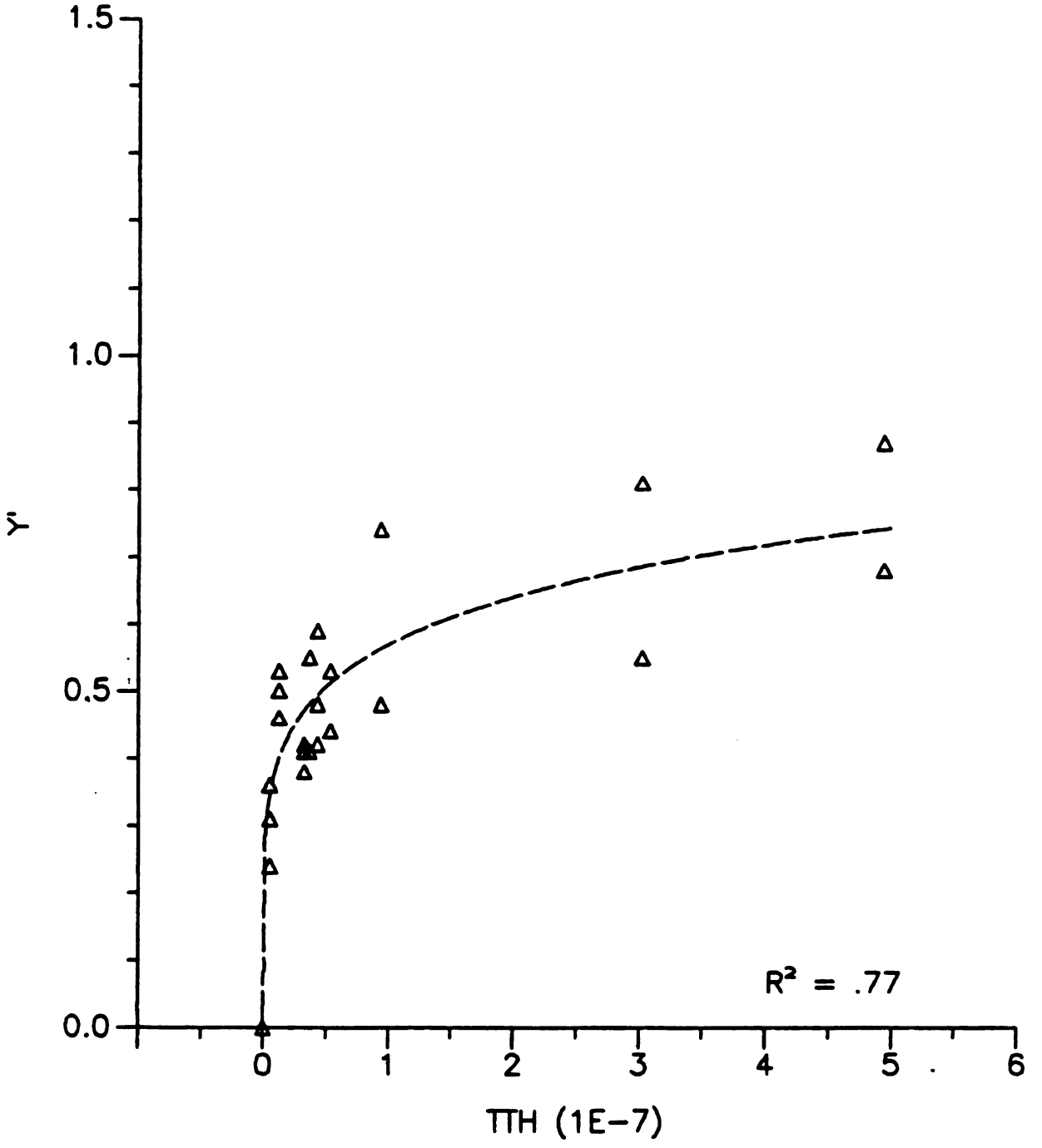


Figure 20. Experimental Y' values for xanthan– protein solution and values predicted by the TTH model.

concentration was calculated as the hypothetical protein concentration which would be required to produce the same value of Y' after an infinite time of cooking.

The calculated equivalent protein concentrations were used to determine their corresponding A' and α values using Equations 45 and 48. Calculated equivalent protein concentration, A' and α for the protein-gum solutions are presented in Table 8. Relative change in α as affected by the different protein-gum combinations is shown in Figure 21.

Carrageenan gum was found to contribute to the protein-gum system by increasing the strength of the cooked gel by 184.8% over that produced by the control. This meant an increase in equivalent protein concentration of 121.2% and a concomitant reduction of α of 19.4% (Table 8).

Carrageenan gum increased the water holding capacity by a 130.7% over the control but had the lowest effect on water holding capacity of any of the gums evaluated in this experiment (Figure 22). This agrees in part with Wallingford and Labuza (1983) who reported that carrageenan gum had the second highest water binding capacity (WBC) of nine gums in low fat meat emulsions studied. Foegeding and Ramsey (1986) reported that carrageenan gum used at 1% with a 13% protein meat batter increased the force to fracture (FF), the hardness (Hi) and the water holding capacity of

Table 8. Calculated values of equivalent protein concentration, α and A' for selected gum-protein combinations.

GUM	Y'	C+	α	A'
Control	1.82675	32.66	0.9186	0.0706
Carrageenan	3.37584	39.58	0.7403	0.2180
Guar	3.54221	40.11	0.7389	0.2305
Locust bean	3.02590	38.39	0.7451	0.1894
Xanthan	0.77900	7.76	43795	1.326E-17

+ Concentration of protein (dry basis)

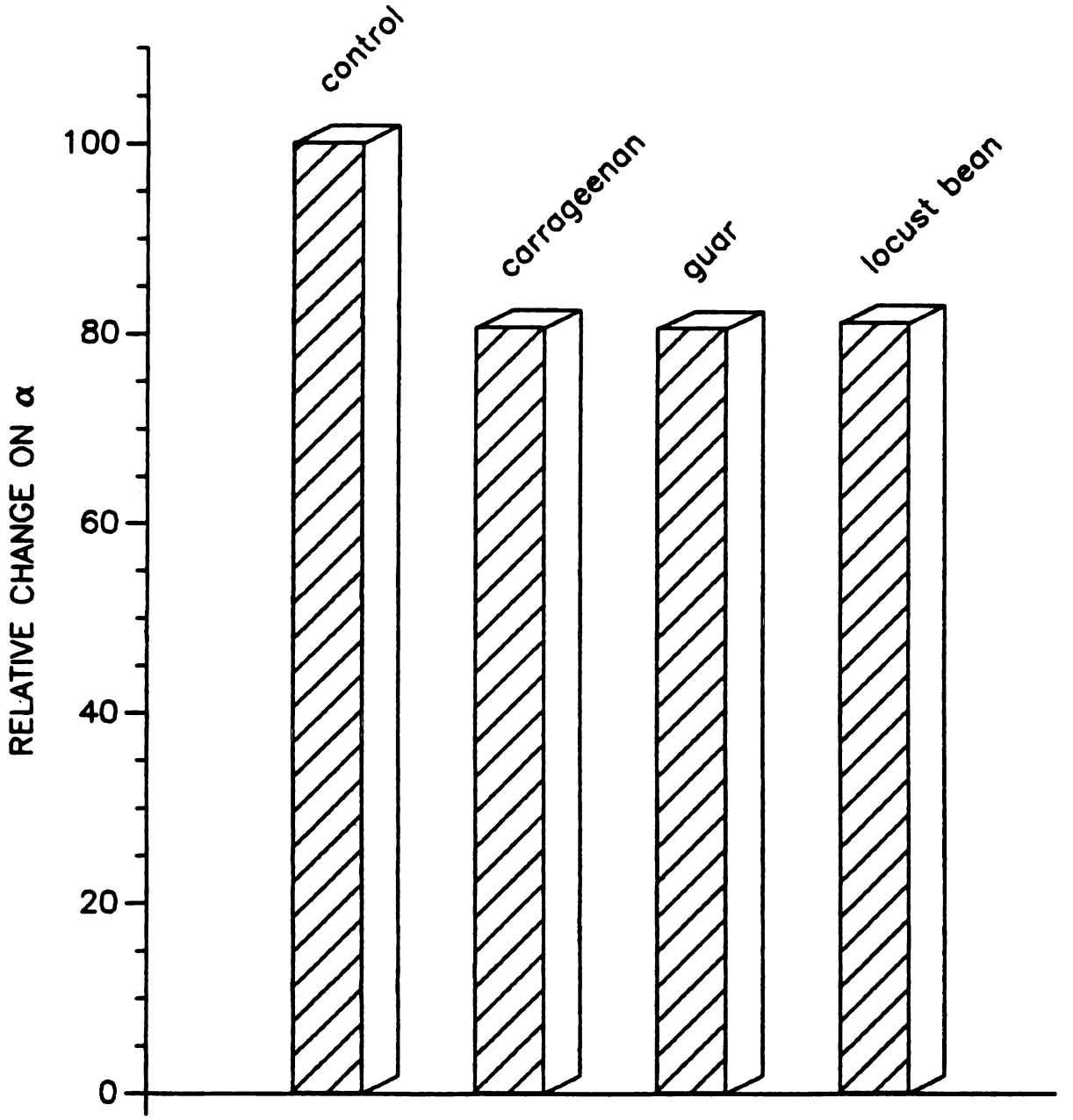


Figure 21. Effect of protein–gum combination on α.

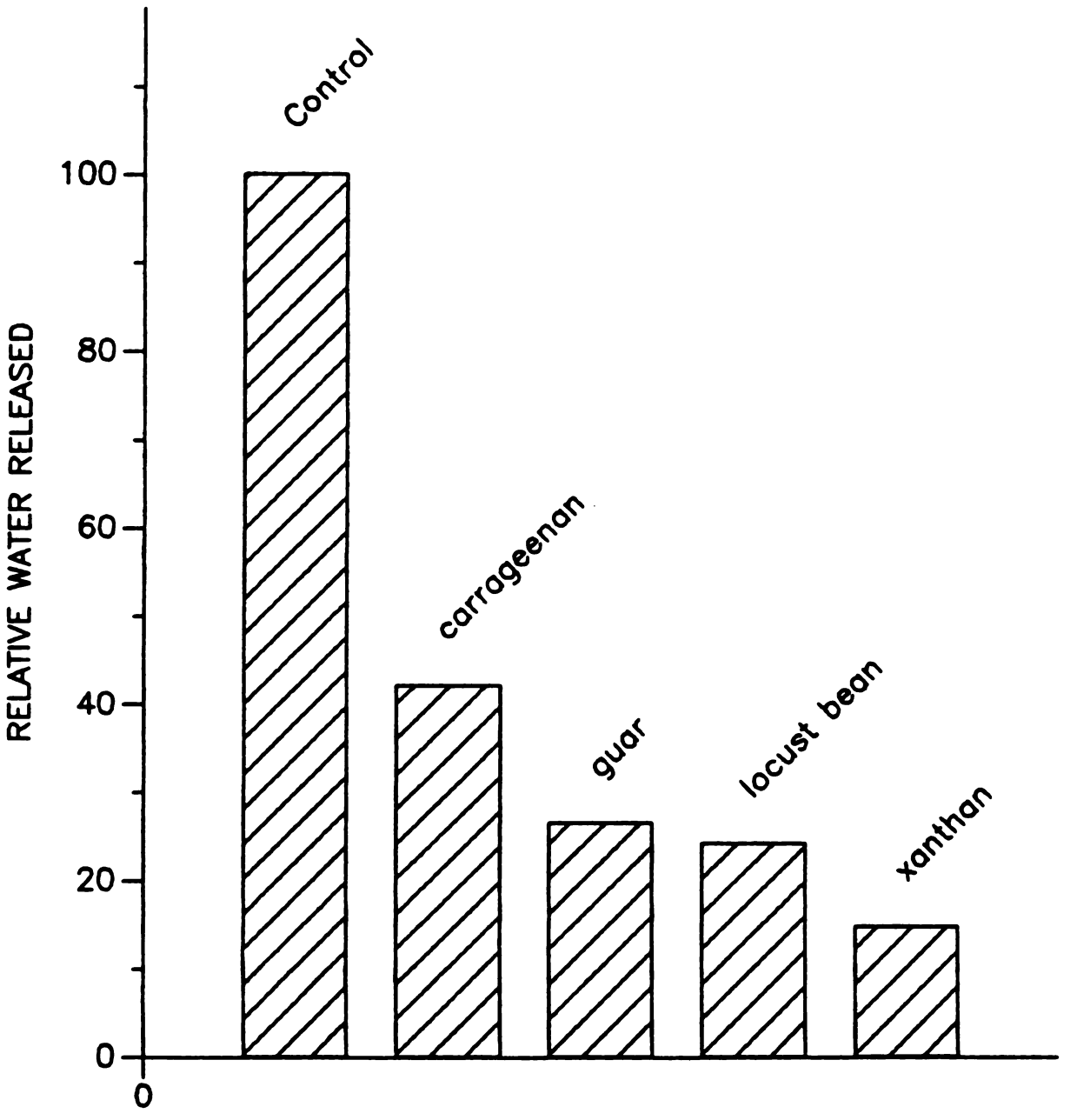


Figure 22. Water released by four gum–protein combinations (75 min at 70°C).

frankfurters. They concluded that among other gums studied, carrageenan was the most beneficial in manufacturing low fat frankfurters. When calculated model constants were introduced in the TTH model it was found that the TTH model predicted reasonably well (R^2=0.912) the change of Y of this protein-gum solution as a function of the time-temperature history of the process, as shown in Figure 17.

Guar gum increased the strength of the cooked gel by 193.9% over that produced by the control. The protein samples containing guar gum had the highest Y' among the gum-protein combinations used in this experiment. The increase in equivalent protein concentration was 122.8% whereas α was decreased by 19.7% (Table 8).

Guar gum increased the water holding capacity by 138.94% over the control and was ranked third. This seems to disagree with Wallingford and Labuza (1983) who reported that guar gum had approximately two-thirds of the WBC of carrageenan, but because carrageenan gum is not a single homogeneous compound but rather a heterogeneous mixture of several different polysaccharides, it is possible that their sample was different than the one used in this experiment. Foegeding and Ramsey (1986) reported that the addition of guar gum to low fat frankfurters reduced force to fracture (FF) and hardness by almost 50%. While our results appear

not to support their findings, a direct comparison between studies is difficult because variations in ingredients and techniques. The calculated TTH model for the protein-guar solution acceptably predicted (R^2= 0.85) the change of Y' over time of cooking as shown in Figure 18.

Locust bean gum increased the strength of the cooked gel by 165.6%. This value is lower than that produced by carrageenan and guar gums but this gum still produced an increase in equivalent protein concentration of 117.5%, with a concomitant reduction in α of 18.9% (Table 8).

Locust bean gum increased the water holding capacity the protein-gum solution by 140.19% (Figure 22). The increase in water-retained during the cooking process induced by this gum was slightly lower than that produced by xanthan gum. This result supports the findings of Foegeding and Dayton (1986) who reported that xanthan and locust bean gums produced the lowest weight loss during cooking among several meat-gums combinations. The calculated mathematical model described reasonably well (R^2= 0.904) the change in Y' due to the time-temperature history of the process as indicated in Figure 19.

Xanthan gum was found to behave as an inhibitor of the gelling phenomenon. Gel strength of the xanthan-protein solution was found to be only 42.6% of that produced by the control. This value of Y' is outside of the limits of the mathematical model described by Equation 49 and therefore the equivalent protein concentration (70.8%) and α (93.3%) were calculated only as an exercise (Table 8).

The water holding capacity of the xanthan gum-protein solution was the best of all experimental units (Figure 22) showing an increase of 145.3% over the control. Similar findings have been reported by Whiting (1984), who found that xanthan gum added at 0.1 or 0.3% decreased cooking losses and gel strength. It also agrees with Wallingford and Labuza (1983) who described xanthan gum as the best water binder among several gums studied.

SUMMARY AND CONCLUSIONS

The objectives of this study were to develop a mathematical model for predicting the effects of time-temperature history and protein concentration in the thermal gelation of myofibrillar beef proteins as well as the interaction of these proteins with selected hydrocolloids.

Thermal gelation of myofibrillar beef proteins was measured as the thermally-induced Instron back-extrusion apparent viscosity (Y'). Water holding capacity was used as parallel method of measuring gelation and to verify the model developed for Y'.

The developed mathematical model has an Ea for the thermal gelation of myofibrillar beef proteins of 20,000 cal/mol. Basic model constants (A', a and α) were found to be mathematically related to protein concentration, these mathematical relationships were developed and integrated into the basic model.

Experimental values of Y' and WHC obtained under the

conditions of this experiment support the hypothesis that the proposed mathematical model (Equation 49) can be used to describe the thermally-induced gelation of myofibrillar beef proteins as a function of the protein concentration (dry basis) and the time-temperature history of the process.

It was also found that the TTH model could be used to predict the water released during the thermal treatment as a function of the protein concentration and the time-temperature history of the process.

The TTH model was shown to describe reasonably well the thermal gelation of solutions of myofibrillar beef proteins and selected vegetable gums. Under the conditions of this experiment xanthan gum was found to inhibit gelation, and therefore, the TTH model could not be applied to this protein-gum solution.

More research is needed to study the effect of other ingredients normally found in meat products (salts, lipids, connective tissue, etc.) in the mathematical model, as well as the relationship between back-extrusion apparent viscosity and sensorial attributes of thermally gelled meat products.

Fundamental knowledge of effects of temperature-time history in meat gels should enhance the understanding of the reaction kinetics involved in the thermally-induced gelation process. This TTH model can be useful in studying and predicting effects of process conditions on product quality and significantly reduce experimental cost and time.

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APPENDICES

APPENDIX A. List of the computer program Rodrigo.

```
100 REM  "***    PROGRAM RODRIGO   ***"
200 CLS @ DISP " PROGRAM RODRIGO BY CARLOS A. LEVER"
300 REM  "* Program to Calculate Area, Apparent Viscosity,
    Apparent Elasticity and Shear Rate at the TWS from
    Back     Extrusion Data  *"
400 DISP "TURN POWER ON" @ GOSUB 9900 @ CLEAR
500 REM **** INSTROM CONTROL COMMANDS ****
600 DISP "Attach Plunger, set distance from envil= 3 cm. " @
    GOSUB 9900 @ CLEAR
700 DISP "Press S1 then 0 <ENTER>" @ GOSUB 9900 @ CLEAR
800 DISP "Set Recorder Paper, Turn on line, CHart & servo."
    @ GOSUB 9900 @ CLEAR
900 DISP "Press LOAD CAL <ENTER>" @ GOSUB 9900 @ CLEAR
1000 DISP "Press LOAD BAL <ENTER> " @ GOSUB 9900 @ CLEAR
1100 DISP "Press IEEE <ENTER> " @ GOSUB 9900 @ CLEAR
1200 PRINTER IS 701
1300 DIM SAMPID$[80],SAM$[15]
1400 DIM A(200),B(200),C(200)
1500 DIM AA(200),CC(200),S(8)
1600 DISP "CALIBRATION IS MANUAL, IF YOU WANT TO MAKE ANY
     CHANGE"
1700 SET TIMEOUT 7;10000
1800 ON TIMEOUT 7 GOTO 9200
1900 GOSUB 9900
2000 I=1
2100 CCP=0
2200 AO=704
2300 CLEAR
2400 GOSUB 25800
2500 OUTPUT AO ;"K25,-30K32,3K24,2.5K31,3"
2600 OUTPUT AO ;"K21K26,0.0K27,-30.0"
2700 REM"***PRINTER CONTROL COMMANDS ***"
2800 DISP "Select % of Printer Scale Desire"
2900 DISP "20 = 20%", "10 = 10%","5 = 5%"
3000 DISP "ENTER #"
3100 INPUT NUMBER
3200 IF NUMBER=20 THEN GOTO 3600
3300 IF NUMBER=10 THEN GOTO 3800
3400 OUTPUT AO; "K13,20K15,6K19,5K20,2"
3500 GOTO 4000
3600 OUTPUT AO ; "K13,20K15,4K19,5K20,2"
3700 GOTO 4000
3800 OUTPUT AO; "K13,20K15,5K19,5K20,2"
3900 GOTO 4000
4000 OUTPUT AO ;"K34,3"
4010 DISP "Enter Sample Identification Name (Up to 40
     characters)"
4015 INPUT SAMPID$
```

APPENDIX A. (continued)

```
4100 CLEAR
4105 DISP "Do you Want to Change Printer Scale"
4200 DISP "Yes=1","No=2"
4300 INPUT BB
4310 IF BB=1 OR BB=2 THEN 4400 ELSE BEEP 400,40
4320 DISP "ENTER 1 OR 2 ONLY" @ GOTO 4105
4400 CLEAR
4500 IF BB=1 THEN 2700
4600 DISP @ DISP "Ready to Start" @ BEEP @ DISP
4700 DISP "Enter Sample Number and Time    ( Up to 10
     characters)"
4800 INPUT SAM$
5000 I=1
5100 A0=704
5200 OUTPUT A0 ;"K25,-30K32,3K24,2.5K31,3"
5300 OUTPUT A0 ; "K2"
5400 CLEAR
5500 OUTPUT A0 ; "R2R27R3"
5600 ENTER A0 ; A(I),B(I),C(I)
5700 I=I+1
5800 IF I>170 THEN GOTO 6100
5900 WAIT 397
6000 GOTO 5500
6100 OUTPUT A0 ; "K1"
6200 GOT 10300
6300 GOTO 6800
6400 DISP "YES=1", "NO=2"
6500 INPUT BB
6600 CLEAR
6700 IF BB=2 THEN GOTO 8000
6800 REM ***** SAVING CONTROL PROGRAM *****
6900 DISP "Insert Disk to Store The Data. **INITIALIZED**"
7000 DISP "Enter File Name    (Up to 8 characters)"
7100 INPUT FILN$
7200 CREATE FILN$,200,50
7300 ASSIGN# 1 TO FILN$
7400 PRINT# 1 ; SAMPID$
7450 PRINT# 1 ; SAM$
7500 FORI=1 TO 170
7600 PRINT# 1 ; A(I),B(I),C(I)
7700 NEXT I
7800 GOTO 7900
7900 ASSIGN# 1 TO *
8000 DISP "Do you Want to Repeat the Test?"
8100 DISP "YES=1","NO=2"
8200 INPUT BB
8300 CLEAR
8400 IF BB=2 THEN GOTO 9100
8500 DISP "Please WAit"
8600 FOR NN=1 TO 170
```

APPENDIX A. (continued)

```
8700 A(NN),B(NN),C(NN)=0
8800 AA(NN),CC(NN)=0
8900 NEXT NN
9000 GOTO 4100
9100 END
9200 FOR TT=0 TO 6
9300 STATUS 7,TT ; S(TT)
9400 PRINT "STATUS BYTE #";TT;"    ="S(TT)
9500 NEXT TT
9600 PRINT "HP-IB Timeout"
9700 GOTO 9100
9800 ! SUBROUTINE: WAIT FOR <K1> TO BE PRESSED..
9850 BEEP 4000,40 @ DISP "ENTER K1 KEY ONLY!"
9900 DISP "When ready, Press <k1> to Continue."
10000 ON KEY# 1 GOTO 10200
10100 GOTO 10100
10200 RETURN
10300 U=0
10400 PRINTER IS 701
10500 FOR I=1 TO 170
10600 AA(I)=ABS (A(I) @ CC(I)=ABS (C(I)
10700 NEXT I
10800 CLEAR
10900 IF CCP=1 THEN 11500
11000 IF CCP=2 THEN 12900
11100 DISP "Do you Want The Computer To EStimate Le and Lp"
11200 DISP "Yes=1","No=2"
11300 INPUT CCP
11400 IF CCP=2 THEN 12900
11500 REM *** Program to Calculate Le and Lp ***********
11600 FOR I=1 TO 170
11700 IF A(I)<-.03 THEN GOTO 11900
11800 NEXT I
11900 R=I-2
12000 IPV=CC(I-2)
12100 FOR I=I TO 170
12200 IF A(I)<A(I+1) THEN 12400
12300 NEXT I
12400 BFV=CC(I)
12500 PF=AA(I)
12600 LPV=CC(165)
12700 I=1
12800 GOTO 18100
12900 DISP "ALL DATA WILL BE DISPLAY AS:"
13000 DISP
13100 DISP "Force (N)","Distance (mm)","Sequential Number"
13200 DISP
13300 DISP "Please take note of:"
13400 DISP "-Sequential Value of Initial Distance Value
      (mm)"
```

APPENDIX A. (continued)

```
13500 DISP "- Sequential Value of Break Points Distance and
      Force"
13600 DISP
13700 DISP
13800 DISP "To continue Press <k1>
13900 ON KEY# 1 GOTO 14100
14000 GOTO 14000
14100 CLEAR
14200 M=20
14300 PAGESIZE 24
14400 DISP "Force (N)","Distance (mm)","Sequential Number"
14500 FOR I=1 TO M
14600 DISP A(I),C(I),I
14700 NEXT I
14800 DISP "Do You Want To See More Values ?","Yes=1";"
      No=2"
14900 INPUT BB
15000 IF BB=2 THEN 16300
15100 CLEAR
15200 IF I>160 THEN 15900
15300 M=M+20
15400 DISP "Force (N)","Distance (mm),"Sequential Number"
15500 FOR I=I TO M
15600 DISP A(I),C(I),I
15700 NEXT I
15800 GOTO 14800
15900 M=M+10
16000 CLEAR
16100 IF I=170 THEN 16300
16200 GOTO 15400
16300 CLEAR
16400 M=M+10
16500 PAGESIZE 16
16600 DISP "DO YOU WANT TO SEE THE DATA AGAIN"
16700 DISO "YES=1", "NO=2"
16800 INPUT BB
16900 IF BB=1 THEN 14200
17000 CLEAR
17100 DISP "Sequential Value of Initial Distance Value=?"
17200 INPUT I
17300 IPV=CC(I)
17400 R=I
17500 CLEAR
17600 DISP "Sequential Number of Break Distance Value=?"
17700 INPUT I
17800 BFV=CC(I)
17900 PF=AA(I)
18000 LPV=CC(165)
18100 DISP "Please Wait Area CAlculations in Progress"
18200 U=U+1
```

APPENDIX A. (continued)

```
18300 SUM1=0
18400 FOR I=R TO 165
18500 SUM1=SUM1+AA(I)*(CC(I+1)-CC(I-1))*.5
18600 NEXT I
18700 FI=(AA(R-1)*(CC(R)-CC(R-1))+AA(166)*(CC(166)-
      CC(165)))*.5
18800 AREA (U)=SUM1+FI
18900 GOSUB 25000
19000 CLEAR
19100 DISP SAMPID$;" ";SAM$
19200 DISP "Area=";AREA (U);"mm^2"
19300 DISP
19400 DISP "Plunger Velocity is="; B(2);"mm/min"
19500 DISP
19600 DISP "Apparent Viscosity is="; VI;"poise"
19700 DISP
19800 DISP "Apparent Elasticity is=";EA;"N/cm^2"
19900 DISP
20000 DISP "Shear Rate at the PWS is=";LW;"1/sec"
20100 DISP
20200 REM
20300 DISP "Do you Want to Print These Values?"
20400 DISP "Yes=1","No=2"
20500 INPUT BB
20600 IF BB=2 THEN 23200
20700 CLEAR
20800 DISP "PRINTIING IN PROGRESS, PLEASE WAIT" @ DISP
20900 DISP "PRINTING IN PROGRESS, PLEASE WAIT"
21000 PRINT "**************************************"
21100 PRINT
21200 PRINT SAMPID$; " ";SAM$
21300 PRINT
21400 PRINT "Area=";AREA (U);"mm^2"
21500 PRINT
21600 PRINT "Plunger velocity is =";B(2);"mm/min"
21700 PRINT
21800 PRINT "Apparent Viscosity is="VI;"poise"
21900 PRINT
22000 PRINT "Apparent Elasticity is=";EA;"N/cm^2"
22100 PRINT
22200 PRINT "Shear Rate at the PWS is=";LW;"1/sec"
22300 PRINT
23100 PRINT
23200 CLEAR
23300 REM
23400 DISP "Do You Want to Print the Raw Data?"
23500 DISP "YES=1","NO=2"
23600 INPUT BB
23700 IF BB=2 THEN 24700
23800 DISP "PRINTING IN PROGRESS,PLEASE WAIT"
```

APPENDIX A. (continued)

```
23900 PRINT"********************************"
24000 PRINT
24100 PRINT SAMPID$
24200 PRINT
24300 PRINT "Force (N)","Distance (mm)","Sequential Number"
24400 FOR I=1 TO 170
24500 PRINT A(I),C(I),I
24600 NEXT I
24700 CLEAR
24800 GOTO 6300
24900 END
25000 CLEAR
25100 DISP "Please Wait Viscosity Calculations in Progress"
25200 REM **BASIC CALCULATIONS****
25300 LP=LPV-IPV
25400 LE=BFV-IPV
25500 F=2*AREA(U)/LP
25600 VP=B(2)
25700 GOTO 28400
25800 REM **VISCOSITY INDEX CALCULATIONS******
25900 VP=20
26000 RI=4.255
26100 RO=7.028
26200 DISP "The Viscosity Constants Used Are:"
26300 DISP
26400 DISP "-Plunger Velocity = 20 mm/min"
26500 DISP "-Plunger Radius = 4.255 mm"
26600 DISP "-Inner Test Tube Radius = 7.028mm"
26700 DISP
26800 DISP "DO YOU WANT TO CHANGE THE VISCOSITY CONSTANTS ?"
26900 DISP "YES=1","NO=2"
27000 INPUT BB
27010 IF BB=1 OR BB=2 THEN 27100 ELSE BEEP 400,40
27020 DISP "ENTER ONLY A 1 OR 2 " @ GOTO 26800
27100  IF BB=2 THEN GOTO 28300
27200 CLEAR
27300 DISP "VALUE OF PLUNGER VELOCITY IS=?"
27400 INPUT VP
27500 CLEAR
27600 DISP "PLUNGER RADIUS, IN mm, IS=?"
27700 INPUT RI
27800 CLEAR
27900 DISP "INNER TEST TUBE RADIUS, IN mm,IS=?"
28000 INPUT RO
28100 CLEAR
28200 RETURN
28300 RETURN
28400 DISP "PLEASE WAIT ELASTICITY CALCULATIONS IN PROGRESS"
28500 REM ****Apparent VIscosity Calculations ********
28600 K=RI/RO
```

APPENDIX A. (continued)

```
28700 ALFA=(1-K^2)/(1+K^2)
28800 VI=1/(2*PI*VP)*(F/LP)*(1-K^2)*LOG(1/K)*(1+ALFA/LOG(K))
      *6*10^8
28900 REM**APPARENT ELASTICITY CALCULATIONS***
29000 SIGMA=SQR ((RO-RI)^2+LE^2)
29100 ROA=PF/(PI*(RI^2+SIGMA*(RI+RO)))
29200 EP=(RI+SIGMA-RO)/RO
29300 EA=ROA/EP*100
29400 REM ***SHEAR RATE AT THE PWS****
29500 LW=((-(ALFA/(LOG(K)+ALFA)))*(VP/RI))/60
29600 RETURN
29700 END
```

APPENDIX B. List of computer program Delia.

```
1000 DISP @ DISP @ DISP
1050 DISP " *** PROGRAM DELIA   BY CARLOS A. LEVER *** "
1100 DISP
1150 DISP "** THIS PROGRAM USES DATA FROM INSTROM CONTROL
     PROGRAM RODRIGO **"
1200 DISP "TO CALCULATE APPARENT VISCOSITY AND APPARENT
     ELASTICITY,"
1250 DISP "      FROM BACKEXTRUSION DATA          "
1300 REM
1350 DISP @ WAIT 5000 @ CLEAR
1400 U=0
1450 CCP=0
1500 PRINTER IS 701
1550 DIM A(200),B(200),C(200),AA(200),CC(200)
1600 DIM AREA(10)
1650 DIM SAMPID$[85]
1700 DISP
1750 GOSUB 10000
1800 DISP "ENTER FILE NAME WITH INSTROM RAW DATA"
1850 INPUT FILNAME$
1900 ASSIGN# 1 TO FILNAME$
1950 READ# 1 ; SAMPID$
2000 DISP SAMPID$
2050 DISP
2100 FOR I=0 TO 170
2150 READ# 1 ; A(I),B(I),C(I)
2200 AA(I)=ABS(A(I)) @ CC(I)=ABS(C(I))
2250 NEXT I
2300 IF CCP=1 THEN 2600
2350 IF CCP=2 THEN 3950
2400 CLEAR
2450 DISP "DO YOU WANT THE COMPUTER TO ESTIMATE Le AND Lp"
2500 DISP "YES=1","NO=2"
2550 INPUT CCP
2600 REM ** PROGRAM TO CALCULATE Le AND Lp DISTANCE **
2650 IF CCP=2 THEN 3950
2700 FOR I=1 TO 165
2750 IF A(I)<-.03 THEN GOTO 2850
2800 NEXT I
2850 IPV=CC(I-1)
2900 R=I-2
2950 FOR I=I TO 165
3000 IF A(I)<A(I+1) THEN 3100
3050 NEXT I
3100 BFV=CC(I)
3150 PF=AA(I)
3200 LPV=CC(165)
3250 I=1
```

APPENDIX B. (continued)

```
3300 GOTO 5800
3350 GOTO 3950
3400 DISP "ALL DATA WILL BE DISPLAY AS:"
3450 DISP "Force (N)","Distance (mm)","Sequential Number"
3500 DISP
3550 DISP "Please take note of:"
3600 DISP "-Sequential Value of Initial Distance Value (mm)"
3650 DISP "- Sequential Value of Break Points Distance and
     Force"
3700 DISP
3750 DISP
3800 DISP "To continue Press <k1>
3850 ON KEY# 1 GOTO 3950
3900 GOTO 3900
3950 CLEAR
4000 M=20
4050 PAGESIZE 24
4100 DISP "Force (N)","Distance (mm)","Sequential Number"
4050 FOR I=1 TO M
4200 DISP A(I),C(I),I
4250 NEXT I
4300 DISP "Do You Want To See More Values ?","Yes=1";" No=2"
4350 INPUT BB
4400 IF BB=2 THEN 5300
4450 CLEAR
4500 IF I>160 THEN 4850
4550 M=M+20
4600 DISP "Force (N)","Distance (mm),"Sequential Number"
4650 FOR I=I TO M
4700 DISP A(I),C(I),I
4750 NEXT I
4800 GOTO 4300
4850 CLEAR
4900 M=M+10
4950 IF I=170 THEN 5100
5000 GOTO 4600
5050 PAGESIZE 16
5100 DISP "DO YOU WANT TO SEE THE DATA AGAIN"
5150 DISO "YES=1", "NO=2"
5200 INPUT BB
5250 IF BB=1 THEN 3350
5300 CLEAR
5350 PAGESIZE 16
5400 DISP "INPUT INITIAL DISTANCE AND BREAK DISTANCE NUMBER"
5450 INPUT I,J
5500 IPV=CC(I)
5550 R=I
5600 BFV=CC(J)
5650 PF=AA(J)
5700 LPV=CC(165)
```

APPENDIX B. (continued)

```
5750 CLEAR
5800 DISP "Please Wait Area CAlculations in Progress"
5850 U=U+1
5900 SUM1=0
5950 FOR I=2 TO 165
6000 SUM1=SUM1+AA(I)*(CC(I+1)-CC(I-1))*.5
6050 NEXT I
6100 FI=(AA(1)*(CC(2)-CC(1))+AA(166)*(CC(166)-
     (CC(165)))*.5
6150 AREA(U)=SUM1+FI
6200 GOSUB 9600
6250 CLEAR
6300 DISP SAMPID$;" ";SAM$
6350 DISP "Area=";AREA (U);"mm^2"
6400 DISP
6450 DISP "Plunger Velocity is="; B(2);"mm/min"
6500 DISP
6550 DISP "Apparent Viscosity is="; VI;"poise"
6600 DISP
6650 DISP "Apparent Elasticity is=";EA;"N/cm^2"
6700 DISP
6750 DISP "Shear Rate at the PWS is=";LW;"1/sec"
6800 DISP
6850 REM
6900 GOTO 7200
6950 DISP "Do you Want to Print These Values?"
7000 DISP "Yes=1","No=2"
7050 INPUT BB
7100 IF BB=2 THEN 8300
7150 DISP "PRINTIING IN PROGRESS, PLEASE WAIT" @ DISP
7200 DISP "PRINTING IN PROGRESS, PLEASE WAIT"
7250 PRINT "************************************************"
7300 PRINT
7350 PRINT SAMPID$; " ";SAM$
7400 PRINT
7450 PRINT "Area=";AREA (U);"mm^2"
7500 PRINT
7550 PRINT "Plunger velocity is =";B(2);"mm/min"
7600 PRINT
7650 PRINT "Apparent Viscosity is="VI;"poise"
7700 PRINT
7750 PRINT "Apparent Elasticity is=";EA;"N/cm^2"
7800 PRINT
7850 PRINT "Shear Rate at the PWS is=";LW;"1/sec"
7900 PRINT
7950 PRINT
8300 CLEAR
8350 GOTO 9350
8400 DISP "Do You Want to Print the Raw Data?"
8450 DISP "YES=1","NO=2"
```

APPENDIX B. (continued)

```
8500 INPUT BB
8550 IF BB=2 THEN 9100
8600 DISP "PRINTING IN PROGRESS,PLEASE WAIT"
8650 PRINT"*****************************************"
8700 PRINT
8750 PRINT SAMPID$
8800 PRINT
8850 PRINT "Force (N)","Distance (mm)","Sequential Number"
8900 FOR I=1 TO 165
8950 PRINT A(I),C(I),I
9000 NEXT I
9050 GOTO 9100
9100 CLEAR
9150 DISP "DO YOU WANT TO READ ANOTHER FILE ?"
9200 DISP "1=YES","2=NO"
9250 INPUT SINO
9300 IF SINO=2 THEN 9550
9350 FOR L=1 TO 165
9400 A(L),B(L),C(L),AA(L),CC(L)=0
9450 NEXT L
9500 GOTO 1800
9550 END
9650 DISP "Please Wait Viscosity Calculations in Progress"
9700 REM **BASIC CALCULATIONS****
9750 LP=LPV-IPV
9800 LE=BFV-IPV
9850 F=2*AREA(U)/LP
9900 VP=B(2)
9950 GOTO 11150
10000 REM **VISCOSITY INDEX CALCULATIONS******
10050 VP=20
10100 RI=4.255
10150 RO=7.028
10200 DISP "The Viscosity Constants Used Are:"
10250 DISP
10300 DISP "CONTANT (1)-Plunger Velocity = 20 mm/min"
10350 DISP "CONTANT (2)-Plunger Radius = 4.255 mm"
10400 DISP "CONSTANT(3)-Inner Test Tube Radius = 7.028mm"
10450 DISP
10500 DISP "IF YOU WANT TO CHANGE ANY OF THEM, INPUT
      CONSTANT NUMBER "
10550 DISP "IF NOT THEN INPUT 0"
10600 INPUT BB@CLEAR @IF BB=0 THEN RETURN
10620 IF BB=1 OR BB=2  OR B=3 THEN 10650 ELSE BEEP 400,40
10730 DISP "ENTER ONLY A 1, 2 OR 3 " @ GOTO 10500
10650 IF BB=1 THEN GOTO 10800
10700 IF BB=3 THEN 11000
10750 IF BB=2 THEN 10900
10780 CLEAR
10800 DISP "ACTUAL PLUNGER VELOCITY IS=";VP;mm/min, INPUT
```

APPENDIX B. (continued)

```
      NEW VALUE"
10850 INPUT VP @ CLEAR @ GOTO 1200
10900 DISP "ACTUAL PLUNGER RADIUS, IS";RI;"mm, INPUT NEW
      VALUE"
10950 INPUT RI @ CLEAR @ GOTO 10200
11000 DISP "ACTUAL INNER TEST TUBE RADIUS IS";RO;"mm, INPUT
      NEW VALUE"
11050 INPUT RO @ CLEAR @ GOTO 10200
11100 RETURN
11150 DISP
11200 DISP "PLEASE WAIT ELASTICITY CALCULATIONS IN PROGRESS"
11250 REM ****Apparent VIscosity Calculations ********
11300 K=RI/RO
11350 ALFA=(1-K^2)/(1+K^2)
11400 VI=1/(2*PI*VP)*(F/LP)*(1-K^2)*LOG(1/K)*(1+ALFA/LOG(K))
      *6*10^8
11450 REM**APPARENT ELASTICITY CALCULATIONS***
11500 SIGMA=SQR ((RO-RI)^2+LE^2)
11550 ROA=PF/(PI*(RI^2+SIGMA*(RI+RO)))
11600 EP=(RI+SIGMA-RO)/RO
11650 EA=ROA/EP*100
11700 REM ***SHEAR RATE AT THE PWS****
11750 LW=((-(ALFA/(LOG(K)+ALFA)))*(VP/RI))/60
11800 RETURN
11850 END
```

APPENDIX C. List of computer program Mariana.

```
1000 CLEAR @ DISP @ DISP
1050 DISP "***   PROGRAM MARIANA BY CARLOS A. LEVER *****"
1100 REM ****
1150 DISP
1200 DISP "** PROGRAM TO CALCULATE THERMAL MODEL VARIABLES
     *********"
1250 U=0
1300 DIM tt(60), Ta(60), TT(15,65), Tt(15,65)
1350 DIM t(100), Y(100), T(100), X(100)
1400 DIM n(100), c(5,65), a(5,65), FO(80), TRA(60), RMU(30),
     Fo(80)
1450 DIM FLN$[8], SID$[80], TFLN$[10]
1500 CCP=0
1550 DIM PSIA (15,65), PSIAV(65), A_PRIME(15,65)
1600 PRINTER IS 701
1650 DISP
1700 REM ****** MODEL CALCULATION ROUTINES ****************
1750 REM
1800 DISP
1850 DISP " If you Want to Calculate the Thermal Process
     (Bi, Fo,etc.), TYPE 1
1900 DISP
1950 DISP " If you Want to Calculate A' and alpha, TYPE 2"
2000 DISP
2050 DISP "If you Want to Calculate First Raw Estimate of
     Ea, TYPE 3"
2100 DISP
2150 DISP "If you Want to Calculate a(kt), TYPE 4"
2200 DISP
2250 DISP "If you Want to Calculate TTH, TYPE 5"
2300 DISP
2350 DISP "If you Want to Calculate Rings Temperature,
     TYPE 6"
2400 INPUT BB
2450 CLEAR
2500 IF BB=1 THEN 2850
2550 IF BB=2 THEN 20000
2600 IF BB=3 THEN 21450
2650 IF BB=4 THEN 23100
2700 IF BB=6 THEN 10950
2750 IF BB=5 THEN 15300 ELSE BEEP @ CLEAR @ DISP "WRONG
     CHOICE " @ GOTO 1700
2800 CLEAR
2850 REM ******* Calculation of Thermal Process ***********
2900 K=1 @ D=2
2950 DISP " Will Data Be Input From Keyboard (k) or from
     Disk (D)"
3000 DISP "Input k OR d" @ INPUT BB
```

APPENDIX C. (continued)

```
3050 IF BB=1 THEN 3700
3100 IF BB=2 THEN 3150 ELSE CLEAR @ GOTO 2950
3150 CLEAR
3200 DISP "Enter File Name"
3250 INPUT FLN$
3300 ASSIGN# 1 TO FLN$
3350 READ # 1 ; SID$
3400 DISP SID$ @ DISP
3450 FOR x=1 TO 30
3500 READ# 1 ; tt(x), T(x)
3550 IF T(x)=0 then 3650
3600 NEXT x
3650 ASSIGN# 1 TO * @ GOTO 4750
3700 DISP "INPUT TIME (sec) and Temperature (C)"
3750 DISP "INPUT 0,0  when finish"
3800 FOR x=1 TO 100
3850 DISP "sample No." ; x
3900 INPUT TT(x), t(x)
3950 IF T(x)=0 THEN 4050
4000 NEXT x
4050 CLEAR @ DISP " Storage of Data is Next" @ DISP
4100 DISP " Insert Disk to Store Data (** Initialized
     Disk **)"
4150 DISP "Enter File Name (up to eight Characters)"
4200 INPUT FLN$
4250 CREATE FLN$, 15,100
4300 ASSIGN# 1 TO FLN$
4350 DISP "Enter Sample Identification (up to 40
     Characters)"
4400 INPUT SID$
4450 PRINT# 1 ; SID$
4500 FOR PP=1 TO x
4550 PRINT# 1 ; tt(PP), T(PP)
4600 NEXT PP
4650 ASSIGN# 1 TO *
4700 DISP "time", "Temperature"
4750 Print "time", "Temperature"
4800 DISP @ FOR PP=1 TO x-1 @ DISP tt(PP), T(PP) @ PRINT
     tt(PP), T(PP)
4850 NEXT PP
4900 REM **** Calculation of FO, [T], Bi, r/r ************
4950 REM *** Constants use in Fo and [T] are Defined
     Next****
5000 Ti=20
5050 Cp= 3.9216375
5100 DEN= 1071.823
5150 MOI= 90
5200 Tin= 70
5250 REM ***** FO CALCULATION**********
5300 CLEAR
```

APPENDIX C. (continued)

```
5350 DISP "FO AND [T] CONSTANTS ARE:"
5400 DISP
5450 DISP "Constant (1) ; Cp="; Cp; "Kj/Kg-K"
5500 DISP "Constant (2) ; Density (RO)="; DEN; "Kg/m^3"
5550 DISP "Constant (3) ; Initial Temperature of Sample
     (To)="; Ti; "C"
5600 DISP "Constant (4) ; Temp, of Water Bath=; Tin; "C"
5650 DISP
5700 DISP "Do you Want to Change any of Them?"
5750 DISP "If YES then input Constant No."
5800 DISP "If NO then input 0"
5850 INPUT BB
5900 IF BB=0 THEN 7000
5950 IF BB=2 THEN 6800
6000 IF BB=3 THEN 6400
6050 IF BB=4 THEN 6600
6100 CLEAR
6150 DISP "To Calculate New Cp, Input Moisture Content of
     Sample"
6200 DISP "as PERCENTAGE  (0 to 100)"
6250 INPUT MOI
6300 Cp=1.675+.025*MOI
6350 GOTO 5300
6400 CLEAR
6450 DISP "Input New Value of To (C)"
6500 INPUT Ti
6550 GOTO 5300
6600 CLEAR
6650 DISP "Input New Value of T (water bath- C)"
6700 INPUT Tin
6750 GOTO 5300
6800 CLEAR
6850 DISP "Input New Value for Density (RO), in Kg/m^3"
6900 INPUT DEN
6950 GOTO 5300
7000 REM **** FO FROMULA ****
7050 RAR= 7.028/1000 !  r from mm to meters
7100 RR=RAR*RAR ! r^2
7150 TOL=1*10^-5 ! For use in Bessel and Bissect Subr.
7200 MAXI=35 ! IDEM than TOL
7250 REM
7300 DISP "Please Wait , FO and [t] calculations are next"
7350 FOR X=1 TO x-1
7400 ThC= (.30776.775*10^-4*T(X)*.001730314961 ! to get
     Kj-m/sec-m^2-C
7450 THC=ThC/ (DEN*Cp*RR)
7500 FO(X)= tt (X)* THC
7550 TRA (X)= (Tin-T(X)))/(Tin-Ti)
7600 REM **** printing option for Checking values******
7650 REM ***
```

APPENDIX C. (continued)

```
7700 DISP FO(X), TRA(x), tt (X)
7750 PRINT USING "3A,2D,2A,D.4D,X,5A,2D,2A,D.4D, X,3D,X,4A";
     "FO(",X,")=",FO(X),TETA (",X,")=",TRA(X),tt (X),"sec."
7800 NEXT X
7850 CLEAR
7900 REM **** CALCULATIONS FOR BI**********
7950 CLEAR
8000 DISP "Calculations for Bi are next"
8150 FOR X=1 TO x-1
8200 FOU=FO(X)
8250 TETA=TRA(X)
8300 XA=1
8350 XB=5
8400 FA=FNFUN (XA)
8450 FB=FNFUN (XB)
8500 JJJ=1
8550 GOSUB BISECT
8600 RMU (X)=RD @ IF RMU (X)=5 THEN 8650 ELSE 8750
8650 DISP "Value of Sample No (";X;") is out of Range"
8700 DISP "Then a Very High Value of Bi (2*10^40) will be
     assumed"
8750 RD=0
8800 NEXT X
8850 WAIT 5000
8900 CLEAR
8950 PAGESIZE 24
9000 CLEAR
9050 DISP "All values of Bi (Experimental) are Displayed"
9100 DISP "Select Range to be Considered (First and Last
     SEQUENTIAL Values)"
9150 DISP
9200 DISP
9250 DISP "Bi ", "Sequential Number"
9300 PRINT @ PRINT @ PRINT
9350 FOR X-1 TO x-1
9400 LBi= .08359*RMU(X)^4.35842959
9450 Bi=EXP (LBi)
9500 DISP Bi, X
9550 PRINT "Bi (";X;")="Bi
9600 NEXT X
9650 DISP @ DISP @ DISP "Press 1 to Continue"
9700 INPUT BB
9750 PAGESIZE 16
9800 CLEAR
9850 DISP "Input First and Last Value of Bi Selected
     (Sequential Value)"
9900 INPUT FIRST, LAST
9950 RMUA=0
10000 FOR X=FIRST TO LAST
10050 RMUA=RMUA+RMU (X)
```

APPENDIX C. (continued)

```
10100 NEXT X
10150 RMUAa=RMUA/(LAST+1-FIRST)
10200 TEM
10250 LBi = .08359*RMUAa^4.35843
10300 CLEAR
10350 CLEAR
10400 DISP "average Bi="; Bi
10450 DISP "Values Utilized Were From "; FIRST;" to "; LAST
      @ PRINT @ PRINT
10500 PRINT
10550 PRINT "Average Bi="Bi,"Average MU=";RMUAa, "Average
      M=";1/Bi
10600 PRINT "Values Utilized Were From"; FIRST; " to "; LAST
      @ PRINT @ PRINT
10650 MUA=RMUAa
10700 FOR X=1 TO x-1
10750 RMU (X) =0
10800 NEXT X
10850 DISP
10900 GOTO 11450
10950 REM **** Calculations for r/R ***************
11000 DISP "Calculations for r/R and [T]s are Next"
11050 DISP @ DISP
11100 Cp=3.9216375 @ DEN+ 1071.823 @ MAXU=35
11150 RAR= 7.028/1000 ! r from mm to meters
11200 RR=RAR*RAR ! r^2
11250 Bi= 15.724 @  Ti=20 @ disp "Bi=";Bi, "Temp.
      Initial=";Ti
11300 DISP "Input Temp of Water Bath " @ INPUT Tin
11350 ThC=(.3077 + 6.775* 10^-4 * Tin)* .001730314961
11400 THC= ThC/(DEN*Cp*RR)
11450 AREA=155.1720074 @ TOL= 1*10^-5
11500 IRADI= 7.028
11550 NA=10 @ TOLE= .01 @ Ea= 20000
11600 CLEAR @ DISP @ DISP "Value Of Ea ="; Ea
11650 DISP "Number of Rings to be considered is";NA @ DISP @
      GOTO 12150
11700 DISP "If you Want to change any of them TYPE Ea or NR"
11750 DISP "To continue TYPE C"
11800 INPUT BB$@ IF BB$="Ea" THEN 12000
11850 IF BB$= "NR" THEN 12050
11900 IF BB$= "C" THEN 12150 ELSE 11950
11950 BEEP @ BEEP @ DISP "Wrong Choice,try Again" @ Wait
      2000 @ GOTO 11600
12000 DISP "INPUT NEW VALUE OF Ea (Cal)"@INPUT Ea@ GOTO
      11600
12050 DISP "INPUT NEW VALUE OF NUMBER OF RINGS" @ INPUT NA @
      GOTO 11600
12100 Ea=2000
12150 DISP
```

APPENDIX C. (continued)

```
12200 DISP "STORAGE OF t(I,J) Data Parameters are Next"
12300 DISP "ENTER FILE NAME ( UP TO 8 CHARACTERS)"
12350 INPUT TFLN$
12400 DISP " Please wait,intense calculations in progress"
12450 DISP @ DISP " Calculation of MU roots (6) is next"
12500 XA= .1
12550 XB= 18
12600 JJJ=2 @ X=1
12650 GOSUB BISECT2
12700 LL=1 @ BEEP @ DISP "The 6 MU's roots calculated"
12750 FOR QQ=1 TO 6 @ PRINT "R(";QQ;")=";R(QQ)@ NEXT QQ
12800 RMU(LL)=R(LL)
12850 DISP @ DISP "Calculation of NODE temperatures are
      next"
12900 FOR TIEMPO =0 TO 1800 STEP 30
12950 SUM1 = 0
13000 X= TIEMPO/30 +1
13050 Fo(X)= THC*TIEMPO
13100 FOR Ii =0 TO NA
13150 ENE=SQR(Ii/NA)
13200 FOMU12=Fo(X)*RMU(LL)^2
13250 ROO=RMU(LL)*ENE
13300 VRMU=RMU(LL)
13350 VFF=FNANJO(VRMU,ROO)
13400 SUM1=SUM1+VFF
13450 DISP @ DISP "** Calculation Round, Time"; TIEMPO;"SEC
      RING";Ii;",ROOT";LL
13500 LL=LL+1 @ IF LL>6 THEN 13700
13550 RMU(LL)=R(LL)
13600 FOMU12=Fo(X)*RMU(LL)^2
13650 GOTO 13250
13700 TETA=SUM1
13750 TT(Ii,X)=TETA*(Ti-Tin)+Tin
13800 BEEP @ DISP
13850 DISP " ** TEMP AT RING";Ii;"AND TIME";TIEMPO;"SEC,
      =";TT(Ii,X);"**"
13900 IF TT(O,X)>=Tin-TOLE THEN 14150
13950 11=1 @ SUM1=0
14000 NEXT Ti
14050 NEXT TIEMPO
14100 CLEAR @ GOTO 14450
14150 FOR RE=X TO 61
14200 FOR IE=0 TO NA
14250 TT(IE,RE)=Tin
14300 NEXT IE
14350 NEXT RE
14400 PRINT SID$
14450 PRINT USING "5A,11(X,5A)";"SEC","RO","R-1","R-2","
      R-3","R-4","R-5","R-6","R-7","R-8","R-9","R-10"
14500 FOR J=1 TO 61
```

APPENDIX C. (continued)

```
14550 PRINT USING "5D,11(X,2D.2D)";J*30-30,TT(0,J),TT(1,J),
      TT(2,J),TT(3,J),TT(4,J),TT(5,J),TT(6,J),TT(7,J),
      TT(8,J), TT(9,J),TT(10,J)
14600 DISP USING "5D,11(X,2D.2D)";J*30-30,TT(0,J),TT(1,J),
      TT(2,J),TT(3,J),TT(4,J),TT(5,J),TT(6,J),TT(7,J),
      TT(8,J), TT(9,J),TT(10,J)
14650 NEXT J
14700 CLEAR @ DISP "STORAGE OF T(i,j) DATA IS NEXT " @ DISP
14750 CREATE TFLN$, 11,600
14800 ASSIGN# 1 TO TFLN$
14850 PRINT# 1;SID$
14900 FOR E=1 TO 61
14950 FOR N=0 TO NA
15000 PRINT# 1;TT(N,E)
15050 NEXT N
15100 NEXT E
15150 ASSIGN# 1 TO *
15200 DISP "TTH CALCULATIONS ARE NEXT" @ DISP @ GOTO 18150
15250 DISP
15300 REM ** CALCULATIONS FOR TTH **
15350 DISP "TTH CALCULATIONS ARE NEXT"
15400 Ea = 20000
15450 DISP @ DISP " VALUE OF Ea ="; Ea
15500 DISP " IF YOU WANT TO CHANGE IT TYPE Ea"
15550 DISP " TO CONTINUE TYPE C"
15600 INPUT BB$ @ IF BB$= "Ea" then 15750
15650 IF BB$ = "C" THEN 15850 ELSE 15700
15700 BEEP @ BEEP @ DISP " WRONG CHOICE, TRY AGAIN"@ WAIT
      2000 @ GOTO 15450
15750 DISP " INPUT NEW VALUE OF Ea " @ INPUT Ea @ GOTO 15450
15800 CLEAR @ DISP "DATA WILL BE INPUT FROM DISK (D) OR
      KEYBOARD (K)"
15850 DISP
15900 INPUT BB$
15950 IF BB$="D" THEN 16050
16000 IF BB$="K" THEN 17850 ELSE BEEP @ GOTO 15300
16050 CLEAR
16100 DISP "ENTER FILE NAME"
16150 INPUT FLN$
16200 ASSIGN# 1 TO FLN$
16250 READ# 1; SID$
16300 DISP SID$ @ DISP
16350 FOR E=1 TO 61
16400 FOR NA=0 TO 10
16450 READ# 1; TT(NA,E)
16500 NEXT NA
16550 NEXT E
16600 ASSIGN# 1 TO * @ GOTO 17850
16650 DISP "INPUT NUMBER OF RINGS CONSIDERED"
16700 INPUT NA @ CLEAR
```

APPENDIX C. (continued)

```
16750 DISP "INPUT NUMBER OF SAMPLES PER RING"
16800 INPUT J @ CLEAR
16850 FOR X=1 TO NA
16900 FOR RR=1 TO J
16950 DISP " INPUT TEMPERATURE (RING,TIME) OF SAMPLE
      (";X;",";RR;")"
17000 INPUT TT(X,RR)
17050 NEXT RR
17100 NEXT X
17150 CLEAR @ DISP " STORAGE OF DATA IS NEXT " @ DISP
17200 DISP "INSERT DISK TO STORE DATA (** INITIALIZED
      DISK**)"
17250 DISP " ENTER FILE NAME (UP TO EIGHT CHARACTERS)"
17300 INPUT TFLN$
17350 CREATE TFLN$,11,600
17400 DISP "ENTER SAMPLE IDENTIFICACTION (UP TO 50
      CHARACTERS)"
17450 INPUT SID$
17500 PRINT# 1 ; SID$
17550 FOR RR=1 TO J
17600 FOR NA=0 TO X
17650 NEXT NA
17700 NEXT RR
17750 ASSIGN# 1 TP *
17800 CLEAR
17850 BB=0 @ T=0 @ R=1.986 @ I=0 @ DT=30 @ PSI=0 @ PEPE=0
17900 PRINT @ PRINT @ PRINT SID$
17950 FOR J=1 TO 61
18000 DISP USING "5D,11(X,2D.2D)" ; J*30-30,TT(0,J),TT(1,J),
      TT(2,J),TT(3,J),TT(4,J),TT(5,J),TT(6,J),TT(7,J),
      TT(8,J), TT(9,J),TT(10,J)
18050 PRINT USING "5D,11(X,2D.2D)" ;J*30-30,TT(0,J),TT(1,J),
      TT(2,J),TT(3,J),TT(4,J),TT(5,J),TT(6,J),TT(7,J),
      TT(8,J), TT(9,J),TT(10,J)
18100 NEXT J
18150 DISP " PLEASE WAIT, INTENSE CALCULATIONS IN PROGRESS"
18200 BB=0 @ T=0 @ R=1.986 @ I=0 @ DT=30 @ PSI=0 @ PEPE=0
18250 PRINT @ PRINT @ PRINT SID$
18300 DISP @ DISP "TRANSFORMATION FORM C TO K IN PROGRESS"
18350 FOR J=1 TO 61
18400 FOR I=0 TO 10
18450 Tt=TT(I,J)+273.2
18500 IF Tt<313.2 then 18550 else 18600
18550 A_PRIME(I,J)=0 @ GOTO 18650
18600 A_PRIME(I,J)=Tt*EXP (-(Ea/R*Tt)))
18650 NEXT I
18700 NEXT J
18750 DISP @ DISP "CALCULATION OF A' IN PROGRESS"
18800 FOR I=0 TO 10
18850 FOR J=2 TO 61
```

APPENDIX C. (continued)

```
18900 PSI=(A_PRIME(I,J)+A_PRIME(I,J))/2*DT
18950 PEPE=PEPE+PSI
19000 PSIA(I,J)=PEPE
19050 NEXT J
19100 PEPE=0 @ PSI=0
19150 NEXT I
19200 DISP @ DISP "TTH CLACULATIONS FOR EACH RING ARE IN
      PROGRESS"
19250 PSI=0
19300 FOR J=2 TO 61
19350 FOR I=0 TO 10
19400 PSI=PSIA(I,J)+PSI
19450 NEXT I
19500 PSIAV(J)=PSI/10
19550 PSI=0
19600 NEXT J
19650 DISP "TTH AVERAGE"
19700 FOR J=2 TO 61
19750 DISP "TTH AT " ;J*30-30; "SEC = ";PSIAV(J)
19800 PRINT "TTH AT " ;J*30-30; "SEC = ";PSIAV(J)
19850 NEXT J
19900 CLEAR
19950 GOTO 1700
20000 REM *** ESTIMATION OF A' AND ALPHA ****
20050 REM
20100 SX,SY,SXY,SXX,SYY=0
20150 FOR X=1 TO 20
20200 DISP "INPUT Y' AT N (INFINITE) AND PROTEIN
      CONCENTRATION (DRY BASIS) NO" ; X
20250 DISP "INPUT 0,0 WHEN FINISH"
20300 INPUT Y,C
20350 IF Y=0 THEN 20550
20400 YY(X)=LGT (Y)
20450 XX(X)=LGT (C)
20500 GOTO 20800
20550 GOSUB LINREG
20600 DISP "A'=" ;10^a,"alpha=",b,";R^2=";r*r,"n=";x-1
20750 GOTO 1700
20800 REM ***** LINEAR REGRESSION SUBROUTINE *****
20850 SX(SX+XX(X)
20900 SYY=SYY+YY(X)*YY(X)
20950 SY=SY+YY(X)
21000 SXY=SXY+YY(X)*XX(X)
21050 SXX=SXX+XX(X)*XX(X)
21100 CLEAR
21150 NEXT X
21200 LINREG:
21250 b=(-((X-1)*SXY)+SX*SY)/(-((X-1)*SXX)+SX*SX)
21300 a=(SX*SXY-SXX*SY)/(SX*SX-(X-1)*SXX)
21350 r-((X-1)*SXY-SX*SY)/SQR (((X-1)*SXX-SX*SX)*((X-1)
```

APPENDIX C. (continued)

```
      *SYY -SY*SY))
21400 RETURN
21450 REM ****** ESTIMATION OF Ea *** ******************
21500 T,t,Y=0
21550 SX,SY,SXY,SXX,SYY=0
21600 FOR x=1 TO 10
21650 FOR X=1 TO 100
21700 DISP " INPUT TEMPERATURE (IN C) AND TIME (SEC) FOR
      EXP. NO. " ; X
21750 DISP "ESTIMATION OF Ea No. ";x
21800 DISP "WHEN FINISH INPUT 0,0"
21850 INPUT T,t
21900 IF T=0 THEN 22150
21950 XX(X)=1/(t+273.2)
22000 YY(X)=LGT (t)
22050 CLEAR
22100 GOTO 20850
22150 GOSUB LINREG
22200 r(x)=r
22250 Ea(x)=b*1.986
22300 DISP "Ea=";Ea(x),"R=;r(x),"VARIABLE No ";x
22350 PRINT "Ea=";Ea(x),"R=;r(x),"VARIABLE No ";x
22400 DISP "DO YOU WANT TO CALCULATE ANOTHER Ea"
22450 DISP "YES=1", "NO=2"
22500 INPUT BB
22550 IF BB=2 THEN 22650
22600 NEXT x
22650 EA=0
22700 FOR X=1 TO x
22750 EA=EA+Ea(X)
22800 NEXT X
22850 Ed=EA/x
22900 DISP "Ea AVERAGE OF";x;"EXPERIMENTS IS =";Ed;"CAL/MOL"
22950 PRINT " Ea AVERAGE IS =";Ed;"CAL/MOL"
23000 CLEAR
23050 GOTO 1700
23100 REM ** SUBROUTINE TO ESTIMATE (a) **
23150 DISP
23200 DISP "CALCULATION OF (a) WILL BE THE AVERAGE OF 3 SETS
      OF VALUES"
23250 FOR m=1 TO 3
23300 b=1
23350 a=0
23400 DISP "INPUT VALUES OF Y'(a),PSI(a), AND Y'(b),PSI(b)"
23450 DISP "OF SAMPLE "' m
23500 INPUT y(1), psia(1),Y(2),psia(2)
23550 CLEAR
23600 DISP "PLEASE WAIT INTENSE CALCULATIONS IN PROGRESS"
23650 DISP "round";m
23700 LHS=y(1)/y(2)
```

APPENDIX C. (continued)

```
23750 FOR X=1 TO 10
23800 FOR x=1 TO 100
23850 c=a+b*x
23900 RHS=(1-EXP (-(c*psia(1))))/(1-EXP (-(C*psia(2))))
23950 IF RHS>LHS THEN 24050
24000 NEXT x
24050 a=c+(x-1_*b
24100 b=b*.1
24150 NEXT X
24200 A(m)=a
24250 DISP "a(";m;")=";A(m)
24300 NEXT m
24350 AV=(A(1)+A(2)+A(3))/3
24400 DISP "(a) AVERAGE IS ="; AV
24450 PRINT
24500 PRINT "(a) AVERAGE IS ="; AV
24550 PRINT
24600 PRINT "a(1)="; A(1),"a(2)=";A(2),"a(3)=";A(3)
24650 PRINT
24700 CLEAR
24750 GOTO 1700
24800 REM *** SUBROUTINE DEFINITION OF BESSEL FUNCTION ****
24850 DEF FNBESJ (ROO)
24900 TOL=1*10^-5 @ DISP @ DISP "CALCULATION OF BESSEL
      FUNCTION, SAMPLE No.";X
24950 X2=ROO*ROO
25000 FNBESJ=0
25050 IF ROO>15 THEN 25600
25100 SUM=1
25150 TERM2=SUM
25200 I=0
25250 I=I+1
25300 TERM+TERM2
25350 TERM2=-(TERM*X2*.25/(I*I))
25400 SUM=SUM+TERM2
25450 IF ABS (TERM2)>ABSD (SUM*TOL) THEN 25250
25500 FNBESJ=SUM
25550 GOTO 25650
25600 FNBESJ=SQR (2/(PI*ROO))*COS (ROO-PI /4)
25650 FN END
25700 END
25750 REM ****** DEFINITION OF FUNCTION FNFUN (RMU) *******
25800 DEF FNFUN (RMU)
25850 RM=RMU
25900 ATE=LOG (TETA)
25950 FNFUN=RM*RM*FOU-.5395*LOG (RM)+ATE-.03974
26000 FN END
26050 END
26100 REM *** SUBROUTINE BISECT ******************
26150 REM
```

APPENDIX C. (continued)

```
26200 BISECT:
26250 RM=0 @ RD=0 @ DISP "CALCULATION IS IN SUBROUTINE
      BISECT, SAMPLE No. ";X
26300 X0=XA
26350 F0=FA
26400 X1=XB
26450 F1=FB
26500 XM+(XA+XB)/2
26550 IF F0*F1>0 THEN 29250
26600 REM
26650 FOR J=1 TO MAXI
26700 IF ABS (F1)>= ABS (F0) THEN 26800
26750 GOTO 27100
26800 X2=X0
25850 X0=X1
26900 X1=X2
26950 F2=F0
27000 F0=F1
27100 REM
27150 XX=X1-F1*(X1-X0)/(F1-F0)
27200 IF XB<XA THEN 27400
27250 IF XX<XA THEN 27900
27300 IF XX>XB THEN 27900
27350 GOTO 27500
27400 IF XX<XB THEN 27900
27450 IF XX>XA THEN 27900
27500 REM
27550 DIFF= ABS (XX-X1)
27600 IF DIFF<ABS (XX*TOL) THEN 29050
27650 X0=X1
27700 F0=F1
27750 X1=XX
27800 GOSUB SELECT1
27850 NEXT J
27900 REM ********
27950 GOSUB SELECT2
28000 IF FM=0 THEN 28850
28050 IF FA*FM<0 THEN 28450
28100 XA=XM
28150 FA=FM
28200 F0=FA
28250 X0=XA
28300 F1=FB
28350 X1=XB
28400 GOTO 28750
28450 XB=XM
28500 X1=XB
28550 FB=FM
28600 F1=FB
28650 X0=XA
```

APPENDIX C. (continued)

```
28700 FO=FA
28750 XM=(XA+XB)+2
28800 IF ABS (XA-XB)>ABS  (XM*TOL) THEN 26600
28850 RM=XM
28900 RD=XM
28950 RETURN
29000 END
29050 RM=XX
29100 RD=XX
29150 RETURN
29200 END
29250 DISP "ROOT NOT IN INTERVAL"
29300 RD=5
29350 RETURN
29400 END
29450 REM *****    FUNCTION FNJ1    **************
29500 DEF FNJ1 (ROO)
29550 X2=ROO*ROO
29600 FNJ1=0
29650 IF ROO >15 THEN 30200
29700 SUM=ROO/2
29750 TERM2=SUM
29800 I=0
29850 I=I+1
29900 TERM= TERM2
29950 TERM2=-(TERM*X2*.25/(I*(I+1)))
30000 SUM=SUM+TERM2
30050 IF ABS (TERM2)>ABS (SUM*TOL) THEN 29850
30100 FNJ1=SUM
30150 GOTO 30250
30200 FNJ1=SQR (2/(PI*ROO))*COS (ROO-.75*PI)
30250 FN END
30300 END
30350 REM ****** SUBROUTINE SELECT1 **************
30400 SELECT1:
30450 IF JJJ=1 THEN F1=FNFUN(X1)
30500 IF JJJ+2 THEN Y=FNFX1(XX)
30550 RETURN
30600 END
30650 REM ******** SUBROUTINE SELECT2 ******************
30700 SELECT2:
30750 IF JJJ=1 THEN FM=FNFUN(XM)
30800 IF JJJ+2 THEN FM=FNFX1(XM)
30850 RETURN
30900 END
30950 REM ****** SUBROUTINE SELECT3 *********
31000 SELECT3:
31050 IF LL=2 THEN XA=3.5
31100 IF LL=2 THEN XB=6
31150 IF LL=3 THEN XA=6.5
```

APPENDIX C. (continued)

```
31200 IF LL=3 THEN XB=9
31250 IF LL=4 THEN XA=9.5
31300 IF LL=4 THEN XB=11
31350 IF LL=5 THEN XA=13
31400 IF LL=5 THEN XB=15
31450 IF LL=6 THEN XA=16
31500 IF LL=6 THEN XB=17
31550 IF LL>6 THEN DISP "LL > 6" ELSE 31650
31600 DISP "VALUE OUT OF RANGE" @ END
31650 RETURN
31700 END
31750 REM **** DEFINITION OF FNANJO(MU,ROO) ****************
31800 DEF FNANJO(MU,ROO)
31850 AN=2*Bi/(FNBESJ(MU)*(MU*MU+Bi*Bi))
31900 FNANJO=AN*FNBESJ(ROO)*EXP (-FOMU12)
31950 FN END
32000 END
32050 REM ** SUBROUTINE VMUROO TO OBTAIN MU FROM ROOT EQ. **
32100 VMUROO:
32150 WW=0
32200 FA=FNFX1(XA)
32250 FB=FNFX1(XB)
32300 GOSUB BISECT
32350 RETURN
32400 END
32450 REM **** DEFINITION OF FUNCTION FNFX1 *************
32500 DEF FNFX1 (MU1)
32550 FNFX1=MU1*FNJ1(MU1)-Bi*FNBESJ (MU1)
32600 WW=WW+1 @ DISP
32650 DISP " MU1="; MU1;" ROUND No."; WW
32700 FN END
32750 END
32800 REM **** SUBROUTINE BISECT2 ****************
32850 BISECT2:
32900 N1+6 @ S=.2 @ DISP "CALCULATION IS IN SUBROUTINE
      BISECT2"
32950 A1=XA @ B=XB @ WW=0
33000 A=A1
33050 N=0
33100 FOR I=1 TO N1
33150 R(I)=INF @ F(I)=INF  @ E(I)=INF
33200 NEXT I
33250 XX=A
33300 IF N>=N1 THEN 35650
33350 N=N+1
33400 GOSUB SELECT1
33450 F+Y
33500 A=A+S
33550 IF A>B THEN 35650
33600 XX=A
```

APPENDIX C. (continued)

```
33650 GOSUB SELECT 1
33700 P=F*Y
33750 IF P>0 THEN 33450
33800 IF P<0 THEN 34300
33850 IF F<> 0 THEN 34000
33900 XX=A-S
33950 Y=F
34000 R(N)=XX
34050 F(N)=Y
34100 A=A+S
34150 Z=10^-12
34200 LET E(N)=Z
34250 GOTO 33250
34300 L=A-S
34350 R=A
34400 C=0
34450 XX=(L+R)/2
34500 GOSUB SELECT1
34550 C=C+1
34600 IF C>MAXI THEN 35300
34650 IF ABS (Y)<TOL THEN 35050
34700 P=F*Y
34750 IF P<= 0 THEN 34900
34800 L=XX
34850 GOTO 34450
34900 IF P=0 THEN 35050
34950 R=XX
35000 GOTO 34450
35050 R(N)=XX
35100 F(N)=Y
35150 Z=R-L
35200 LET E(N)=Z
35250 GOTO 33250
35300 DISP "ROOT NOT FOUND IN"; MAXI; "ITERATIONS"
35350 DISP "FOR ROOT No.";N
35400 Z=R-L
35450 R(N)=(L+R)/2
35500 F(N)=Y
35550 LET E(N)=Z
35600 GOTO 33250
35650 RETURN
35700 END
```

APPENDIX D. Experimental values of Y' and TTH utilized in testing the model (Figure 13).

TEMPERATURE OF PROCESSING

64 °C		70 °C	
Y'	TTH	Y'	TTH
0.04	1.53E-10	0.55	5.0E-10
0.08	1.53E-10	0.63	5.0E-10
0.10	1.53E-10	0.64	5.0E-10
0.71	6.46E-10	0.97	2.0E-9
0.71	6.46E-10	1.18	2.0E-9
0.71	6.46E-10	1.19	2.0E-9
0.78	1.58E-9	2.35	5.22E-9
0.88	1.58E-9	1.98	5.22E-9
0.77	1.58E-9	2.25	5.22E-9
2.21	4.50E-9	3.00	1.21E-8
1.80	5.37E-9	3.23	1.21E-8
1.95	6.27E-9	3.21	1.21E-8
2.77	1.40E-8	3.77	2.0E-8
3.42	1.40E-8	4.09	2.0E-8
3.13	1.40E-8	4.58	2.0E-8
4.05	2.40E-8	3.85	3.0E-8
3.79	2.40E-8	4.72	3.0E-8
3.58	2.40E-8	4.86	3.0E-8
4.13	5.70E-8	3.93	3.0E-8
4.43	5.70E-8	3.44	3.0E-8
4.87	5.70E-8	3.68	3.0E-8
4.19	9.00E-8	3.71	5.0E-8
3.95	9.00E-8	4.14	5.0E-8
4.17	9.00E-8	3.56	5.0E-8
4.86	1.23E-7	4.13	7.0E-8
3.28	1.23E-7	4.42	7.0E-8
4.20	1.23E-7		

APPENDIX D. (continued)

TEMPERATURE OF PROCESSING

80 °C		84 °C	
Y'	TTH	Y'	TTH
0.26	3.0E-10	0.18	6.0E-10
0.35	3.0E-10	0.19	6.0E-10
0.66	1.14E-9	1.50	2.19E-9
0.51	1.14E-9	0.86	2.19E-9
0.76	1.14E-9	1.54	2.19E-9
0.67	3.0E-9	1.87	4.72E-9
0.83	3.0E-9	1.85	4.72E-9
1.25	3.0E-9	2.23	4.72E-9
2.06	4.53E-9	3.44	8.21E-9
1.69	4.53E-9	3.27	8.21E-9
1.80	4.53E-9	2.72	8.21E-9
3.16	1.00E-8	3.49	1.77E-8
3.43	1.00E-8	3.12	1.77E-8
2.56	1.00E-8	3.38	1.77E-8
3.72	2.0E-8	3.54	3.0E-8
3.72	2.0E-8	4.10	3.0E-8
3.72	2.0E-8	3.20	3.0E-8
5.05	5.0E-8	3.47	8.0E-8
3.89	5.0E-8	3.85	8.0E-8
4.91	8.0E-8	4.23	8.0E-8
4.10	8.0E-8	4.20	2.0E-7
4.55	8.0E-8	4.58	2.0E-7
4.09	1.0E-7	4.96	2.0E-7
3.97	1.0E-7	3.68	2.0E-7
4.03	1.0E-7	4.08	2.0E-7