The characterization of the functional properties of three cold alkali extracted meat protein concentrates and the relationship between functional properties determined using model systems and the properties of an emulsion-type sausage in which they were incorporated

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ABSTRACT

The feasibility of marketing three meat protein concentrates (MassMPC, MyoMPC and SarcoMPC) on the commercial protein market was studied. Solubility, heat gel strength, water binding and emulsification properties of these proteins were investigated under different temperature, protein to water ratio and salt treatments using model systems.

Model systems were designed so that they approximated the physical and chemical conditions found in an emulsion-type sausage. The properties of the meat proteins were compared to those exhibited by a soy concentrate (GL750) and three sodium caseinates (SV07, SV02 and CasN) measured under the same conditions. In addition the performance of the meat, soy, and caseinate proteins in an emulsion-type sausage was evaluated.

MassMPC and MyoMPC exhibited superior heat gelling properties compared to the commercial protein products, while SarcoMPC was more soluble and exhibited foaming properties similar to these products. All three proteins could be used successfully in sausages which had 3% of the total protein replaced with additive protein. At a 30% replacement level the addition of MassMPC and SarcoMPC produced undesirable effects on sausage properties. MyoMPC did not adversely affect the characteristics important in a commercial sausage, at this replacement level. However, in terms of overall functional properties and performance in a food system, MassMPC, MyoMPC and SarcoMPC were inferior to the commercial protein products.

Data collected from this study were analyzed using the Pearson's correlation test to identify relationships between: functional properties assessed using model systems; sensory and instrumental measurements of emulsion-type sausage rheological properties; and model system functional properties of proteins and the properties of sausages

incorporating these proteins.

A negative correlation between emulsion stability moisture loss and water binding capacity was identified but this relationship was dependent on the methods used to measure these properties. Solubility was found to be negatively related to the water binding capacity of proteins.

The Warner Bratzler Shear test, multiple compression test and extrusion test were the most useful instrumental measurements for evaluating textural properties considered important in a commercial emulsion-type sausage. However, the relationships between sensory properties and these instrumental measurements were dependent on the level of incorporation of protein additives in the sausages.

Important correlations were identified between functional properties of proteins measured in the presence of 2% salt, and properties of sausages which had 30% of the total protein replaced by additive proteins. The usefulness of these relationships are discussed, the reasons why some relationships were not significant are suggested, and a new approach to studying the functional properties of proteins is proposed.

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CHAPTER 1

INTRODUCTION

The loss of edible skeletal meat protein, resulting from hand boning of beef in a freezing works, has been estimated to be 7-8 kg per carcass (Fried, 1976). Currently this meat is processed into inedible meat and bone meals used as a protein supplement for pig and poultry feeds. Beef kill figures for New Zealand during the years 1972 to 1978 ranged from 1.8-2.6 million cattle per annum (Anon., 1979), so the processing losses could have amounted to 12.6-20.8 metric tonnes of potentially high quality edible meat protein each year. In economic terms, this is an appreciable loss to the N.Z. Meat Industry.

Extensive technical and economic developmental work has been carried out over the past six years on both mechanical deboning processes and cold alkali recovery of skeletal meat protein for edible purposes (Duerr and Earle, 1974; Field, 1974, 1974a, 1974b; Dudley, 1975; Hamilton, 1978; Jelen et al., 1978, 1979). In 1978 it became apparent that the technological objectives of a cold alkali process were close to being met but there was a lack of knowledge concerning the acceptability of the end products as food ingredients. The next logical step in this research programme was to investigate the properties governing their acceptability.

The purpose of this study was to examine the properties of three meat protein concentrates; one produced at Massey University (called MassMPC) and two (called SarcoMPC and MyoMPC) produced at C.S.I.R.O. Meat Division, Cannon Hill, Australia, produced using alkali extraction.

Use of conventional product development analysis techniques has led to the view that the potential applications of these meat protein concentrates could be as protein additives in multicomponent food systems. It was therefore desirable that they possessed properties, apart from their added nutritional value, which would make them compatible with the foods in which they could be incorporated. These additional properties are known as "functional properties".

Functional properties have been defined (Kinsella, 1976) as "any physicochemical property which affects the processing and behaviour of proteins in food systems, as judged by the quality attributes in the final product". These reflect complex interactions between the:

i) composition, structure and conformation of the proteins and other food components,

ii) intrinsic physicochemical properties of the proteins and other food components,

iii) the nature of the environment in which these are associated and measured.

Protein concentrates which exhibit high quality functional properties are always in demand on the world protein market because of their numerous potential applications.

Traditionally the functional properties of new protein ingredients have been assessed by incorporating them into food products under practical conditions (Hermansson, 1979). While these tests are ultimately necessary they have some serious drawbacks. These have been summarised by Hermansson (1979) as follows:

i) they require large quantities of raw material,

ii) limited information is obtained about the function of the ingredient being investigated,

iii) minor changes in processing conditions or the recipes may have unexpectedly large effects on

the final food properties,

iv) and it is difficult to control the fat and water content of the final product without proper knowledge of the function of the ingredients.

Alternatively, model systems can be used to determine the functional properties of new protein materials. Methods used in this field are numerous, have been developed piecemeal and are empirical. They do provide useful information regarding the behaviour of a protein in its native state. However, the data obtained is of limited use for predicting the behaviour of the protein in food systems which have different environments and components (Kinsella, 1976; Randall, 1978). The empirical nature of these tests dictates the necessity for comparisons between new and existing materials. An improved approach combines the use of model systems and real but controlled food systems to measure functional properties. This technique was used in the present study and will be discussed in more detail in Chapter 2.

Protein concentrates are very important in the meat smallgoods industry for the production of emulsion-type sausages. However their functional properties are crucial and it was envisaged that the three meat protein concentrates recovered from the boning room waste could be important in this industry. In order to establish which non-meat protein concentrates were currently being used in the production of sausages a survey was carried out.

In smallgoods manufacture the most frequently used non-meat protein concentrates would be in direct competition with meat protein concentrates should they ever reach the commercial market. It was assumed that in the context of value for money these non-meat proteins exhibited the required functional characteristics and measurement of their properties could be used as bench marks for assessing the functional properties of the meat proteins. As a result of the survey the choice of non-meat concentrates to be used in the comparative study with the new meat protein concentrates was made. Thus a soy concentrate was selected for the study as it was used most frequently in the industry. Sodium caseinates were also

used, especially the low viscosity material which was freely available. Furthermore the European meat smallgoods industry (C. Towler, personal communication) employs high viscosity caseinates because of their good functional properties and these could become important to the New Zealand smallgoods industry in the future. For these reasons three sodium caseinates of varying viscosity were included in this study.

The aim of the study was twofold:

i) To rate the quality of the functional properties of the three meat protein concentrates when compared with competitive materials which are sold on the commercial protein market.

ii) To set up model systems which would identify the interactions of the selected proteins with other food components in such a way that the results could be used to predict the possible effects of the added proteins in a multicomponent food system.

The multicomponent food system chosen for this study was a cooked emulsion type sausage, since the quality of this product is dependent upon the functional properties of the proteins in the system. These properties include the water binding capacity, emulsion capacity, emulsion stability, protein solubility and heat gel strength of the suspended proteins. Model systems were developed to measure each of these properties.

Foaming characteristics of the meat protein concentrates are not important in a sausage system but could be important in other food systems. For this reason foam stability and foam capacity were assessed for these materials and compared with the values for dried egg albumin which is one of the most widely used foaming agents in the food industry.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This general review attempts to pull together information on the principal categories of functional properties of proteins and leads onto a discussion of the functional properties that are important in emulsion type sausages. The theoretical aspects of each of these properties is briefly discussed and methods used to measure them are critically reviewed.

Approaches to characterizing the functional properties of new protein products are limited and have been used with varying degrees of success. These approaches are outlined and their limitations are discussed. Particular emphasis is placed on the use of model systems combined with the trial-and-error approach and the results of such investigations have been summarized.

Complete characterization of the functional behaviour of new proteins necessitates studying their foaming properties and the theoretical aspects associated with these properties and methods used to assess them have also been reviewed.

2.2 Functional properties of proteins

In a multicomponent food system, proteins have been shown to have a significant effect on both the sensory (e.g. texture) and functional properties of the product (Kinsella, 1976).

Typical functional properties include emulsification which is important in sausage-type processed meats and coffee whiteners; hydration and water binding, which are critical in doughs and meat products; viscosity, important for beverages; gelation, required in marshmallows and cold meat products; foaming/whipping properties vital in whipped

toppings; and cohesion, which is important in manufacture of textured products. The broad range of functional properties of proteins that are important in food products can be divided into five categories according to the organoleptic, hydration, surface, structural and other miscellaneous properties of the protein as shown in Table 2.1 (Adapted from Kinsella, 1976).

TABLE 2.1

The functional properties of proteins important in food applications

General Category	Specific functional term
1. Organoleptic	Color, flavor, odor, texture, mouth-feel, smoothness, grittiness, turbidity etc.
2. Hydration	Solubility, dispersibility, wettability, water absorption, swelling, thickening, gelling, rheological, water holding capacity, syneresis, viscosity, dough formation, etc.
3. Surface	Emulsification, foaming, aeration, whipping, protein/lipid film formation, lipid binding, flavor binding, stabilization, etc.
4. Structural and Rheological	Elasticity, grittiness, cohesion, chewiness, viscosity, adhesion, network cross-binding, aggregation, stickiness, gelation, dough formation, texturizability, fiber formation, extrudability, elasticity, etc.
5. Other	Compatibility with additives, enzymatic, inertness, modification properties

Within each of these categories there can be many specific functional properties. These will vary with processing conditions, such as pH, temperature, protein concentration, ionic strength and dielectric constant of the medium, protein source and other treatments, i.e. macromolecules (carbohydrates, lipids) in the medium. This complex dependence of protein functional properties on these various factors is illustrated in Figure 2.1.

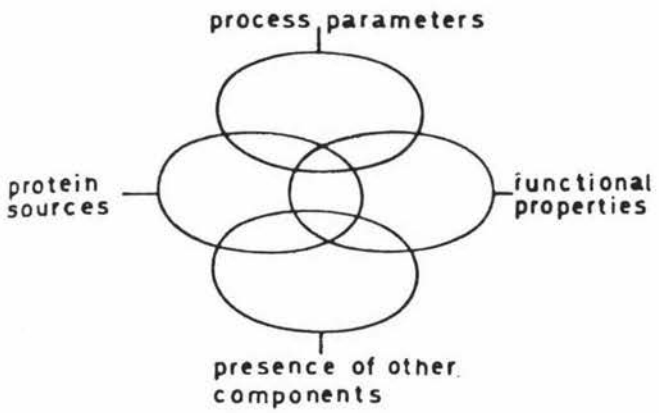


Figure 2.1 A Venn-diagram illustrating the complex dependence of functional properties on various factors (Hermansson, 1979)

2.3 The manufacture of emulsion-type cooked sausages and the importance of protein functional properties

A finely comminuted meat sausage (known as an emulsion-type sausage henceforth) has been defined as "a two phase system consisting of a dispersion of a solid in a liquid in which the solid is not miscible" (Saffle, 1968) and possesses somewhat different characteristics from classically defined emulsions with which they have been compared in the past. Research over the past decade (Brown, 1972; Hamm, 1973; Van den Oord and Visser, 1973; Schut, 1976) led to the opinion that an emulsion-type sausage is a fairly coarse dispersion of solid fat (dispersed phase) in an aqueous solution of salts and proteins (continuous phase). The continuous phase, often referred to as the emulsion sausage matrix (Schut, 1976), also contains insoluble proteins and particles of muscle fibre and connective tissue. The addition of fine

fat particles to the matrix results in a very complex multi-phase system which is probably even more complex when non-meat protein materials are added (Randall, 1978).

The first step involved in the manufacture of an emulsion-type sausage is to chop the meat, water and salt to a fine protein homogenate (i.e. the matrix). Salt is generally added as 2% of the raw ingredients (Randall, 1978; B. Wilkinson, personal communication). It's addition is considered to be vitally important in the production of this type of product as it aids in (Randall, 1978):

i) the solubilization of the myofibrillar muscle proteins particularly myosin and actomyosin which participate in emulsification of the fat. (The latter are often referred to as the salt soluble proteins), and

ii) increases the ability of the meat proteins to retain/bind water.

Changes in the water binding properties of the proteins in the homogenate are also brought about by alterations of the interactions between adjacent protein molecules and between charged groups of the proteins and small polar particles (such as salt ions and water molecules) which arise during the comminution operation (Schut, 1976). The greater the water binding capacity of the proteins the more stable is the matrix and a stable matrix is a prerequisite for the production of a stable emulsion-type sausage. Thus the homogenate consists of swollen insoluble fractions and soluble protein fractions which are suspended in the water trapped between the network of protein fibres.

Animal fat, generally pork fat (B. Wilkinson, personal communication) is chopped into the matrix resulting in the disintegration of some of the fat cells (Schut, 1976).

The salt-soluble proteins are thought to act as emulsifying agents (Schut, 1976) for the free fat released from the disintegrated fat cells, migrating to the fat-matrix boundaries to form a film structure. Schut (1976) proposes

that only a part of the salt solubilized myofibrillar segments are absorbed at the surface of the fat particles while the remaining parts reach out sideways into the continuous phase where they are able to interact with the insoluble actomyosin molecules which are not directly involved in film formation. According to this model the protein molecules of the interfacial film could participate in the gel structure of the actomyosin molecules of the matrix leading to the formation of the coherent system that is observed.

Emulsion-type sausages are preserved by heat. Upon heating, however, the protein films around the fat particles are disrupted resulting in a number of definite pores and openings (Borchert et al., 1967), although no data are available revealing the exact temperature at which this occurs. It has been assumed that disruption of the membrane must occur at a temperature at which the matrix has been solidified (i.e. the meat proteins coagulate to a network) to such an extent that the fat particles are immobilized. At the same time vacuoles are formed in which the free water is immobilized. If the matrix is not stable and coherent enough the resulting cooked sausage will have a crumbly, unacceptable texture and sensory appeal (Schut, 1976). Matrix instability is a result of water separation from the raw emulsion and fat exudation during cooking.

Therefore, the characteristics of a cooked emulsion-type sausage are dependent on the water binding ability, solubility and gelation properties of the proteins present for the formation of a stable matrix and the emulsion capacity and emulsion stability properties of these materials when fat is added to the matrix. The success of the proteins at performing these tasks can be assessed by measuring fat and moisture losses from the raw mix. In addition, stability upon heating can also be assessed by measuring these parameters and will depend on the heat gelling ability of the proteins and their water and fat

binding abilities at the processing temperatures employed. Heat gel strength and the degree of fat and moisture loss will affect the texture of the cooked product and thus textural parameters of cooked sausages can also be evaluated to assess the functional properties of the proteins present.

2.4 The effect of additives on the properties of emulsion-type sausages

Non-meat proteins and carbohydrates are frequently added to emulsion-type sausages affecting the matrix and emulsion structure and stability.

2.4.1 Non-meat protein additives

The products of this group can be divided into two subdivisions: animal proteins, which include blood plasma and milk proteins and vegetable proteins which include soy proteins, leaf proteins, cereal proteins and miscellaneous proteins such as grape seed proteins, nut proteins etc.

In N.Z. sodium caseinate is one of the most commonly used animal non-meat protein additives. This is a product which contains approximately 90% protein, is very soluble in water and, depending on the type of caseinate used, produces solutions showing different viscosities. Some special types even form gels. The viscosity of these solutions and gels is inversely related to temperature (Schut, 1976). Sodium caseinate is considered to be the best known non-meat protein emulsifier (Kutscher and Pfaff, 1961; Pearson et al., 1965; Pfaff, 1968; Schut, 1968). Since it does not coagulate at normal cooking temperatures the protein film does not shrink, no fat is liberated and no coalescence of emulsified fat particles occurs at heating. For the same reason (no coagulation) the direct contribution of sodium caseinate to the water retention of meat emulsions is not very pronounced. It has been found, however, that when sodium caseinate has been used to emulsify the fat, the content of myofibrillar meat protein in the matrix remains higher which means that emulsification of fat by sodium caseinate "saves" meat proteins

(Schut,1976).This favours stability of the matrix of meat emulsions so, in an indirect way, sodium caseinate can also contribute to water retention and texture. There are three methods for the incorporation of sodium caseinate into emulsion-type sausages (Schut, 1969):

i) The total amount of sodium caseinate may be added dry, or dispersed in part of the water,before adding to the lean meat at the beginning of the chop.

ii) If the amount of fat to be incorporated is high, a part of the fat can be emulsified in a part of the water by means of a part of the sodium caseinate. The other part of the sodium caseinate is used as described in i). The hot-made - and subsequently cooled-emulsion is added together with the balance of the fat after the lean meat, salt, water and remaining caseinate have been chopped together.

iii) If the amount of salt-soluble meat protein is expected to be small the best results have been found to occur when water- and fat-binding is done completely by sodium caseinate instead of the meat proteins. This involves making an emulsion of all the water and fat with sodium caseinate and subsequently chopping the lean meat part into the emulsion.

The most important vegetable protein additives used in emulsion-type sausages are the soy proteins which can be classified according to their protein content as soy flour (50%), soy protein concentrate (70%), and soy protein isolate (90%). The latter is the most suitable for use in this type of meat product (Schut, 1976) but has very little use in this field in N.Z. It is not water soluble, but when dispersed in water sets to a gel at heating. This property improves the water retention of the matrix and contributes to the stability and texture of the product. Soy concentrates are the most commonly used vegetable protein additives in N.Z. although no reports on the functional properties of this type of material have been

seen. It contains approximately 15% carbohydrate (Griffith Laboratories N.Z. Ltd., in litt.) and this constituent is likely to contribute to the overall properties observed for this material (Wolf, 1970), and especially water absorption properties. The emulsifying properties of soy proteins are not very pronounced and it is even doubtful if soy proteins emulsify fat at all in meat emulsions (Schut, 1976). Soy concentrate is generally added dry to the fine meat homogenate (Griffith Laboratories N.Z. Ltd., in litt.).

2.4.2 Carbohydrate additives

The most commonly used carbohydrate additives in New Zealand are starches and flours derived from cereals. The main purpose of this additive is to bind or absorb excess water (Schut, 1976) thereby increasing the stability of the matrix. Carbohydrates neither participate in the emulsifying process nor improve the water binding capacity of the meat itself. The use of these binders enables the producer to add more water than the meat protein matrix itself can hold. Personal experience has shown that the addition of excess carbohydrate has undesirable effects on the cooked product texture.

2.5 Theoretical aspects and measurement of the functional properties important in emulsion-type sausages using model systems

2.5.1 Percent soluble protein

The percent soluble protein, referred to as protein solubility, is an important characteristic of proteins and is often the first functional property to be studied. Most functional properties are determined by the balance between forces underlying protein-protein interactions and protein-solvent interactions (Hermansson, 1979). Generally soluble proteins possess superior functional attributes for most applications (Kinsella, 1977).

2.5.1.1 Theoretical aspects

Solubilization of a protein molecule is a process which simultaneously involves wetting, swelling, solvation and dissolution (Chou and Morr, 1979). Complete dissolution is dependent on there being sufficient water present and a swollen semi-solid material may result if the amount of water available is limited (Hermansson, 1972).

The degree of solubility of proteins in a test solvent (usually water for food applications) is dependent on the balance of attractive and repulsive forces of the proteins in the system (Hermansson, 1972). Protein association leads to a reduction in solubility while dissassociation results in an increase (Hermansson, 1973a) The latter will be affected by the conformational changes of the protein molecule under-study (Chou and Morr, 1979) and the availability of hydration sites.

2.5.1.2 Factors affecting protein solubility

Protein solubility is dependent on factors such as pH, salt and temperature, which will influence the balance between attractive and repulsive forces (Hermansson, 1973a) as well as concentration, the presence of other components and protein type (Hermansson, 1979). This study was limited to investigating the effects of common salt and temperature on solubility, while all other variables were held constant.

i) Salt

The effect of salt on protein systems is complex and differs with protein type (Hermansson, 1973a). It is thought to be due to changes in the macromolecular structure of proteins brought about by the chloride binding to the protein and altering the electrostatic charge. Chloride has generally been found to bind more firmly than sodium ions (Hamm, 1972). Hydrogen ion concentration (pH) has an important role in salt solubility. At a pH below the isoelectric point proteins have a net positive charge and would usually be soluble. When chloride ions are added they bind to the protein, thereby reducing the

net postive charge and solubility (Figure 2.2).

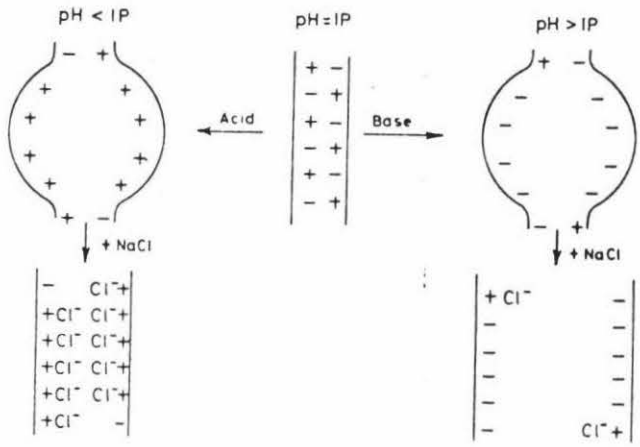


Figure 2.2 The effect of salt and pH on the electrostatic charge of proteins in solution (Hamm, 1975)

At a pH close to the isoelectric point, the net charge is very small, attractive forces dominate and the proteins are associated. Binding of chloride ions at this pH would result in a net negative charge with consequent repulsive forces. The solubility could also be increased by dissociation due to replacement of electrostatic protein-protein interactions by protein-chloride interactions.

At a pH above the isoelectric point it would be expected that the addition of salt would increase the solubility by increasing the net negative charge and subsequent repulsive forces.

It is important when discussing the effect of salt on the solubility of proteins to consider ionic strength, rather than percent salt addition, since most foods are complex ionic systems (Kinsella, 1976).

ii) Temperature

Increasing the temperature of protein has a disordering effect both on the proteins and the solvent in which they are dispersed. The proteins may unfold, new interaction possibilities occur and at a certain temperature aggregation and coagulation takes place (commonly termed thermal

denaturation) as shown in Figure 2.3.

Folded structure (helix etc.) $\xrightarrow{1}$ random coil $\xrightarrow{2}$ aggregation

Figure 2.3 The changes in protein structure relating to increases in temperature (adapted from Hermansson, 1973a)

In the course of unfolding hydrophobic groups are exposed to water which is thermodynamically unfavourable, and leads to the hydrophobic interactions partly responsible for the aggregation in step 2 of Figure 2.3. The oxidation of sulphydral groups (-SH) to intra- and intermolecular disulphide bridges (S-S-) can contribute to aggregation, but oxidation can also lead to the formation of(-SOH), ($-SO_2H$) and ($-SO_3H-$) groups. The effect of temperature on protein solubility will depend on the specific temperature at which unfolding and aggregation of the protein molecules under study occurs.

2.5.1.3 Measurement of percent soluble protein

Percent soluble protein is easily determined and relatively standardized methods are used in contrast to other functional property test methods. The test basically involves dispersing the protein material in water, centrifuging the dispersion and determining the nitrogen content of the supernatant.

The protein concentration of the dispersion must be chosen to enable solvation and dissolution of the proteins to occur. Protein concentrations of 0.2% (Betschart and Kinsella, 1973), 0.5% (Mattil, 1971) and 1% have been used with 1% being the most popular (Lawhon and Carter, 1971; Lu and Kinsella, 1972; Tybor et al., 1973; Betschart, 1974; Crenwelge et al., 1974). Betschart (1974) has reported that the solubility of leaf proteins decreases as protein concentration increases which may be due to the intermolecular associations between the protein chains when the distance between them is reduced.

Dispersion of the proteins can be manual or mechanical, however with either of these modes care must be taken to avoid foaming which may cause the albumins to become insoluble. The addition of antifoam may overcome this problem.

Centrifugation conditions vary considerably and sample sizes range from 10 ml (Betschart, 1974) to 100 ml (Lawhon and Carter, 1971). Conditions such as 4,300 rpm for 20 minutes (Tybor et al., 1973), 2,000 rpm for 20 minutes (Lawhon and Carter, 1971) and 35,000 rpm for 60 minutes (Macfarlane & McKenzie, 1976) have been reported. Higher forces and longer centrifugation times have been reported to result in apparently lower solubility values (Defremery et al., 1972). This is probably due to the higher force causing the finely dispersed insoluble material to be forced into the sediment layer.

Following centrifugation, the supernatant is generally filtered (Lawhon and Carter, 1971; Betschart, 1974; Tybor et al., 1975; Macfarlane and MacKenzie, 1976) and the percentage protein in this fraction is calculated using either the Kjeldahl, Biuret or Folinphenol Reagent tests. The latter two tests have an advantage over the Kjeldahl determination in that they can be performed more quickly. For the standard dairy products solubility test the residue (insoluble solids) is reported in millilitres on a calibrated centrifuge tube for a 50 ml sample of known solids content. This approach is of little value for determining the percent soluble protein for individual protein materials and between materials of varying composition.

2.5.2 Gelation

One of the most important structures in semi-solid and solid food products, including sausages, is the protein gel or three dimensional network structure (Hermansson, 1979). The network structure determines many rheological parameters and acts as a matrix holding water, lipids, dissolved proteins and carbohydrates (Bull, 1947). The most common

food gels are induced by heat treatment, however, they can also be formed spontaneously (for example, by swelling at high protein concentrations) (Hermansson, 1979).

2.5.2.1 Theoretical aspects

"True" gels consist mostly of fluid (often water) but behave as a rigid solid. They are characterized by a relatively high viscosity, plasticity and elasticity and are dependent on the presence of some protein components with tri- or higher functional units. Bifunctional units alone result in the formation of a very viscous solution but not a gel (Bull, 1947). The characteristic textural and mechanical properties of gels are related to the spacing and frequency of the crosslinks along the polymeric chains of the proteins. "True" gelation differs from flocculation or coagulation in that the complete system becomes solidified and there is no visible separation of the solid and liquid phase.

Ferry (1948) considered gelation to be a two stage process involving the initial denaturation of native globular proteins into unfolded polypeptides which gradually associate to form the gel matrix if attractive forces and thermodynamic conditions are suitable. The first stage is accelerated due to the high temperature coefficient of denaturation and occurs most readily in the presence of water (Chou and Morr, 1979). Upon cooling the uncoiled polypeptides associate to form the network crosslinks. The network may be stabilized by primary bonds (largely disulphide), by secondary forces localized on the protein, or by non localized secondary attractive forces (Chou and Morr, 1979). These may include multiple hydrogen bonds, ionic attractions, hydrophobic associations or a combination of these (Kinsella, 1976). If attractive forces predominate the result will normally be an insoluble precipitate. When disulphide crosslinkages become significant an irreversible coagulated gel results (Chou and Morr, 1979).

The bond types vary quantitatively and qualitatively with different protein materials and processing conditions thereby giving rise to different types of gel structures

(Hermansson, 1979). Denatured proteins can form gels only under specific conditions with just the right balance of attractive and repulsive forces (Hermansson,1973a). Aging or repeated heating of a true gel may increase the protein-protein interactions resulting in a "tighter" gel structure which exudes solvent by syneresis.

2.5.2.2 Factors affecting gelation

Gelation studies have demonstrated the effect of protein type (especially water binding and solubility characteristics), pH, concentration, temperature, rate of heating and cooling and the presence of salts, sugars and lipids. The literature in this field has been extensively reviewed by Kinsella (1976).

In this study the effect of common salt was studied for each protein type while all other variables were held constant.

i) Salt

Salt has been found to affect the temperature required to induce gelation (Kinsella, 1979) and the heat gel strength of soy isolates (Circle et al. 1964; Hermansson 1975a) although results are conflicting. Sodium caseinate (Sodinol V LA/S Lidano) did not form a gel and the addition of salt to 10% and 12% dispersions of this caseinate did not influence the absence of gelation. Viscosity of the heat treated ((80°C) for 30 minutes) dispersions, on the other hand, showed a marked increase with salt concentration.

2.5.2.3 Measurement of heat gelation

Rheological test methods used to measure heat gelation properties of protein dispersions have been divided into three classes, fundamental, empirical and imitative (Scott Blair, 1958). Fundamental tests measure well defined parameters such as elastic modulus of viscosity. Empirical tests measure parameters which cannot be expressed in terms of fundamental rheological quantities, and, therefore, the

results obtained will depend upon the geometry of the instrument employed. Imitative tests measure various properties under test conditions similar to those to which the material is to be subjected in practice. Fundamental and empirical tests have been used most frequently to measure the heat gelation properties of proteins.

Trautman (1966) developed a test to assess the heat gel strength of proteins known as the Least Concentration End-point Test (LCE). This test involves heating a series of tubes containing protein solutions of varying concentration to 80°C for 10 minutes, cooling them to 0°C and evaluating the strength of the coagulum by inverting each tube. The LCE is the lowest protein concentration which will form a stable gel which will remain in the inverted tube. Trautman (1966) used this method to rank the processing potential of muscle tissue from various sources. A modification of this method has been used by Kijowski and Niewiarowicz (1978) to study the gelling properties of broiler breast muscle proteins.

Tsai et al. (1972) heated 5 ml aliquots of sarcoplasmic and myofibrillar proteins to 70°C for 5 minutes. The material was cooled and centrifuged at 1,000 rpm for 15 minutes. The tubes containing the heat set gels were then inverted over flasks and an examination of the heat gelling properties of the proteins was made. Centrifugation will cause very weak gels to break up causing them to exhibit no gelling properties. This approach may not be satisfactory if the results are to be extrapolated to those found in real food systems which are not subjected to severe centrifugal forces.

Hermansson (1972), Hermansson and Akesson (1975), Hermansson and Tornberg (1976) and Torgersen and Toledo (1977) have measured the viscosity (p) of heat treated protein dispersions using a Brookfield viscometer as an indicator of heat gel strength. T-spindles and the Helipath stand were used and the torque required to cut through the gel was recorded. It has been suggested

(H. Cooper, personal communication) that most sample sizes used with this apparatus are too small and the final viscosity measurements are affected by eddy formation.

Mitchell (1976) described the Marine Colloids Gel Tester which is used commercially to evaluate the heat gel strength of protein dispersions. It is capable of measuring the force to rupture the gel, a property which is often measured to indicate the strength of the heat gel matrix in a multi-component food system (Voisey et al., 1975). An Instron Texture measuring system could also be used to measure this property of a protein gel.

The objectives associated with the heat gel evaluation should dictate the choice of test method. Fundamental test methods should be used when the aim is to use rheological experiments to elucidate gel structure (Mitchell, 1976). For quality control purposes, where ease of operation and reproducibility are important, generally only one quantity needs to be determined using an empirical test. If, however, it is desired to measure the heat gel strength of a protein dispersion so that the results can be related to the gelation properties of the food system in which it is to be incorporated, then a test should be chosen which measures the same property as the test used on the food system.

2.5.3 Water binding capacity

The ability of proteins to bind water is an important attribute when considering their application in many multi-component food systems including sausages, doughs, processed cheeses and custards (Kinsella, 1976). In these foods, proteins permanently imbibe water but do not dissolve because of insufficient water. This functional property must be determined to facilitate adjustment in existing food formulations when interchanging protein sources. Water binding by proteins may be caused by any of the following properties (Hermansson, 1979):

a) the ability to swell and take up water

b) a high viscosity caused by soluble molecules, swelled particles or a mixture

c) the ability to form a gel network during processing

2.5.3.1 Theoretical aspects

Kuntz and Kauzmann (1974) reviewed the literature concerning the physicochemical aspects of water with proteins while Berendsen and Migchelsen (1968) discussed the influence of protein structure on water absorption. Hamm (1960, 1972, 1973, 1975) has studied the mechanisms of water binding by proteins using meat proteins.

Proteins are apparently surrounded by a "loose" hydration shell composed of several layers of water: viz. an "innermost" layer consisting of water (10 to 20 molecules water per molecule of protein) tightly bound to specific sites on or in the protein molecule; another layer of water (10^2 to 10^5 molecules) more loosely bound covering the immediate surface of the protein molecule (i.e. adsorbed, hydrogen bounded, non freezable water); and second, third and additional layers of water with properties graduating to bulk water in physical properties (i.e. essentially non structured water surrounding the adsorbed layers).

The hydration shell is not influenced significantly by changes in the protein structure and thus it cannot be altered by manipulating processing conditions. In contrast the amount of "free" water bound by a protein material can be altered by altering the protein structure (Kinsella, 1976) and spatial molecular arrangements (Hamm, 1975). For example, initial denaturation of soy proteins which results in dissociation and unfolding of the molecule to expose additional "free" water binding sites will result in an increase in the water binding capacity (Kinsella, 1979) provided the viscosity remains unchanged. Viscosity has been found to be positively related to water binding capacity (Hermansson, 1972; Kinsella, 1979).

By decreasing the cohesion between adjacent protein molecules or filaments (as is caused by increasing the electrostatic repulsion between similar charged groups or by weakening the hydrogen bonds) the network is enlarged and the molecules can imbibe and immobilize more water within the larger meshes as illustrated in Figure 2.4.

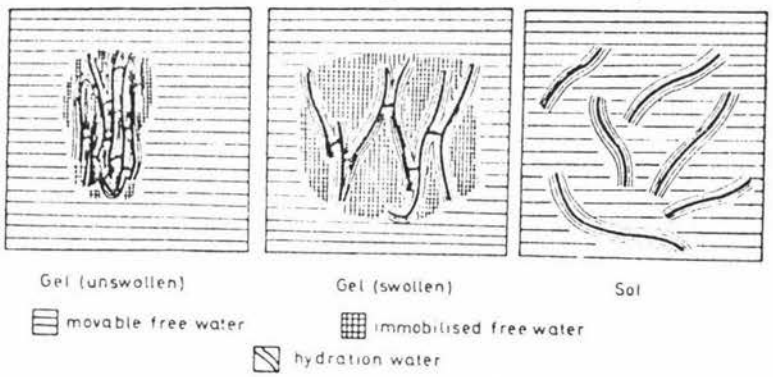


Figure 2.4 The degree of cohesion between adjacent protein molecules and its effect on the amount of immobilized water (Hamm, 1975).

As the intermolecular cohesion becomes more and more loose, the network finally collapses and the gel becomes a colloid solution of the protein. On the other hand, by increasing the attraction between adjacent molecules (as occurs in thermal denaturation of meat myofibrillar proteins) less space is available for the retention of immobilized water.

2.5.3.2 Factors affecting the water binding capacity of proteins

According to Briskey (1970) and Hermansson (1973, 1975) the degree of protein hydration is directly influenced by pH, ionicity, temperature and protein concentration. Water binding capacities of protein slurries also will vary with protein source, composition and the presence of carbohydrates and lipids (Kinsella, 1976). In this study only the effect of temperature, protein concentration and the addition of common salt on the water binding capacity of each test protein was considered while all other parameters were held constant. Therefore this discussion will be limited to these three factors.

i) Salt

The addition of salt causes a change in the water binding properties of soy proteins (Hermansson, 1972; Fleming et al., 1974), caseinates (Hermansson, 1972) and meat proteins (Hamm, 1963; Hermansson and Akesson 1975a)although effects are different for different proteins and ionic strengths. The effect of the addition of salt on the water holding capacity of a protein is thought to be mainly due to chloride ion which has been described in Section 2.5.1.2.

ii) Temperature

Subjecting proteins to heat treatments in the range 10°C to 80°C generally results in a change in the water binding capacity although the form of the change depends on the type of protein under investigation. Hamm (1975) reports that a considerable decrease of water holding capacity occurs during the heating of meat, which is due to the tightening of the myofibrillar network by heat denaturation of the proteins.

Kinsella (1979) reported that heat treatment between 25°C and 80°C enhanced the water binding capacity of soy isolate which may be due to increasing viscosity (Kinsella, 1979) or thermal unfolding of the soy molecules (Wu & Inglett, 1974). Caseinates do not undergo thermal aggregation at processing temperatures but exhibit a decrease in viscosity. The latter phenomenon is likely to result in a decrease in the water holding capacity of this material following heat treatment, although verification of this has not been found in the literature.

iii) Protein concentration

Grabowska and Hamm (1978) report that there is an optimum protein to water ratio at which the water binding capacity of a meat protein homogenate is maximized (this represented a 9.0% protein concentration for the conditions they used). For soy products, water binding capacity has been reported to increase with the protein concentration of the raw material (Kinsella, 1979).

The effect of protein to water ratio and raw material protein concentration on the water binding capacities of proteins is likely to be confounded with viscosity effects.

2.5.3.3 Measurement of water binding capacity

Measurement of water binding capacity of a protein denotes the water which is retained by a protein following filtration and/or the application of mild pressures and/or centrifugal forces.

Assessment of the water binding capacity of meat proteins has mainly employed pressure methods (e.g. Grau and Hamm, 1953; Pohja and Niinivaara, 1957; Wierbicki and Deatherage, 1958; Zessin et al. 1961) although a filtration method has been used on several occasions by Jay (1964) and Shelef & Jay (1969) to measure the water released from meat protein homogenates of varying microbial quality. Most of the work on vegetable, and non-meat animal protein concentrates and isolates used centrifugal force to determine water binding properties (Marsh, 1953; Wierbicki et al., 1957; Swift and Berman, 1959; Bendall and Wismer-Pedersen, 1962; Wierbicki et al., 1962; Bouton et al., 1971, 1972; Schults et al., 1972; Thomas et al., 1974; Hermansson and Akesson, 1975).

i) Pressure methods

The most widely used method in this category was originally devised by Grau and Hamm (1953). A meat sample of about 0.3 g is pressed on filter paper, between two plexi-glass plates, to a round thin film and the water released is quickly absorbed by the filter paper around the meat film. The area of the wetted area around the film is proportional to the amount of free water in the meat. Several modifications, associated with controlling the applied force, have been made to this method in order to improve the reproducibility of results. Pohja and Niinivaara (1957) applied the pressure to the meat sample by placing a load on the plates. Wierbicki & Deatherage (1958) used a hydraulic press operating at 35.2 kg/cm^2 while Zessin

et al. (1961) used a higher pressure of 70.5 kg/cm^2.

ii) Centrifugal methods

Centrifugal water binding capacity determinations have been carried out on samples where no extra water has been added (e.g. meat homogenates) as well as protein slurries where water is added to a sample of known weight and the amount that remains bound following the application of force is recorded (Marsh, 1953; Swift and Berman, 1959; Bendall & Wismer-Pedersen, 1962; Thomas et al., 1974). Varying centrifugal forces have been used in these tests: (3,000-5,000 rpm for 15 minutes (Marsh, 1953), 15,000 rpm for 20 minutes (Swift and Berman, 1959), 18,000 rpm for 30 minutes (Hermansson and Akesson, 1975), and 36,000 rpm for 1 hour (Bouton et al., 1972)) the choice of which condition appears not to be critical and is probably governed by the equipment that is available.

iii) Filtration method

The filtration test used by Jay and associates, known as the Extract Release Volume (ERV) test, involves mixing the protein sample with water, pouring the mixture directly into a filter funnel equipped with a sheet of No. 1 Whatman filter paper; collecting the released fluid in graduated measuring cylinder and determining the volume collected after 15 minutes. No reports of the suitability of this method for non-meat proteins materials have been found.

The choice of method and operating conditions for determining the water binding capacity of protein materials should be made bearing in mind the type of materials under test and the reason for carrying out the determination. If the aim is to assess the water binding capacity of a material for use in a given food system then a test should be chosen with physical and chemical condition resembling those of the processed food system. Subjecting a meat sample to centrifugal forces of 36,000 rpm for 1 hour would seem inappropriate and would be likely to provide misleading results if the

water released from the product after overnight chill was of interest. In this case the use of the filtration method would seem more rational.

Goutefongea (1966) made a comparison between the centrifugation and pressure methods for determining water binding capacity. He used the methods of Swift and Berman (1959) and Wierbicki et al. (1957) and a modified method by Grau and Hamm (1953) which involved pressing the meat sample between two pieces of filter paper and recording the weight difference of the sample. For repeatability of results and considerations of speed and simplicity the plate pressure method was preferred. Hermansson & Akesson (1975), however, found this pressure plate method to be unsuitable for water binding capacity studies on powdered protein materials such as caseinate and whey protein concentrates.

2.5.4 Emulsification properties

The ability of protein to aid the formation and stabilization of emulsions is critical in many applications in chopped, comminuted meats, cake batters, coffee whiteners, milks, mayonnaise, salad dressings and frozen deserts (Kinsella, 1979). In these products varying emulsifying and stabilizing capacities are required because of the differing composition and process treatments to which these products are subjected.

The two most commonly measured emulsifying properties of proteins are emulsion or emulsifying capacity, usually defined as the volume of oil (ml) that can be emulsified by protein (g) before phase inversion or collapse of the emulsion occurs, and emulsion stability which refers to the ability of a protein to form an emulsion that remains unchanged for a particular duration, under specific conditions (Kinsella, 1976).

An emulsion is defined as a dispersion of one liquid in a second immiscible liquid with the dispersed phase consisting of macroscopic droplets usually within the size

range of 0.1-100 μ in diameter (Randall, 1978). In food systems, oil and an aqueous phase constitute the two phases forming either an oil in water type emulsion, where the oil droplets are dispersed in the continuous water phase, or a water in oil type emulsion where the reverse occurs. This discussion will be limited to the first of these two emulsion types.

2.5.4.1 Theoretical aspects

Only the theoretical aspects of classical emulsions will be considered as the theory of a sausage emulsion has already been discussed in Section 2.3. The theory of food emulsification has been reviewed by Friberg (1976).

Emulsions are thermodynamically unstable because of the positive free energy caused by the interfacial tension between the oil and water phases. Stabilization of emulsion droplets is achieved by the formation of a charged layer around the fat globules causing mutual repulsion and/or the formation of a membrane film around the droplets by solutes, e.g. protein, which lowers the interfacial energy and physically prevents droplet coalescence. This latter effect may be further enhanced by a hydration layer around the interfacial material (Kinsella, 1979).

The ability of proteins to stabilize an emulsion is dependent on their ability to lower the interfacial tension between the two phases. This is a function of the ease with which the protein can migrate to, adsorb at, unfold and rearrange at the interface (Bull, 1972). When proteins arrive at the boundary of the two phase system they take up a configuration with low free energy in which hydrophobic segments of the polypeptide are exposed to the lipid interface and the polar ionic segments to the aqueous phase (Schut, 1976; Webb, 1976; Kinsella, 1979). As a consequence interfacial protein denaturation occurs, the extent of which depends on the flexibility of the protein, the stability of the native conformation (i.e. extensive

intermolecular disulphide bonds would tend to retard unfolding), and conditions prevailing in the environment (Friberg, 1976; Phillips, 1977).

Factors favouring stability in emulsions are (J. Le lievre, in litt.):

i) low interfacial tension

ii) strong mechanical strength in the interfacial film and resistance to outside forces which depends mainly on the viscoelasticity property of the film (Schut, 1976). Viscoelasticity of a protein film is higher the greater the length of the side chains and the more polar the characteristics of the protein molecule.

iii) relatively small volume of dispersed phase which results in a decreased probability of a collision between stabilized dispersed phase particles.

iv) small droplet size (i.e. high surface area and low protein concentration per unit area of film). Sedimentation and creaming occur more readily with the larger droplets while film elasticity is reduced at high surface surface concentrations (Schut, 1976). Droplet size will depend on the splitting mechanism caused by the emulsion processing method, and the ability of the protein membrane to prevent coalescence of oil droplets (Hermansson, 1979).

v) high viscosity. The more viscous the dispersion medium the slower the rate of collision and the rate of creaming and sedimentation

The underlying mechanisms of emulsion instability are flocculation and coalescence as illustrated in Figure 2.5 (Hermansson, 1979).

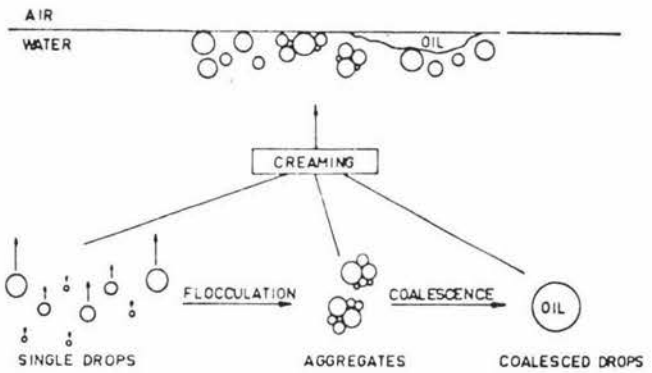


Figure 2.5 A schematic representation of emulsion stability

Inversion of an emulsion occurs when there is a sudden change from an oil in water emulsion to a water in oil emulsion or vice versa. The mechanism of inversion is uncertain however it is known that the following factors are important (J. Le lievre, in litt.):

i) phase volume ratios

ii) concentration of the emulsifying agent. When there is insufficient emulsifying agent present a monolayer cannot be formed around all the dispersed particles and the emulsion inverts

iii) temperature. As temperature increases inversion occurs at a lower concentration of the dispersed phase which may be due to thermal denaturation of the emulsifying agent making it unavailable for film formation.

The emulsion capacity of a protein material is generally considered to be related to the percentage salt soluble proteins in the emulsifying agent(Nilsson et al., 1971).

2.5.4.2 Factors affecting emulsification

As pointed out in Section 2.5.4.1 the extent at which a protein can denature at the oil/water interface and thus stabilize the emulsion is dependent on the conditions in which the emulsion is formed in particular pH, temperature and ionic effects (Friberg, 1976; Phillips, 1977). In this study the effect of addition of salt on emulsion capacity and emulsion stability was studied along with the effect of temperature on emulsion stability. Therefore this discussion will be limited to these two parameters.

i) Temperature

Emulsion stability has been reported to decrease with increasing temperatures for meat proteins (Schut, 1976) although the method of destabilization by heat is not completely understood. Elevated temperatures cause phenomena such as coagulation, and shrinkage of the proteins which is thought to influence stability in an unfavourable way (Schut, 1976).

It would be expected that thermal treatment of an emulsion stabilized by the non heat coaguable protein caseinates would also lead to destabilization which would not be due to coagulation of the emulsifying agent but the decrease in viscosity which results when this material is heated.

ii) Salt

Swift et al. (1961) and Swift and Sulzbacher (1963) determined the influence of salt on the effectiveness of meat proteins as emulsifiers. The emulsifying capacity was found to increase with increasing salt concentration (0.5, 1.0 and 2.0 M) and this was thought to be due to the salt rendering the proteins more soluble in water. A critical relationship, however exists between pH and salt concentration in assessing the emulsion capacity of proteins (see Section 2.5.1.2). Under conditions where salt addition results in increased protein solubility the stability of the emulsion

is also likely to be enhanced unless decreases in viscosity also result as has been demonstrated for caseinate and whey protein concentrates (Hermansson and Akesson, 1975).

2.5.4.3 Methods used to measure emulsion capacity

Many modifications of the model system developed by Swift et al. (1961) have been used to assess the emulsion capacity of proteins. Essentially oil is added at a given rate to a constantly stirred protein dispersion until the emulsion inverts into a water-in-oil emulsion. The registration of the inversion point varies from a visual estimate of the reduction in viscosity (Swift et al., 1961; Carpenter and Saffle, 1964; Pearson et al., 1965) to changes in amperage required to drive the blender (Crenwelge et al. 1974), or to changes in electrical resistance of the emulsion (Webb et al., 1970; Satterlee and Free, 1973; Smith et al., 1973). Most conditions used in determining the emulsion capacity have been chosen arbitrarily by various investigators which makes the comparison of data on the emulsifying capacity of different proteins from various studies very difficult. Conditions such as equipment design, shape of container, speed of blending, rate of oil addition, temperature, pH, protein source, solubility, concentration, kind of oil used, salt (type and concentration), sugar, and water content have all been found to affect the emulsion capacity of proteins (reviewed by Saffle, 1968). All these influencing factors raise doubts about the possibility of evaluating the field of application of a protein in food emulsions by this type of method.

The results of emulsion capacity tests may, however, serve to rank a series of proteins against each other if their emulsifying properties are markedly different from each other which, under certain circumstances, may be useful.

2.5.4.4 Methods used to measure emulsion stability

Emulsion stability measures the ability of an emulsion product to remain durable and unchanged. Instability will appear as creaming and fat separation from the bulk of the product (Figure 2.5). Thus emulsion stability has been measured from oil separation (Inklaar and Fortuin, 1969; Morrison et al., 1971; Neelakantan, 1971; Tsai et al., 1972, Smith et al., 1973) with centrifugation being used in some cases to separate the oil from the treated material (Inklaar and Fortuin, 1969; Morrison et al., 1971). In the study of Tsai et al. (1972) no distinction was made between the amount of fat/oil and water lost, their combined loss being taken as a measure of emulsion stability. It is questionable whether centrifugation provides a valid stability test for highly stable emulsions (Kitchener & Musselwhite, 1969). Measurement of the size distribution of fat particles as a function of time is time consuming but more informative (Mita et al., 1973).This test may not be warranted if the intention is to determine the amount of fat and water that may be released from an emulsion food product containing a test protein.

Another approach is to measure the extent of creaming as a rapid test to characterize the stability of the emulsion produced. Various parameters can be chosen to evaluate creaming. Such as depth of cream layer, percentage of the total fat collected in the cream layer (Tornberg & Hermansson (1977) or the percentage of the total fat left in the aqueous lower phase (Vakaleris and Sabharwal, 1972). The percentage of water (Acton and Saffle, 1970, 1971) or of total solid (Smith and Dairaki, 1975) in the aqueous lower phase can also be recorded. Mild centrifugation is often used to accelerate the creaming rate of the fat particles. The tests vary depending on storage temperatures and storage time of the emulsions.

With all these test methods the method of emulsion formation will affect the observed stability results (Tornberg and Hermansson, 1977; Kinsella, 1979).

2.6 Characterization of the functional properties of new protein products

Evaluation of new protein products in terms of their functional properties is complicated by the many different test methods that are employed, the empirical nature of many of these methods; the substantial array of functional properties that are important for proteins to be used in food systems, and the many combinations of physical and chemical conditions found in multicomponent food systems. Three approaches have been applied in characterisation studies of functional properties of new protein products: the trial-and-error approach using industrial formulae and equipment; the use of model systems; and a combination of both of these.

Traditionally new protein ingredients were tested using the trial-and-error approach which involves testing the ingredient using industrial equipment in various types of foods (Hermansson, 1979). This approach is time consuming and in the long run, turns out to be expensive and inefficient for the reasons discussed in Chapter 1. An example (Hermansson, 1979) readily illustrates these drawbacks. To assess the functional properties of two new protein products for use in an emulsion-type sausage, using the trial and error technique, would involve testing them directly in an industrial recipe. This would probably mean a rather low concentration of the ingredients and the presence of several other food components. The meat used would be a mixture and thus quality variations may be considerable. If a difference between the sausages containing the two proteins was noted, it would be very difficult to say whether it is due to different functionality of the protein ingredients; minor variations in processing conditions, e.g. temperature; variations in the meat quality; or

variations due the other ingredients. Consequently, a large number of tests would have to be done to establish a difference between the two ingredients as additives in the sausage formulation. This type of test gives no information on the specific function of a protein and lack of knowledge makes it difficult to handle the protein in the right way. The requirement of large quantities of raw material for this technique may be reduced if pilot plant industrial equipment is used to prepare the test batches but care must be taken to ensure the same processing conditions prevail. The trial-and-error approach was taken by Thomas et al. (1973) who used fresh sausage and cooked luncheon sausage characteristics to determine which of the three types of non-meat proteins produced superior water retention and sensory properties in these products. Care was taken to ensure that fat and protein levels for all formulations were equivalent although the moisture level was not controlled. Therefore differences between the proteins may have been due to differences in moisture content rather than protein type. Preparation of the sausages was carried out in a silent cutter. However, selective addition of ingredients was not carried out as is done on a commercial basis which may have caused results to be different than would be observed if the proteins were incorporated into an equivalent commercially processed product. Randall et al. (1976) used a simplified commercial sausage formulation to evaluate the properties of some plant and animal proteins. However the results of this study are questionable as although the moisture and fat levels were the same for all formulations, protein levels differed between formulations and the latter effect could have been confounded with the effect of protein type. The emulsions were prepared using a Polytron homogenizer which would produce a product with different properties compared to one manufactured using a silent cutter. Thus extrapolation of these results to those expected in a food product could be misleading. Meyer et al. (1964) used a commercial formulation and commercial equipment in their study However in all these studies no understanding of why the addition of various

proteins produced different responses in the final products was obtained.

An alternative approach to the trial-and-error technique is to use model systems (e.g. Lawhon and Carter, 1971; Hermansson and Tornberg, 1976; McWatters & Cherry 1977; Rahma and Narasinga, 1979) in which the behaviour of proteins can be measured objectively. With a model system the property of the protein is determined in a simple experimental system with a controlled environment so that the function of the ingredient is more easily understood and the number of large scale tests can therefore be restricted. This approach can reveal properties that are important for certain food application at an early stage (Hermansson, 1979).

For a proper functional characterization combinations of several data are necessary as it is the pattern of data rather than individual measurements which give information on functionality (Hermansson, 1979).

The number and complexity of food systems and the wide range of functional properties of proteins would mean that many test model systems would be required to determine the suitability of a new protein for general food applications. The empirical nature of these tests also dictates the use of commercially available proteins as comparative benchmarks for assessing the functionality of the new protein. Therefore, it is important that a series of model systems be designed to test critical criteria which would be important in the type of food product in which the test material is likely to be used. For example, it would be pointless to determine only the emulsion characteristics of a protein if its thermal gelling properties were also important for the food in which it were to be used. The simplicity of many of the model systems make it very difficult for valid extrapolation of the performance of the protein in food applications with different food components and environments. Matill (1971) has emphasized the

deficiencies and misleading information that is associated with many empirical functional tests, many which cannot be related to practical conditions.

Such problems arise when synergistic or antagonistic relationships between food components and test proteins occur.

An improved approach is to combine the use of model systems with controlled test food systems. Under these conditions the results from the model systems can be used to help identify and estimate relationships of the test materials with components in the food system, and can be useful as predictors of possible effects of added proteins in processing. At the same time data from the food system can have direct meaning to the processing industry. Generally pilot plant equipment is used to prepare food systems when this approach is taken.

The multiplicity of interacting variables and parameters that require determination (i.e. different protein environments, functional properties and applications) means that an essential component of studies using this combined approach for evaluating new proteins is the employment of appropriate statistical methods that will simplify experimental design, data reduction and presentation. In addition these techniques will help in the development of mathematical models to describe the behaviour of the protein. The use of statistics in this field is discussed in the following section which also reviews the combination approaches that have been used and the relationships that have been identified between functional properties measured using model systems as well as between these properties and those observed in food systems.

2.6.1 The use of model systems combined with test food systems to characterize the functional properties of protein products

2.6.1.1 Choice of the test food system

The most frequently used food systems have been emulsion-type sausages although formulations have varied in complexity (Smith et al. 1973; Lauck 1975; Torgersen and Toledo, 1977; Kijowski and Niewiarowicz, 1978, 1978a; Chattoraj, et al., 1979). A commercial meat ball recipe was used by Hermansson (1975). Processed meat systems have been chosen because they rely on the presence of proteins with several important functional properties as outlined in Section 2.3 and also because new protein additives have the potential to be used in these products on a commercial basis. When comparing the performance of different protein additives, achieved by replacing a certain percentage of the meat there is often no attempt made to ensure that the fat, protein and moisture contents of the prepared formulations are the same. Achieving this criteria is complicated by the fact that the protein additives under comparison have markedly different compositions. Torgersen and Toledo (1977) controlled the water and fat levels of all formulation but differences in protein content existed. Their results could, therefore, be used only to assess the commercial viability of a test protein product on a weight for weight basis compared to an existing product but they are of little value in understanding the functional properties of the protein fraction of the new product.

The simplest formulations use one type of lean meat, fat or oil, water and salt (Torgersen and Toledo, 1977; Kijawski and Niewiarowicz, 1978, 1978a; Chattoraj et al., 1979) and can hardly be compared to the commercial emulsion sausage. The advantage of this simplicity is that results can be interpreted easily. Complex commercial formulations have been used by Smith et al. (1973); Hermansson (1975); and Lauck (1975). Smith et al. (1973) included additives

into the mix so that the finished product would contain them at approximately 3.5% of the total weight of finished frankfurters. Lauck (1975) used least cost formulations to compare the performance of sausage emulsions with and without various meat binders however fat and protein levels varied between formulations containing the different binders. A modification of this mathematical technique, goal programming, could be used to overcome this problem (A.Anderson, personal communication) and could also be used to ensure that the base formulations of sausages containing the additives would have the same functional properties.

The advantage of keeping the protein to water ratio quotient constant have previously been discussed for model calculations on sausages (Hennig, 1971; Lindner and Stadelmann, 1961). If the composition is not constant it is difficult to tell whether any differences observed between the test systems are due to the functional properties of the protein additives or to compositional variations, and what relationships exist between the functional properties determined using model systems and the properties exhibited by the test food product.

2.6.1.2 Measurement of food system properties related to the functional properties of protein additives

The effect of adding proteins to emulsion-type sausage systems is frequently determined by measuring fat and water losses of fresh and cooked sausages and/or the texture of the cooked sausage. The latter is usually done using both objective (i.e. instrumental) and subjective (i.e. taste panel) procedures (Smith et al., 1973; Hermansson, 1975; Lauck, 1975; Torgersen and Toledo, 1977; Kijowski and Niewiarowicz, 1978, 1978a; Chattoraj et al., 1979).

Smith et al. (1973) used the method of Morrison et al. (1971) to determine the cooking losses which involves weighing a sample of the raw mix into a glass bottle,

heating it and decanting off the fluid. Emulsion stability is then expressed as the ratio of weights before and after cooking. The disadvantage of this method is that no distinction is made between fat and moisture losses which are related to different functional properties of the proteins in the formulation. The procedure of Saffle and Helmer (1963) was used by these researchers for high fat content frankfurters. This method is similar to that already described except that the mix is weighed into a Paley bottle and the percent fat separation can be read directly from the scale on the neck of the bottle following heat treatment. Torgersen and Toledo (1977) determined the amount of fat and water lost from sausages after cooking them in disposable foil loaf pans using a Thelco Laboratory oven. After cooking the pans were removed from the oven, the loafs transferred to a tray and allowed to drain for 30 minutes at room temperature. All the fluids that separated from the loaves were transferred into a graduated cylinder and the volumes of the white top layer of fat and the yellowish bottom layer of water were recorded. These volumes were divided by the total weights of uncooked batter in the loaves and expressed as a percentage (v/w). This method of collecting the fluid depends on large volumes of fluid release and may not be sensitive enough to measure cooking loss differences between sausages containing proteins with good water binding and emulsification properties. No mention of controlling moisture and fat losses within the oven was made and this parameter could contribute significantly to the results. The use of a closed container could be of assistance in this respect. Lauck (1975) used a press method described by Ackerman (personal communication) to assess stability parameters of cooked emulsion-type sausages.

The texture of emulsion-type sausages can be measured using instrumental methods or a taste panel. The objectivity of instrumental methods often gives them the advantage over taste panels.

Objective measurement of texture has employed the use of many instruments as reviewed by Voisey et al. (1975) and Huang and Robertson (1977). Instrumental methods measure a variety of rheological properties and it is important to be able to identify what these are. Voisey et al. (1975) carried out a study in which a series of instrumental tests were compared to sensory evaluations of wiener texture, the results of which can be used for selection of instrumental test methods. Quinn et al. (1979) have studied the effectiveness of the Universal Food Rheometer and the penetrometer to predict sensory firmness and chewiness of wiener sausages.

Voisey et al. (1975) evaluated wiener texture using the Warner-Bratzler Shaw, compression to rupture, relaxation, force per unit deformation and resilience tests and using sensory analysis as a standard for comparison. From their results they recommended that a simple instrumental texture profiling technique for testing wieners would be to use a combination of compression tests if equipment is available: first to compress the sample non-destructively to record resilience and then a second compression cycle to compress the sample to rupture to establish firmness and chewiness. Such a test could be easily accomplished automatically on an instrument such as the Instron Universal Testing Machine or the Ottawa Texture Measuring System by appropriate arrangement of the crosshead cycling controls. In this study sausage skins affected the observed textural properties and therefore should be removed if this characteristic is not important.

Untrained taste panels have been used to rank their preference for sausages (sometimes specifically for textural properties) containing different protein additives (Smith et al., 1973; Lauck, 1975). The use of this system of textural analysis does not give any indication of what textural parameters are affected by the inclusion of the test proteins and how they are affected and is, therefore, not a satisfactory evaluation system to use in these circumstances. Trained panels should be used in preference.

Torgersen and Toledo (1977) used both the compression test and Warner-Bratzler Shear test to evaluate the texture of their sausages. Hermansson (1975) combined the use of objective tests (using the Instron Universal Testing Machine) of extrusion force (Kp) and compression work (g cm) with sensory evaluation. She determined the relationship of these test results when determining the textural properties of meat balls containing test proteins. This approach allows a check to be made between the property measured by the objective tests and their sensory equivalents.

2.6.1.3 Mathematical approaches for use when employing model systems and the trial-and-error technique when studying functional properties

To determine relationships between model system functional properties and between these properties and parameters measured in real food systems the use of several mathematical techniques have been suggested; some of which have been implemented.

Toda et al. (1971) used a multivariate technique - principle component analysis, to clarify relationships between measurements obtained using instrumental texture measuring devices. Correlation coefficients were used to determine correlations among the objective tests. Principle component analysis was used to summarize the objective and sensory data and determine which components were important in explaining the texture of test proteins and which components differentiate different groups of protein gels. Correlation coefficients were also used to determine relationships between the sensory ratings and objective test measurements. When studying functional properties of new proteins principle component analysis could possibly be used to determine which functional properties are most important in explaining the textural properties of a food system. The use of correlation coefficients has already proved to be

an efficient way of determining the relationships between model system results and between these physical properties and the functional characteristics of test meat systems (Smith et al., 1973; Torgersen and Toledo, 1977).

Response surface methodology (Henika,1972)has been suggested for use in this area (Kinsella, 1976). This technique is likely to be of most value when studying the effect of physical and chemical conditions on a specific functional characteristic of proteins, similar to the study of Hermansson (1973).

Hermansson and Akesson (1975, 1975a) and Hermansson (1975) used a number of statistical techniques, hierarchical clustering methods and multiple regression models to obtain a general description of the principal correlations between functional properties of proteins and their performance in model meat systems. Step-wise multiple regression techniques have also been described by Gillet et al. (1977). These techniques warrant carefully designed experimentation.

2.6.1.4 Relationships identified between empirically determined functional properties of proteins

The most significant work on the identification of relationships between empirically determined functional properties of proteins has been completed by Hermansson and her associates (Hermansson, 1972, 1975; Hermansson and Akesson 1975, 1975a).

Hermansson (1972) found that the viscosity of protein dispersions is related to their solubility and swelling properties. Using model systems Hermansson and Akesson (1975) found that solubility was positively correlated with moisture loss while swelling and viscosity were negatively correlated with this property. In heat treated model systems the properties measuring gelation were highly correlated with moisture loss.

Emulsion capacity is generally considered to be positively related to the salt solubility of the proteins in the test material (Nilsson et al., 1971).

2.6.1.5 The relationships identified between empirically determined functional properties of proteins and the properties of meat products in which they are incorporated

Research into determining the relationships between empirically determined functional properties of proteins and the properties of meat products in which they are incorporated has been limited.

In the study by Torgersen and Toledo (1977) a significant positive correlation was found between the strength of a 5% protein solution at 10°C and the mechanical properties of the cooked product. Gel strength showed no significant correlation with either the consistency of the raw batter or with fat and water binding characteristics. Negative correlation was found between the solubility of the proteins and the raw batter consistency while a positive correlation existed between the protein solubility at any temperature between 4.5 and 72°C and fat lost on cooking. The spontaneous water adsorption capacity showed significant correlation with raw batter consistency and also with fat and water release or cooking.

Hermansson (1975) found good correlation between moisture loss and textural properties of meat balls with much of the variance being explained by the swelling and gel strength properties of the various protein additives.

2.7 The properties of meat proteins

Red muscle proteins can roughly be divided into three groups (Schut, 1976): the sarcoplasmic proteins, soluble in salt solutions of low ionic strength (≤ 0.1), the myofibrillar or structural proteins, soluble in concentrated salt solutions (ionic strength 0.5 to 0.6), and the connective tissue proteins, insoluble in both, at least at low temperatures.

The properties of the myofibrillar and sarcoplasmic groups of proteins are of particular importance in this study.

i) The sarcoplasmic proteins. This protein group consists of globular proteins, i.e., proteins soluble in solutions of low salt concentration but not in water, and the really water-soluble albumins. The sarcoplasmic proteins amount to about 30% of the total muscle proteins (Schut, 1976).

ii) The myofibrillar proteins. Five protein fractions make up the myofibrillar proteins: myosin, actin, troponin, tropomyosin and actomyosin and represent about 60% of the total muscle protein.

The functional properties of these groups of meat proteins in their native state are likely to change markedly if they are subjected to severe alkali conditions or hot or cold temperature, if the proteins undergo partial or complete denaturation (Kinsella, 1976; Schut, 1976).

2.7.1 Solubility

The solubility of these proteins is discussed in Section 2.7.

2.7.2 Heat gelation

The myofibrillar proteins are important in the structure of processed meats (Kinsella, 1976). The gelling properties have been studied by several groups (reviewed by Briskey, 1970). Tsai et al. (1972) reported that the myofibrillar proteins exhibit better gelling properties at 75°C when compared to the sarcoplasmic proteins.

2.7.3 Water binding

Hamm (1963) has estimated that 70% of the water content of fresh meat is located in the myofibrillar proteins, 20%

in the sarcoplasmic proteins and 10% in the connective tissue proteins. It has been estimated that the sarcoplasmic proteins account for only 3% of the total water binding capacity of meat (Pederson, 1971). As a general consequence, the myofibrillar protein are considered to be the main water binding constituents of meat.

2.7.4 Emulsification properties

Almost all research workers report that the solubilized myofibrillar proteins are superior to the sarcoplasmic proteins in the amount of fat/oil which can be emulsified and that these proteins also contribute to the stability of the uncooked emulsion (Kinsella, 1976). Hegarty et al. (1963) presented data on the emulsion capacity of isolated meat proteins and ranked them from greatest to least emulsifying capacity as: actin (in the absence of salt), myosin, actomyosin, sarcoplasmic and actin in 0.3 m NaCl.

2.7.5 Foaming properties

The foaming properties of meat proteins have not been studied.

2.8 Theoretical aspects and measurement, using model systems of protein foaming properties

In the food industry the capacity to form stiff, high volume, stable foams is an important requirement for proteins to be used in angel cakes, whipped toppings, desserts and souffle-like products. These properties can be undesirable if the protein is to be used in the manufacture of foods such as meat smallgoods.

The three most common foam properties measured for experimental foams are:

i) Whippability

This is the ability of a protein dispersion to form a foam when subjected to a high whipping/blending force, usually employing a household type mixer. In whipping

bubbles are formed by cutting the surface, whereby atmospheric gas is incorporated into the liquid (Richert, 1979). A coarse foam is formed initially and is made more dispersed by continued cutting of the bubbles.

ii) Foamability or foam capacity

Measurement of this property involves sparging or shaking air or gas through the liquid. Foam bubbles are formed and broken by shear forces during shaking while shear forces are apparently of little importance in the sparging process Waniska & Kinsella, 1979). In the latter process once the foam is formed the rate of rupture of the bubbles is a function of the lamella thickness and interfacial viscoelasticity (Cumper, 1953; Mita et al., 1977).

iii) Foam stability

This is a measure of the rate of breakdown (i.e. decrease in foam volume)or the rate of leakage of fluid from a foam.

2.8.1 Theoretical aspects

A true foam is formed when discrete air bubbles are enveloped in a liquid containing a soluble surfactant (Kinsella, 1976). The latter is known as the continuous phase. Protein acts as the surfactant and is absorbed at the air-liquid interface lowering the interfacial tension between these two phases. Soluble proteins orientate, unfold and spread (i.e. undergo partial denaturation) around the gas droplets which are being introduced into the liquid phase. The mechanism by which absorbed protein layers at the air-liquid interface stabilizse foams was not understood until recently. It has been suggested that once the protein has absorbed at the interface the protein chains link to form an elastic membrane possibly consisting of 2-dimensional networks (Manvelt, 1976) around the droplets (Wang and Kinsella, 1976). This will establish gradients in composition and therefore in viscosity

(Richert, 1979). Film thickness, viscosity and surface properties are important determinants of the rate of drainage from a foam (i.e. stability).

Proteins which form foams must exhibit a critical balance between their ability to engage in the limited intermolecular cohesions required to form a stable elastic membrane and the tendency to self-associate excessively (i.e. a critical degree of spontaneous denaturation is required). The latter results in aggregation and breakdown of foams. The rate at which proteins absorb at the interface is related to the composition and conformation of the protein (Graham and Phillips, 1976) as well as the test conditions (Richert, 1979). Flexible proteins such as β-casein absorb faster than globular proteins. The foams formed from faster absorbing proteins are generally coarse, large volume foams compared to those formed by slow absorbing surfactants (Graham and Phillips, 1976).

The presence of protein in the film, in addition to reducing the free energy at the interface, increases the dilational modulus and the resistance to shear of the film. This means that the thin films between adjacent air bubbles are able to withstand mechanical deformation while shaking continues (Graham and Phillips, 1976).

The overall volume of foam increases as long as the rate of creation of new cells is greater than the rate of collapse. Prolonged shaking leads to surface coagulation and precipitation of the proteins making them unavailable for film formation. This in turn decreases the dilation modulus of the remaining absorbed protein layers, thus decreasing the stability of the foam.

Four mechanisms by which foams breakdown have been described by Bikerman (1973). These include evaporation of the solvent from the surface, lamella film rupture, drainage from the plateau borders and film rearrangement. Flexible proteins form the least stable foams due to the low resistance to shear and dilational modulus of the films

they form (Graham and Phillips, 1976).

In certain cases coagulation upon heating is an important foam property if it is wished to preserve the original foam structure in the cooked food.

2.8.2 Factors affecting protein foam properties

Numerous factors have been reported to influence protein foam properties. These include temperature, pH, protein concentration, the presence of salts, metals, sucrose and lipids, the origin of the protein and the method used to assess the foaming properties. In this study only the effect of the test parameter, shaking time, was investigated and is discussed in Section 2.1 For a detailed discussion of all other factors the reader is referred to Shaw (1970), Bikerman (1973) and Graham and Phillips (1976).

2.8.3 Measurement of foaming properties

A protein foam can be formed by shaking a protein dispersion in a closed container, (i.e. shaking tests) mixing it in a high speed blender (i.e. whipping tests), blowing gas through a glass siniter into a protein solution (i.e. pneumatic tests) or by pouring liquids or solids into another liquid (i.e. pour tests). From reports in the literature it would appear that only the first three of these tests are used to evaluate the foaming properties of food proteins. Whipping, the most commonly used method, produces protein foams that can be measured by the increase in foam volume, specific gravity and/or viscosity (McKeller and Stadelman, 1955; Eldridge et al., 1963; Lawhon and Carter, 1971; Baldwin and Sinthavalai, 1974). Rapid shaking of a horizontal graduated cylinder containing a protein solution (1%) produces a foam that can be measured by its volume (Yatsumatsu et al., 1972; Graham and Phillips, 1976; Wang and Kinsella, 1976). Foaming capacity, resulting from the sparging of gas into the protein solution, can be measured by the ratio of the volume of gas in foam to the

volume of gas sparged, or by the maximum volume of foam divided by the gas flow rate (Cumper, 1953; Mangan, 1958; Buckingham, 1970; Bikerman, 1973; Mita et al., 1977).

The stability of protein foams is usually measured by the volume of liquid drained from a foam during a specific time at room temperature (McKeller and Stadelman, 1955; Eldridge et al., 1963; Mita et al., 1977), or by the decrease in foam volume over time (Yatsumatsu et al., 1972; Wang and Kinsella, 1976). Dynamic methods have also been employed to measure foam stability which include the rate of fall of a perforated weight through a column of foam (Mangan, 1958; Buckingham, 1970), the penetration of a penetrometer cone (McKeller and Stadelman, 1955) or the ability to support a series of specific weights (IAPI, 1956).

Thus a variety of methods have been used to produce and characterize protein foams. Whipping methods require large amounts of protein which limits their usefulness for testing experimental proteins and large increases in temperatures are observed during whipping which may be undesirable as this would affect the observed foaming properties. The smaller amount of protein required for shaking tests would be suitable for most experimental situations and as this test is carried out in a closed container the results are not affected by evaporation. A temperature increase is also observed using this method. Sparging apparatus can be designed to maintain the protein solution and foam column at the desired temperature (Waniska and Kinsella, 1979) and can be adapted to require small amounts of protein. The effect of gas type on foam formation can also be monitored using this technique. The choice of apparatus for foam property characterization should be determined by the protein end use. If it is to be used as a food foaming agent it would seem valid to choose either the whipping or shaking method as foam formation occurs in the same manner in these apparatuses as it would using commercial food mixers (i.e. bubbles are formed by cutting the surface whereby atmospheric gas is

incorporated into the liquid (Richert, 1979)). Of these two methods the shaking procedure would seem most suitable for reasons already discussed (i.e. it requires a smaller amount of protein than the whipping test and overcomes problems associated with evaporation).

Physical and chemical conditions used in shaking tests are varied making it difficult to compare foaming data from different sources.

The choice of protein concentration used in shaking tests appear to be arbitrary although concentration will affect the observed foaming properties (Richert, 1979). From a commercial point of view it would be desirable to investigate the minimum concentration required for the material to exhibit foaming properties.

Shaking tests have employed different liquid to air ratios, the choice of which will affect the observed foaming properties of material (Bikerman, 1973). Graham and Phillips (1976) used 25 ml graduated cylinders and 5 ml aqueous solutions (a liquid to air ratio of 1:4) while Yatsumatsu et al. (1972) and Wang and Kinsella (1976) shook 50 mls of the protein dispersion in 100 ml flasks yielding a gas to liquid ratio of 1:1. In order that results in the literature can be used in comparative studies it would be desirable to use a liquid to air ratio the same as one already reported.

The choice of shaking rate is also likely to affect the observed foaming results and should be gentle enough to minimize denaturation of the soluble proteins which could result in lowered foaming capacity and foaming stability results.

Graham and Phillips (1976) discussed the effect of shaking time on the foaming capacity of different proteins and found that it was related to protein configuration. From these results it would seem important to establish foaming capacity profiles with shaking time when evaluating the foaming properties of different protein types if information concerning the protein configuration is desirable.

CHAPTER 3

MATERIALS AND METHODS

3.1 Selection of non-meat protein concentrates

Non meat protein concentrates used in comparative evaluation with the three meat protein concentrates were selected after carrying out a postal survey (Appendix I) of 17 meat smallgoods manufacturers. This survey was conducted to establish which protein concentrates were currently being used in the production of meat smallgoods and amounts of these protein added.

3.2 Production of meat protein concentrates

3.2.1 MyoMPC and SarcoMPC

A modification of the extraction method developed by Hamilton (1978) was used to produce the MyoMPC and SarcoMPC. This extraction was carried out at the Meat Research Division, C.S.I.R.O., Brisbane, Australia.

Chilled beef topside was collected from Thos. Borthwick and Sons Ltd., Brisbane (approximately 24 hours post mortem) and stored at $+1^{o}C$ for 18-24 hours. Free fat was trimmed away and the topside was minced with a Kenwood Chef mincer, using the 3 mm die plate. Six kilograms of minced topside and 30 kilograms of tap water (ambient temperature) were weighed into a bottom draining plastic bucket. Dispersion was facilitated using a mechanical overhead stirrer.

A 30% (m/m) aqueous sodium hydroxide solution was added slowly until the slurry equilibrated to pH 11.0 (5 to 10 minutes). Mixing was continued for a further 60 minutes to solubilize the myofibrillar proteins. Collagenous material and large fat masses were separated from the solubilized protein by passing the dispersion through a fine kitchen sieve. Separation was hastened by manual agitation. Free fat that passed through the sieve was removed by passing the sieved extract through cheese cloth.

Neutralization of the defatted extract was achieved by the slow addition of 10% (v/v) hydrochloric acid until the pH of the extract equilibrated to pH 6.5. The extract was agitated throughout this operation with an overhead mechanical stirrer.

This neutralized extract was poured into plastic bags and frozen in approximately 1" slabs in a -20°C freezer for 16 hours. Following freezing, the slabs were thawed in a 20°C water bath and separated into two protein fractions using an Alfa Laval 12" basket centrifuge (De Laval/ATM Model 12) at 1000 rpm for 2-5 minutes. The grade III synthetic bag was used. Fibrous protein, known as the MyoMPC was retained within the bag. It was placed in 2 kg lots in double lined plastic bags, sealed and frozen at -20°C.

Soluble protein collected in the supernatant was freeze concentrated using a pilot plant scrape surface drum freezer (designed and built at Meat Research Division, C.S.I.R.O., Brisbane, Australia). The drum was refrigerated with glycol at -10°C. This step was carried out in a -15°C controlled temperature room. Ice flakes collected from the drum freezer, at -2.5°C were spun in the Alfa Laval basket centrifuge at 3,000 rpm until the ice cake retained in the basket turned white (5-7 minutes). The supernatant (which contained the soluble protein) was collected, refrozen, centrifuged twice more, increasing the protein concentration to 10% (m/m). (The reduced bulk of this material limited further concentration using this method).

Further concentration was achieved using a rotary vacuum evaporator in a -15°C controlled temperature room. Glycol at -10°C was pumped through the condenser coils. Evaporation was hastened by continually heating the round bottom flask containing the extract using a heated water bath (80°C). Foaming of the extract was minimized by carefully deaerating the system initially. Evaporation was continued (approximately 2 hours) until the protein concentration of the extract was increased to 20% (m/m). This soluble protein concentrate, known as the SarcoMPC, was placed in 500 g

lots in double lined plastic bags and placed in a -20°C freezer.

Batch sizes were limited by equipment availability. Two batches were produced over an 8 day period.

These extracts were stored at C.S.I.R.O. (Brisbane) for several weeks. After this time they were packed in dry ice and air freighted to Massey University in a frozen state. On arrival they were immediately transferred to a -20°C freezer and held for at least 3 weeks before experimentation.

3.2.2 MassMPC

A modification of the method described by Jelen et al. (1978) was used to produce the MassMPC. Production was carried out at the Food Technology Department, Massey University, New Zealand.

Boner cow brisket trimmings were collected from the boning room of Thos. Borthwick and Sons Ltd., Longburn Freezing Works, and stored at +3°C for 16-18 hours. This material was minced with a Berry Meatmaster Junior (Henry Berry Ltd.) using the coarsest plate (2 cm) and returned to the +3°C chiller until required for further processing (0-6 hours). One and a half kilograms of minced trims were placed in a motorized plastic concrete mixer and 3 kg of chilled (+3°C) tap water added. Mixing commenced (40 rpm) and 10% (m/m) aqueous sodium hydroxide solution was slowly added, until the slurry equilibrated to pH 10.5 (5-10 minutes). Tumbling was continued for a further 30 minutes.

Separation of the solubilized proteins from the fat and collagenous material was achieved by centrifuging for 7 minutes in a domestic clothes spinner (Hoovermatic) lined with a perforated nylon mesh basket. The supernatant was held for 30 minutes in a bottom draining settling tank to enable the free fat to float to the surface. After this time the defatted solution was drained out of the tank and adjusted to pH 6.8 by the slow addition of 10% (v/v) hydrochloric acid solution, using constant manual agitation. Finally 2 kg lots of the extract known as the MassMPC were frozen in double lined sealed plastic bags in a blast freezer at -20°C for 24 hours. The frozen extract was stored in a -20°C storage freezer for at least one month before experimentation.

Batch sizes were limited by equipment availability. To overcome differences between batches , the extracts from each batch were combined, for a given day's production, following the neutralization step. Twentyfive batches were produced over a period of 3 days.

3.3 Raw material analysis and storage conditions

3.3.1 Storage of dry materials

GL750 (Griffith Laboratories, N.Z. Ltd., Auckland) CasN, SV02, SV07 (N.Z. Dairy Research Institute, Palmerston North), egg white albumin (Egsol Products, Tauranga), and cornflour (Fielder's Cornflour, Neill Cropper and Co. Ltd., Auckland) were stored in plastic bags which were sealed in screwtop jars. Storage conditions were chosen to minimize moisture changes throughout experimentation.

3.3.2 Protein analysis

All ingredients were analysed for protein concentration (%) by the AOAC Kjeldahl method - 2.049 using the following nitrogen conversion factors: meat, cornflour and egg white albumin x 6.25; caseinates x 6.38; and GL750 x 5.71 (AOAC, 1975).

3.3.3 Fat

All ingredients were analysed for fat concentration (%) by the AOAC Ether Extraction Method - 7.045 (AOAC, 1975), except for GL750 which was analysed using a modified method - 14.081 (AOAC, 1975).

3.3.4 Moisture

Test ingredients were analysed for moisture concentration (%) using the AOAC methods detailed in Table 3.1(AOAC, 1975).

TABLE 3.1

Methods used to determine moisture concentration (%) in test ingredients.

Material	AOAC Method
MassMPC, MyoMPC, SarcoMPC and all meat ingredients	24.003 a)
Egg white albumin	17.007 b)
Cornflour and GL750	14.004
CasN, SV02, SV07	16.032

3.3.5 Salt (NaCl)

SarcoMPC was analysed for salt concentration (%) by the standard AOAC (1975) method - 24.007.

3.4 Dispersion methods for test proteins

3.4.1 GL750

Dispersion of GL750 was carried out in a 10°C controlled temperature room using 10°C distilled water. The required amount of GL750 was weighed (± .01 g) into a stainless steel household mixing bowl (bowl size depended on the quantity being prepared). An appropriate amount of water was then weighed (± .01 g) and added slowly, mixing to a smooth paste first. Even mixing was achieved using a stainless steel tablespoon. This mixing procedure was followed to avoid the foaming and agitation caused by mechanical mixing. The latter may lead to some protein denaturation and this was considered undesirable. Following dispersion the bowl was covered with a light gauge polyethylene film and stored in the 10°C controlled temperature room for greater than 1 hour, but less than 4 hours, until required.

3.4.2 SV07, SV02, CasN

Dispersion of the sodium caseinates was carried out at ambient temperature using 80°C distilled water. (Complete dispersion of these materials with protein to water ratios of 1:5 (m/m) at 10°C was practically impossible). The dispersion and storage method used was the same as that described for GL750. After 1 hour in the 10°C controlled temperature room the dispersions had equilibrated to 10°C.

3.4.3 MyoMPC and SarcoMPC

Twentyfour hours prior to experimentation these concentrates were removed from the -20°C freezer and placed in the 10°C controlled temperature room and allowed to thaw to +2 to +5°C. These meat protein concentrates were dispersed and stored in the same manner as GL750.

3.4.4 MassMPC

Twentyfour hours prior to experimentation this material was removed from the -20°C freezer and placed in the 10°C controlled temperature room and allowed to thaw to +2 to +5°C. The concentrate was dewatered by centrifuging (MSE 300, Speed 10) for 5 minutes. The supernatant was discarded and the concentrated material dispersed and stored in the same manner as GL750.

3.4.5 Sausage ingredients

Meat ingredients (frozen for 1 month prior to experimentation and thawed as described for MyoMPC and SarcoMPC) and cornflour were dispersed in the same manner as GL750 and used as soon after dispersion as possible.

3.5 Determination of percent soluble protein

3.5.1 Protein determination

Determination of the protein content of the supernatant was carried out using a modification of the biuret method developed by Gornall et al. (1949). The biuret method was utilized in favour of the more laborious Kjeldahl determination because of the large number of estimations that were required.

3.5.1.1 Reagents

The following reagents were prepared:

i) Biuret Reagent A

Three grams of cupric sulphate (Hydrated) (A.R.) and 12.0 g of sodium potassium tartrate (Hydrated) (A.R.) were dissolved in about 1 litre distilled water. Then 600 ml of 10% sodium hydroxide (A.R.) solution (m/m) was added with constant swirling. The solution was made up to 2 litres with distilled water, mixed and stored in a brown glass bottle.

ii) Biuret Reagent B

Biuret Reagent B was prepared in the same manner as Biuret Reagent A without the addition of cupric sulphate.

3.5.1.2 Protein standards

In order to provide calibration curves for these proteins, a series of determinations had to be carried out on non-heat treated samples as described below. One percent (m/m) protein slurries of GL750, SV07, SV02 and CasN, SarcoMPC, MassMPC and MyoMPC were prepared as described in Section 3.4 except that those of MyoMPC and MassMPC were adjusted to pH 11.5 in order to partially solubilize the proteins. These slurries were added to 50 ml Nalgene tubes and the insoluble components were removed by centrifuging for 5 mins. at 800 rpm (Universal Model U.V., International Centrifuge). In the cases of MyoMPC and

MassMPC the protein in the supernatants was assumed to be solubilized myofibrillar protein.

For each protein solution a duplicate series of nine test tubes was prepared as described in Table 3.2.

TABLE 3.2 Protein standards for biuret test

Test tube	1	2	3	4	5	6	7	8	9
ml of 1% Test Protein Solution (i.e. Supernatant)	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6
ml of distilled water	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4
ml of Biuret Reagent A	8	8	8	8	8	8	8	8	8

A similar duplicate series of nine tubes was prepared for each protein substituting Biuret Reagent B for Biuret Reagent A. Once the Biuret reagent was added the solutions were mixed thoroughly by swirling and held for 30 minutes at room temperature before transfer to plastic 1 cm cuvettes. Absorbance was determined at 540 nm in a Spectronic 20 spectrophotometer against distilled water.

Absorbance values of the solutions prepared with reagent A were subtracted from those of corresponding tubes containing reagent B to correct for nonspecific absorbance (probably attributable to pigments).

To determine the actual amount of protein in these standard solutions Kjeldahl nitrogen determinations were carried out on the remainder of the 1% protein slurry supernatants according to AOAC method - 2.049 (AOAC, 1975). These protein levels were plotted against the corrected Biuret absorbance values to construct calibration curves for each test protein material.

Each time a new batch of Biuret reagent was made up, new calibration curves were prepared in a similar manner.

3.5.2 Measurement of percent soluble protein

The protein solubility test used in this study was based on the Dairy Division Solubility Index test (N.Z. MAF Dairy Division Manual, Chemistry-Standard Laboratory Methods). All preparations were carried out in a 10°C controlled temperature room. Single one percent (m/m) protein slurries, with and without the presence of 2% salt (m/m) (except for SarcoMPC which was not studied after the addition of salt because this protein had a natural salt concentration of 3.87%) were prepared for each test protein material as described in Section 3.4 One hundred grams of the test slurry were transferred to a standard glass mixing jar (as specified by American Dry Milk Institute) then three drops of Antifoam (A.F. Emulsion, Dow Corning for NDA, distributed by Swift Consolidated N.Z. Ltd.) were added and mixed for exactly 90 seconds using a Solindex Mixer (made according to American Dry Milk Institute specifications by Crusade Electrical Ltd., Auckland, N.Z.) (shown in Plate 3.1). After mixing the slurry was allowed to stand for 15 minutes. The slurry was stirred with a stainless steel tablespoon for 5 seconds then transferred into two preweighed 50 ml Nalgene conical centrifuge tubes. The tubes were capped to avoid evaporative losses (respective caps had also been preweighed) and reweighed. Solubility tests were carried out at 10, 20, 30, 40, 50, 60 and 70°C. For test temperatures greater than 10°C the capped tubes were immersed to within 1 cm of the tube top in a water bath set at the test temperature. The tubes were held in the water bath for a predetermined time to allow the material to equilibrate to the test temperature and then centrifuged for 5 minutes at 800 rpm (International Centrifuge, Universal Model U.V., International Equipment Co., U.S.A.). Immediately after centrifugation the supernatant was aspirated off, 15 ml being retained for protein analysts and the tube containing the sediment (including the insoluble protein) was reweighed.

To measure the protein concentration of the supernatant duplicate test tubes were prepared containing 1 ml supernatant, 1 ml distilled water and 8 ml of Biuret Reagent A. These values were corrected (as with standard determinations)

Plate 3.1. Mixing apparatus used in the percent soluble protein determinations.

by preparing another set of duplicate tubes using Biuret Reagent B instead of Reagent A. The tubes were mixed thoroughly by swirling, left for 30 minutes, mixed again and the absorbance recorded at 540 nm against distilled water.

To calculate the % soluble protein for each material Equation 3.1 was used.

Equation 3.1

$$\% \text{ soluble protein} = \frac{\text{mass of protein in supernatant after heat treatment \& centrifugation}}{\text{total mass of protein in sample taken for analysis}} \times \frac{100}{1}$$

After every 48 readings protein determinations were carried out on a reference CasN solution as a between-assay check.

Values obtained from this method may have slightly underestimated protein solubilities as it was:

i) impossible to remove all the supernatant from the centrifuge tubes, and

ii) any soluble protein entrapped within the residue could not be measured

However, errors arising from these sources were assumed to be constant and it was considered that the results produced using this method were adequate for the purpose of this study.

The decision to perform these measurements of solubility with single mixtures of the test proteins was necessitated by the limited amount of time available and was probably justified as the within-assay relative standard deviation was only $\pm$ 2.87% for SV02 (Calculation shown in Appendix II).

3.6 Heat gel strength

The method used to measure the heat gel strength was adapted from the Marines' Colloids gel test described by

Mitchell (1976). Aqueous slurries of GL750, SV07, SV02, CasN, MassMPC, MyoMPC and SarcoMPC (with a protein to water ratio of 1:5 (m/m)) were prepared using the methods described in Section 3.4. All test preparations were carried out in a 10°C controlled temperature room. Five cans (66 x 78 mm, 235 ml capacity, supplied by Alex Harvey Industries, N.Z.) each containing 225 g of protein dispersion, were prepared for each protein type. One of the cans contained a nickel copper thermocouple for temperature profile measurement. In the cases of MyoMPC and SarcoMPC only three and two cans respectively were used, due to the limited quantities of these materials.

Aqueous dispersions with a protein to water ratio of 1:5 (m/m) containing 2% (m/m) common salt also were prepared for all test proteins except SarcoMPC. Five cans, each containing 225 g of protein dispersion, were prepared for each protein type. One of the cans contained a nickel copper thermocouple for temperature profile measurement. In the case of MyoMPC only three cans were used due to the limited availability of this material.

Each can was steam exhausted and seamed using a commercial seaming apparatus (John Heine, Model 710, Series 2, Sydney), and the can containing the thermocouple was connected to a Honeywell temperature recorder (Honeywell/Electronik 15, Honeywell Controls Ltd., Great Britain). All five cans from one trial were placed in an aluminium saucepan (15 litre capacity) half filled with boiling water and a weighted mesh screen was placed over them to ensure the cans were completely immersed. At this point the time was recorded on the Honeywell temperature printout and the cans were held under these conditions until the internal temperature in the can containing the thermocouple reached 70°C. The time was then recorded again and all five cans were immediately plunged into an ice bath until the internal temperature dropped to 10°C whereupon they were transferred to a 10°C controlled temperature room and held overnight (i.e. approximately 18 hours).

On the following day the cans were opened using a kitchen can opener, except for the can containing the nickel copper thermocouple which was discarded. A 1 cm diameter stainless steel flat probe was attached to the drive shaft of an Ottawa Texture Measuring System (OTMS FL 1U-3SG, Canners Machinery Ltd., Simcoe, Ontario, Canada) which had been set to a cross head speed of 10 cm/minute as described in the operating instructions with a chart speed to cross head speed ratio of 2:1. Each can was centred on a platform under the driveshaft and the OTMS switched on. The probe moved into the sample, compressing it until it ruptured and the heat gel strength was recorded as the force (g) required to rupture the gel under these conditions. One reading per can was taken and visual observations of the gel characteristics were also recorded.

When a true gel was not formed the resistance force to penetration (g) was recorded as an estimate of the heat gel strength. This was taken to be the first peak on the force deformation curve.

Not all the proteins formed a true gel. Some exhibited flow properties after heating and cooling so heat gel strength determinations had to be carried out in a container with rigid walls. It is recognized that the forces exhibited by the walls under the test conditions would have had an effect on the observed readings but this effect should be constant for each sample.

Nevertheless, in other respects this test method was found to be satisfactory, it:

i) was sensitive enough to detect subjectively observed differences in the heat gel strengths of the protein,

ii) it approximated the method used to measure the heat gel strength of the real sausage systems,

iii) it approximated the physical and chemical conditions used in the real sausage systems,

iv) it had the ability to measure the heat gel strength of proteins which did not form a true heat set gel,

v) and it had a relative standard deviation of 5.0% for SV02 protein (calculated as shown in Appendix II).

3.7 Water binding capacity (WBC)

A modification of the Extract Release Volume method developed by Jay (1964) was utilised to measure the water binding capacity of the test proteins and the ingredients for the real sausage system.

This test was performed on the test proteins over a temperature range of 10°C to 70°C, with and without the presence of 2% (m/m) added common salt using aqueous dispersions of these materials with protein to water ratios of 1:5 (m/m) and 1:10 (m/m). Water binding capacity determinations for SarcoMPC were not carried out in the presence of added salt. The WBC of the sausage ingredients were tested at 70°C, in the presence of 2% (m/m) salt using a protein to water ratio of 1:5 (m/m) except for cornflour where protein to water ratios of 1:10 (m/m) and 1:20 (m/m) were also investigated.

All dispersions were prepared as described in Section 3.4.

Immediately after dispersion a weighed sample was transferred to the filter funnel and covered with a watch glass to minimize evaporative losses. The filter funnel previously had been lined with No. 1 Whatman Filter paper and a fine nylon mesh screen which extended over the edge of the filter funnel to prevent an air lock forming when the watch glass was in place (Plate 3.2). For WBC determinations at 10°C the sample was held in this apparatus for 15 minutes in a 10°C controlled temperature room. (This holding time was required to allow the free water to drain

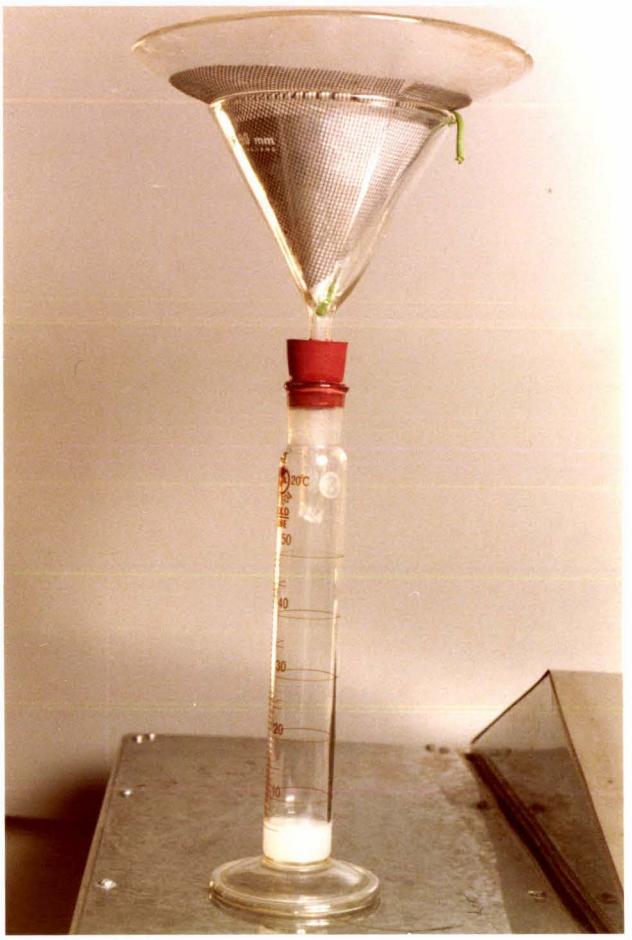


Plate 3.2. Apparatus used to measure water binding capacity.

into the measuring cylinder). In the cases of WBC determinations at 20, 30, 40, 50, 60 and 70°C the apparatus was immersed to within 1 cm of the top of the filter funnel in a water bath set to the test temperature. Preliminary investigations had established the immersion time required for the test materials to reach the test temperature. After the correct heat treatment had been applied the apparatus was removed from the water bath and the volume of solution released into the graduated cylinder known as the extract release volume was recorded. In this test water and soluble constituents were released (as would be the case in a real sausage system) and it was the total volume of these components which was used to calculate the WBC. This test was duplicated and the average extract release volume recorded.

From the extract release volume the water binding capacity was calculated as the g water bound/g protein using Equation 3.2.

Equation 3.2

$$WBC = \frac{A - B}{C}$$

Where,

A = mass of water in the prepared dispersion

B = average mass of water released during test (assuming 1 ml of the aqueous release material is equivalent to 1 g)

C = mass of protein in the original sample

Fat did not appear to be released during this test.

Preliminary investigations indicated that this test was adequate for the assessment of WBC for all test proteins and the sausage ingredients because it:

i) measured both water and soluble solids released; this measure being economically important in the production of emulsion type sausages,

ii) approximated the physical and chemical conditions used in the real sausage system,

iii) and had a low relative standard deviation of 3.07% for SV02 proteins. (Calculation shown in Appendix II).

3.8 Emulsion capacity

Emulsion capacity (EC) of the test materials was measured using a method developed by Webb et al. (1970) and modified by R. Mawson (personal communication). This method measures emulsifying capacity by utilising the fact that a large change in electrical resistance occurs when an emulsion inverts.

Protein dispersions with a protein to water ratio of 1:5 (m/m), with and without the presence of 2% added common salt (m/m), were prepared for all protein materials except SarcoMPC using the method described in Section3.4. For the latter material dispersions were prepared in the absence of salt only.

All emulsion capacity testing was carried out in the 10°C controlled temperature room. Emulsions were prepared using commercial soybean oil (Liquid-maid Products, Wellington) chilled to 10°C.

Two and a half grams of the protein dispersion under test was weighed into a glass centrifuge tube (10 cm long, 3 cm internal diameter (Schott & Gen., MAINZ, Jenner Glas)). The tube was placed under the mixing shaft (2 cm diameter) of a Polytron laboratory mixer (PT 157) and an oil delivery tube (3 mm internal diameter, fed from a graduated burette) was attached to the mixing shaft. Two copper wires, connected to a multimeter (Kyoritsu KEW 66), were also attached to the shaft. This set up is illustrated in Plates 3.3 and 3.4.The multimeter was set to read 1,000 x the resistance in ohms and the Polytron set at a fixed speed of 6.5 units (on the scale). Oil delivery (0.102 ml/sec) commenced immediately and the entire process of emulsion formation and collapse was monitored in terms of electrical resistance.

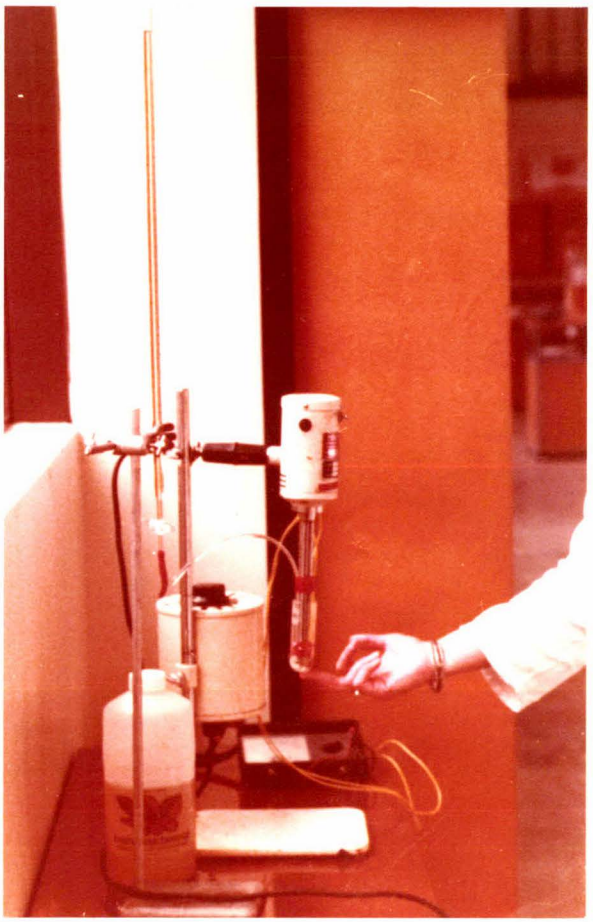
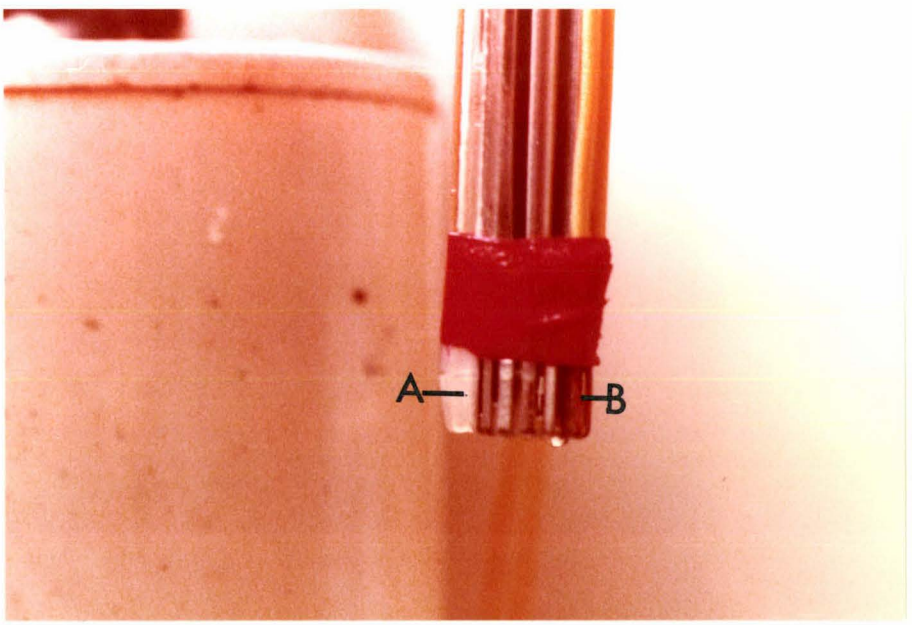


Plate 3.3.(above) Apparatus used to measure emulsion capacity.
Plate 3.4.(below) Mixing shaft showing oil delivery tube (A) and copper wires (B).



When the emulsion collapsed (i.e. the protein reached its maximum oil binding capacity) a large increase in resistance was observed. At the moment that this occurred, the oil delivery was stopped and the amount of oil added was determined by the volume difference, measured on the graduated burette.

Emulsion capacity was expressed as ml oil emulsified/g protein for each test material, calculated using Equation 3.3.

Equation 3.3

$$EC = \frac{\text{ml oil added to reach emulsion collapse}}{\text{g protein in 2.5 g of test dispersion}}$$

All trials were replicated four times. Between successive evaluations the copper wire polarity was reversed to avoid protein deposition which was found to interfere with resistance measurements. The shaft was removed and washed in warm soapy water, rinsed in 10°C water and towel dried after each run. Satisfactory quantitative and repeatable EC results were obtained using this method. The test had a relative standard deviation of 3.46% for SV02 proteins as calculated in Appendix II.

3.9 Determination of emulsion stability

A test, to measure emulsion stability characteristics which are important in an emulsion type sausage system, was developed to suit the requirements of this study.

In this test the emulsion stability was defined as a measure of the ability of the prepared emulsion to hold water and fat under conditions similar to those experienced in the production and storage of an emulsion type sausage. For this reason the stability of the emulsion was evaluated using pork back fat.

Pork back fat (supplied by Kiwi Bacon Co., Longburn) was minced in a Bauknecht mincer (AL 2-1) using the 6 mm die plate and minced again using the 2 mm die plate.

It was held at +3°C overnight to equilibrate to this temperature.

Protein dispersions were prepared as described in Section 3.4 and all further preparation steps for this test were carried out in the 10°C controlled temperature room. Protein to water ratios were selected to produce a protein to water to fat ratio of 1:5:1.67 (m/m/m) when the pork back fat was added.

Minced pork back fat and 2% (m/m) common salt (if required) were added to these dispersions to give the desired protein to fat to water ratio. The fat (and common salt) were initially blended into the protein dispersions for 2 minutes using a Kenwood minimixer (at speed 3).). This mixture was emulsified with a Polytron Laboratory Mixer (4.5 minutes at speed 6.5) using the 3 cm diameter shaft (the temperature of the emulsion was approximately 14°C at this stage) and 15 mls of the emulsion was stuffed into 21 mm Devro reconstituted collagen casings (Johnson & Johnson (N.Z.) Ltd., Auckland) using a 30 ml wide end graduated syringe. This produced a "sausage" occupying approximately 4.5 cm of Devro casing which was secured by tying knots at each end. Each "sausage" was weighed and had 20 pin pricks made in it to facilitate moisture and fat loss. The "sausages" were stored at +3°C overnight (18-24 hours) in sealed bags to prevent evaporative losses. On the following day they were wiped with absorbant preweighed tissue paper and reweighed and visual observations of the emulsion were recorded.

Stability of emulsions prepared in this manner was determined after overnight storage and at 20, 30, 40, 50, 60 and 70°C using a Heatway Upright Grill Master (Model GJ-1) (shown in Plate 3.5.)

The standard cooking procedure involved:

i) covering the Grill Master reflector plate with new aluminium foil between each cooking,

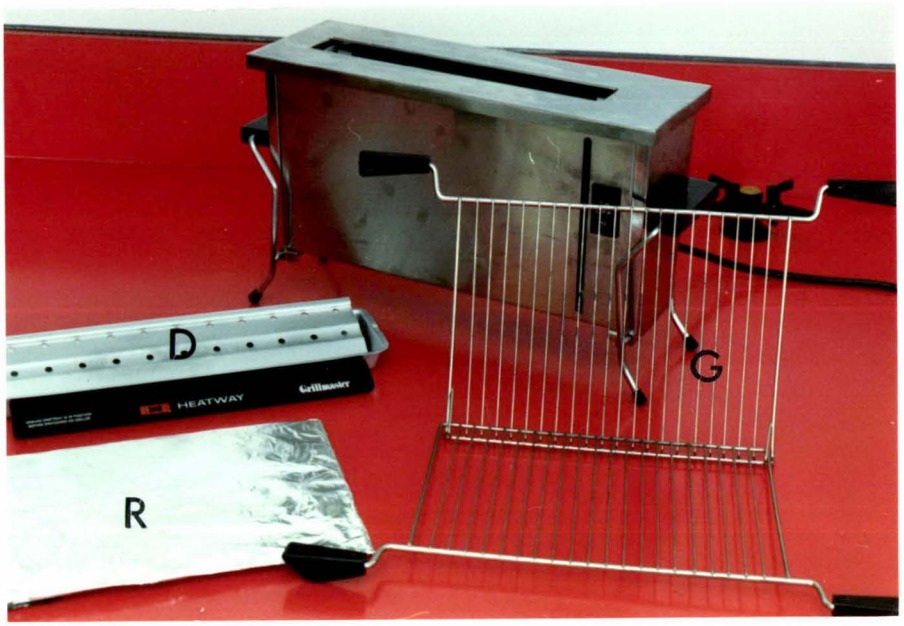
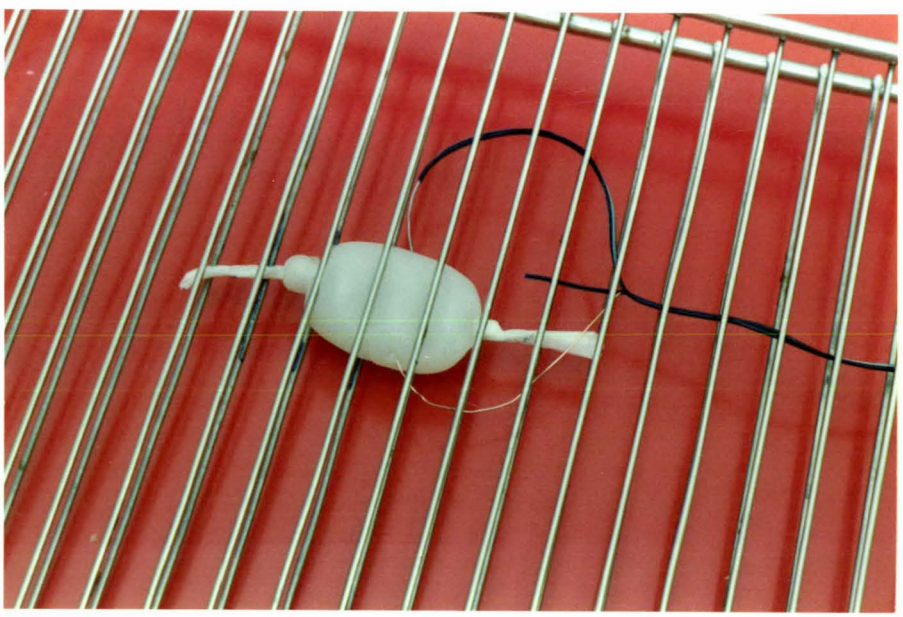


Plate 3.5.(above) Heatway Upright Grillmaster showing grill bars (G),reflector plates (R),and drip tray (D).

Plate 3.6.(below) The system employed to determine heating times for test sausages used in the emulsion stability study.



ii) pre-heating the Grill Master element at the low temperature setting for 4 minutes,

iii) placing the test "sausage" on a premarked area in the centre of the grill bars,

iv) and placing the grill bars in the Grill Master and cooking the "sausage" at the low temperature setting for the predetermined time required to reach the test temperature.

Heating times for each test protein were determined by inserting a copper nickel thermocouple (as shown in Plate 3.6) into the centre of a test sausage prepared as described. The thermocouple was then connected to the Honeywell temperature recorder and the sausage was heated to an internal temperature of 70^{o}C using the previously described cooking procedure. From the temperature recorder printout the time for the centre temperature to reach 20, 30, 40, 50, 60 and 70^{o}C could be calculated for each trial. It was assumed that these heating times would apply to all the sausages in a trial because they should have had similar heat transfer properties since stuffing conditions were standardised.

Before each cooking test the drip tray and grill bars and reflector plates covered with aluminium foil were cooled and weighed. After cooking the grill bars containing the sausages were removed from the Grill Master and left for 15 minutes to allow surface moisture to evaporate. After this time the sausage was wiped with preweighed absorbant tissue paper and both the sausage and paper were weighed. The grill bars, reflector plates covered with foil and drip tray were also cooled and weighed. The weight change of the sausage, absorbant tissue paper, grill bars, reflector plates and drip tray were known as ΔS, ΔP, ΔG, ΔR and ΔD respectively. All trials were carried out in triplicate.

It was assumed that cooling the drip tray, reflector plate covered with foil and grill bars after cooking would allow evaporation of moisture from these surfaces. There-

fore any weight difference in these pieces of cooking equipment would be due to the accumulation of fat released from the sausage under test.

For each set of test conditions the emulsion stability of the protein materials was assessed by calculating the moisture loss (Equation 3.4) and fat loss (Equation 3.5) after heat treatment as a percentage of the total moisture and fat present in the freshly prepared sausages.

Equation 3.4 Calculation of moisture loss

$$\% \text{ moisture lost} = \frac{\text{moisture loss after treatment}}{\text{mass of moisture in the freshly prepared sausage}} \times \frac{100}{1}$$

where:

$$\text{moisture lost after treatment (g)} = \text{mass of freshly prepared sausage} - \text{mass}^{*} \text{ cooked sausage} + \Delta G + \Delta D + \Delta P + \Delta R)$$

* For temperatures greater than $10^{\circ}C$ this was calculated assuming there was no weight loss overnight.

Equation 3.5 Calculation of fat loss

$$\% \text{ fat lost} = \frac{\Delta P + \Delta G + \Delta R + \Delta D}{\text{mass of fat in freshly prepared sausage}} \times \frac{100}{1}$$

This method was found to be satisfactory because it:

i) approximated chemical and physical conditions used in the real sausage system,

ii) was the same method used to measure the stability of emulsion type sausages,

iii) and had a relative standard deviation of 13.72% for moisture losses for SV02 proteins (calculation shown in Appendix II).

3.10 Sausage formulation

Sausage formulae were developed using the goal programming technique described by Anderson and Earle (*in litt.*) and the TEMPO linear programming computer system. Goal programming was chosen to develop these formulae because it allowed for the simultaneous solution of complex objectives thus providing a means of:

i) picking up changes in the sausage which were related to the functional properties of the added test protein,

ii) ensuring the composition of all sausage formulations were the same, thus overcoming the difficulties associated with some of the individual test proteins having markedly different compositions from each other,

iii) developing a complex but controlled multicomponent food system similar to that in which the proteins could be incorporated on a commercial scale.

Formulae were developed for three control sausages WBC (60), WBC (56) and WBC (40), which it was predicted would bind 60 g water/100 g mix, 56 g water/100 g mix and 40 g water/100 g mix, out of a possible 60 g water/100 g mix. For each test protein two formulae were developed; one where 3% of the total protein (12 g/100 g mix) was replaced by the test protein and in which all other ingredients had a predicted binding capacity of 56 g water/100 g mix out of a potential 60 g water/100 g mix. The other had 30% of the total protein (12 g/100 g mix) replaced by the test protein with all other ingredients having a predicted binding capacity of 40 g water/100 g mix out of the 60 g water/100 g mix that were available. Sausages with 3% of the protein replaced with test protein were referred to as SVO7 (3), SV02(3), CasN (3), GL750 (3), Mass (3), Myo (3) and Sarco (3) for the respective test proteins. Similarly sausages with 30% protein replacement were referred to as SV07 (30), SV02 (30), CasN (30), GL750 (30), Mass (30), Myo (30) and Sarco (30). The binding levels of 56 g water/100 g mix and 40 g water/100 g mix assigned to the formulations with 3% and 30% protein replacement

respectively were chosen so that the effect of addition of test protein on the water binding of the system could be monitored.

Five ingredients which are used in commercial cooked emulsion type sausages (B. Wilkinson, personal communication) were made available for the formulations. Preliminary investigations showed that the use of a greater number of ingredients made control and interpretation of the results too difficult. The five ingredients chosen were pork heart, pork trim, pork back fat, beef chuck and cornflour. These were chosen because of their varying WBC's in order that the binding levels of 40 g water/100 g mix and 56 g water/ 100 g mix could be achieved. Cornflour was added because most commercial sausage formulations in New Zealand contain carbohydrate (B. Wilkinson, personal communication).

3.10.1 The goal program

3.10.1.1 The objective

The objective of the program was to minimize the absolute deviation (percent) of the Goal program solution values for protein moisture, fat, meat, batch and water binding from the required values (i.e. constraints) for these parameters. The form of this objective function is shown in the goal programming matrix in Table 3.3.

3.10.1.2 The constraints

Equality constraints were set on protein level, fat level and moisture level, water binding, batch size and the total amount of meat in the final formulation. Fixed limits were placed on cornflour and the relevant amount of test protein to be added to achieve 3% or 30% protein replacement. All constraint levels are shown in the goal programming matrix in Table 3.3.

a) Protein constraint

This was set at 12% which is in line with the protein level in cooked sausages currently

TABLE 3.3

Goal Program Matrix

Objective Function

Minimize:

$$\left|\frac{Y_1^+}{20} \times \frac{100}{1}\right| + \left|\frac{Y_1^-}{20} \times \frac{100}{1}\right| + \left|\frac{Y_2^+}{12} \times \frac{100}{1}\right| + \left|\frac{Y_2^-}{12} \times \frac{100}{1}\right| + \left|\frac{Y_3^+}{60} \times \frac{100}{1}\right| + \left|\frac{Y_3^-}{60} \times \frac{100}{1}\right|$$

$$+ \left|\frac{Y_4^+}{75} \times \frac{100}{1}\right| + \left|\frac{Y_4^-}{75} \times \frac{100}{1}\right| + \left|\frac{Y_5^+}{xi} \times \frac{100}{1}\right| + \left|\frac{Y_5^-}{xi} \times \frac{100}{1}\right| + \left|\frac{Y_6^+}{100} \times \frac{100}{1}\right| + \left|\frac{Y_6^-}{100} \times \frac{100}{1}\right|$$

Constraints		Test protein*	Chuck	Pork trim	Pork backfat	Pork heart	Corn-flour	Water	Slack Y_i^+ variable	Slack Y_i^- variable		Constraint levels
Subject to:	Fat	see Table 4.2	0.088	0.3311	0.8423	0.0671	0.01	0	1	-1	=	20
	Protein	see Table 4.2	0.2089	0.1506	0.0387	0.1571	0.02	0	1	-1	=	12
	Moisture	see Table 4.2	0.6952	0.5141	0.1190	0.7695	0.05	1.00	1	-1	=	60
	Meat	caseinates and soy = 0 meat proteins = 1	1	1	1	1	0	0	1	-1	=	75
	Water binding		1.04	0.55	0.12	0.79	0.32	0	1	-1	=	xi**
	Batch	1	1	1	1	1		1	1	-1	=	100
	Fixed Bounds	see Table 3.4					4.00					

* Not included in the three control sausages

** xi = 60 or 56 or 40

on the market (B. Wilkinson, personal communication).

b) Moisture constraint

Sixty percent moisture was used as this level is also used in commercial sausages, making the protein to water ratio equal to 1:5 (m/m).

c) Fat constraint

A level of 20% was chosen for this constraint which is used in some commercial sausages (B. Wilkinson, personal communication) giving a protein to water to fat ratio of 1:5:1.67 (m/m/m).

d) Meat constraint

Meat constraint was fixed at 75% to comply with the N.Z. Food and Drug Regulations (1973).

e) Batch size

A batch size of 100 g was fixed.

f) Water binding

Water binding was set at 60 g water/100 g mix (i.e. binding of 100% of the available water), 56 g water/100 g mix and 40 g water/100 g mix for the three control sausages. For sausages with 3% of the protein replaced by test protein a value of 56 g water/100 g mix was used while 40 g water/100 g mix was used for sausages with 30% protein replacement.

g) Ingredient limits

Four percent cornflour was included in all formulations. The amounts of each test protein added to achieve 3% and 30% protein replacement in a 100 g batch are detailed in Table 3.4.

h) Slack variables

Two slack variables were assigned to each constraint to allow for the possible deviation above and below the constraint value (Table 3.3).

TABLE 3.4

Addition levels of the test proteins used to achieve 3% and 30% protein replacement in a 100 g batch.

Protein	g added/100 g batch	
	3% protein replacement	30% protein replacement
SV07	0.395	3.95
SV02	0.410	4.10
CasN	0.414	4.14
GL750	0.508	5.08
MassMPC	1.990	19.90
MyoMPC	1.980	19.80
SarcoMPC	1.800	18.00

3.10.1.3 Compositional data

All compositional data are shown in the linear programming matrix in Table 3.3. Water binding values reported in Table 4.5 were converted to g water bound/g raw material but values for test proteins were not included in the matrix. Fat, protein and moisture contents of all raw materials were entered into the matrix in decimal form. All meats were given a value of 1.00 and all non-meat ingredients a value of zero.

3.11 Manufacturing of the emulsion sausage systems

The method chosen to manufacture the emulsion sausages was based on observations of commercial practices used in the production of this type of sausage . Bulk quantities (25 kg) of pork trim, pork heart, pork back fat and beef chucks were purchased and each material was minced through the Henry Berry mincer using the coarsest plate. Following mincing representative samples were removed for compositional analysis. The remaining minced material was packaged in 1 kg lots in plastic bags, sealed and frozen in a -20^{o}C freezer and kept under these conditions for at least one

month before use in trials. After one month a sample of each material was removed for WBC analysis.

On the day before production the required number of 1 kg packs of each meat ingredient and the meat protein concentrates were removed from the freezer and placed in the 10°C controlled temperature room for a period of 18-24 hours. (After this time the temperature of the meats ranged from +2°C to +5°C). Before use the MassMPC was concentrated as described in Section 3.4.4.

Each caseinate was dispersed, as described in Section 3.4, with a small proportion of the water designated for use in the sausage formulation in which it was to be incorporated.

The required amounts of pork heart and/or pork trims and/or beef chuck were weighed and placed in a single speed bowl chopper (Scharfen Cutter 60302). Half of the total volume of water required for the formulation (chilled to +3°C) was added along with the salt and these ingredients were chopped for 1 minute.

Next, the required amount of test protein was added and mixed for a further 1 minute. If no test protein was to be added the initial chop time was extended to 2 minutes. The remaining water was added and the chopping continued for another minute. After addition of pork back fat the mix was emulsified for 5 minutes. Finally, the cornflour was added and the chopping continued for another minute. Emulsion temperatures ranged from 11.5°C to 13°C at this stage.

Freshly prepared sausage emulsions were then stuffed (Ansal, ABR Food Machinery) into 21 mm reconstituted Devro collagen sausage casings. Although an attempt was made to keep the stuffing pressure as constant as possible, no measurable control over this variable was practicable. Following stuffing the sausages were linked into approximately 15 cm lengths. The entire batch was weighed, pin

pricked, placed in a plastic bag (to avoid evaporative losses) and chilled at +3°C for 18-24 hours.

Emulsion sausages were not prepared in the 10°C controlled temperature room because of technical difficulties. However, by carefully controlling the temperature of the raw materials, preparation of the sausage emulsions under ambient conditions led to end products with acceptable temperatures. The meat ingredients were frozen for at least one month prior to this stage because meat used in commercial emulsion type sausages is treated in a similar manner. Freezing is known to affect the ability of meat proteins to form a "meat emulsion" (Schut, 1976). Sodium caseinates were prepared and added in the manner described as this was consistent with commercial handling practices for this material in emulsion type sausage systems. (Schut, 1976). This procedure was found to be satisfactory for producing the sausage systems used in this study.

3.12 Measurement of cooking losses in the emulsion sausage system

Following overnight chilling the sausages were wiped and reweighed and the weight differences were recorded as the percentage moisture loss after chilling. In all cases no fat appeared to be lost.

Cooking losses were determined in quadruplicate after heating the sausages to an internal temperature of 70°C using the methods described in Chapter 3.9. However the moisture lost overnight was used in this calculation.

From this data the WBC of the sausage in g water bound/ 100 g mix was calculated using Equation 3.6.

Equation 3.6

$$\text{WBC of sausage} = 60 - \frac{60 \times \text{\% moisture loss}}{100}$$

Further the absolute value of the deviation of the predicted WBC (calculated from the goal program results) from the observed WBC was calculated. The remaining sausages were cooked to an internal temperature of 70°C in the Grillmaster using the predetermined cooking times. They were allowed to cool before being placed on a plastic tray, covered with a light gauge polyethylene film and chilled to +3°C for 18-24 hours.

3.13 Subjective and objective assessment of emulsion sausage systems

Following chilling selected properties of test sausages were evaluated subjectively using a trained taste panel, and objectively using the Ottawa Texture Measuring System (OTMS). Skins were manually peeled off the sausage before testing to avoid interference with textural evaluation.

3.13.1 Taste panel evaluation

Ten experienced taste panelists were selected for the subjective evaluation of emulsion sausage systems. (This number was limited by the number of people who would be available consistently over the six weeks training and testing period).

3.13.1.1 Taste panel training

During the first two training sessions the panelists were presented with a wide variety of commercial cooked and chilled, skinned emulsion-type sausages. They were asked to identify the organoleptic properties of these products that would be affected by protein quality and to note down words or expressions that adequately described these properties. Group discussions held following these sessions determined the properties to be evaluated and

the evaluation sheet format. From this a prototype evaluation sheet was drawn up and used in the next session. Further modifications were made and an acceptable evaluation sheet, using an equal interval scale (Stone et al., 1974), was drawn up for evaluating emulsion sausage systems. A copy is included in Appendix III.

For the next three weeks the panel was presented with a wide range of emulsion type sausages (the range that was envisaged would be seen in the test sausages) and asked to evaluate them under test conditions. After each session a group discussion was held which endeavoured to achieve group concensus regarding the grading of all test sausages for each property evaluated. Three such sessions were held each week in preparation for emulsion sausage system evaluations.

3.13.1.2 Taste panel evaluation of emulsion sausage systems

Taste panel evaluation of emulsion sausage systems was carried out in the sensory evaluation room in the Food Technology Department at Massey University. Testing sessions were held either mid-morning or early afternoon as people are most able to discriminate product differences at these times. Panelists assessed three test sausage samples, presented at random with random code numbers, and a standard sausage sample at each session. The standard used was Kiwi Bacon Company "Ham and Chicken" luncheon sausage, produced in one batch. It was included in an attempt to ensure that scoring of sausages was consistent between sessions. This standard was assigned a constant score for each property throughout the evaluation of the real sausage systems as shown on the score sheet (Appendix III). The test sausages and the standard were presented to panelists on a white crockery dinner plate which had been sub-divided into four with a marking pen and panelists were able to assess the properties of the sausages independently. Water was provided for panelists if desired.

When all panelists had completed their evaluation a group discussion led by a different person each session was held and a concensus decision made concerning the grading of each test sausage for the properties that were assessed. The values agreed on will be referred to as the group discussion mean. (This procedure was carried out to overcome between-panelist differences in perception of the gradations of the equal interval score lines).

The distance from the left hand edge of the score line to the point where the panelist or group scored the test sausage was measured in centimeters and this distance divided by 0.83 to convert the score to value on a scale of 0 to 10.

3.13.2 Objective evaluation of emulsion sausage systems

Objective evaluations were chosen which were presumed to measure sausage properties that:

i) would be affected by protein quality, and

ii) were similar to those properties evaluated by the trained taste panel.

Five objective tests were carried out on sausages from each trial. For all tests a crosshead speed of 10 cm/minute and a chart speed to crosshead speed ratio of 2:1 were used. Six replicates were carried out for each sausage in all five tests.

3.13.2.1 Compression to rupture

A 2 cm length of the test sausage was compressed under the moving shaft until rupture occurred. This force was applied in the direction of the longitudinal axis of the sausage, as shown in Plate 3.7. The force to rupture, the slope of the force-deformation curve and the deformation distance before rupture were recorded.

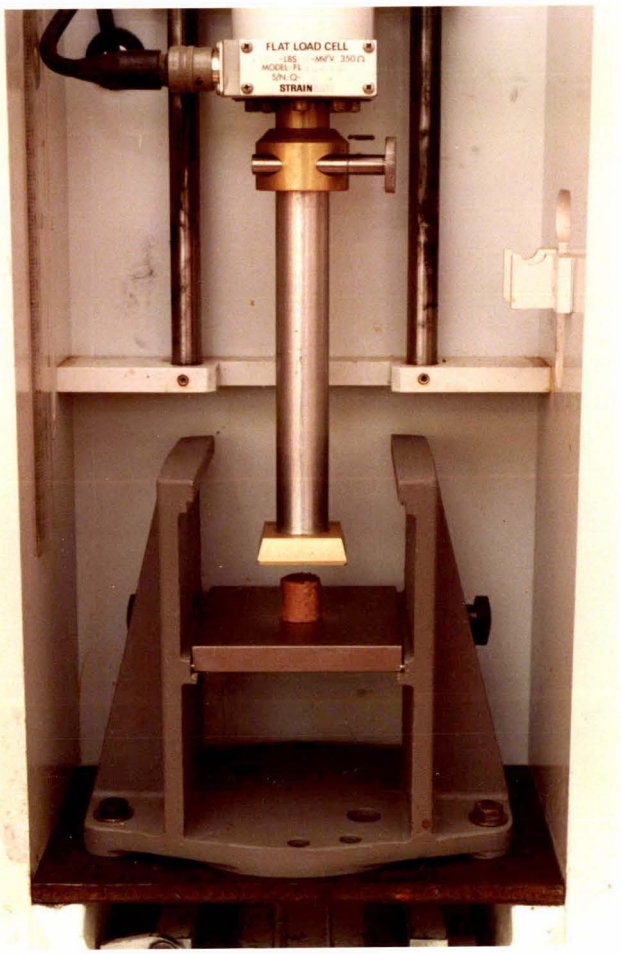
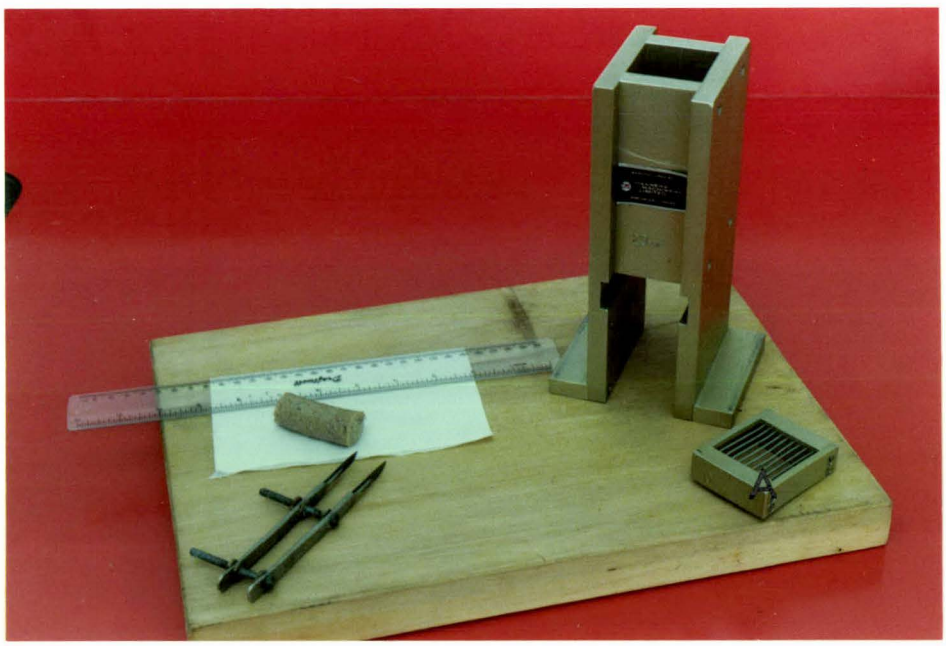


Plate 3.7.(above) Apparatus used in compression tests.
Plate 3.8.(below) Extrusion test apparatus showing wire grid (A).



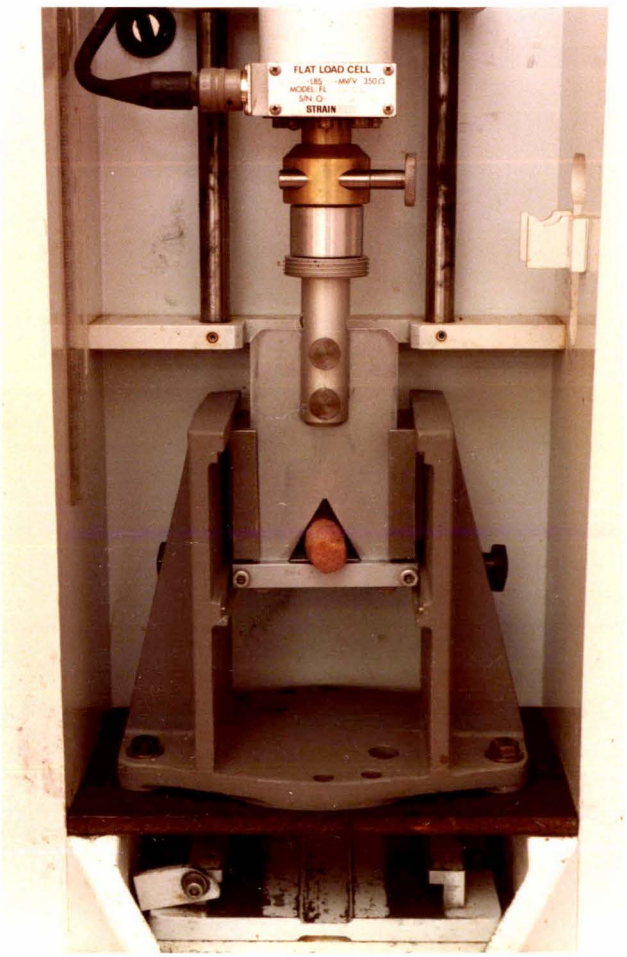


Plate 3.9. Warner Bratzler shear test apparatus.

3.13.2.2 Multiple compression test

A 2 cm length of the test sausage was compressed as in 3.13.2.1 until a 1.0 kg force was applied. Immediately the shaft direction was reversed for 2½ minutes, whereupon the direction was changed again and the sausage section re-compressed until rupture occurred. The force to rupture, the deformation distance before rupture and the slope of the force deformation curve before rupture were recorded.

3.13.2.3 Extrusion through a wire grid

A 2 cm length of the test sausage was placed on a 20 cm^2 wire grid before being subjected to compression as in 3.12.2.1 . This wire grid had 9 wires of 1.6 mm diameter spaced 3 mm apart and is shown, together with its holder, in Plate 3.8. During this test the sausages appeared to rupture along their longitudinal axes before they were extruded through the grid. This initial rupture force, the corresponding deformation distance and the slope of the force deformation curve up until this point were recorded. Then the extrusion force (thought to occur immediately prior to extrusion through the wire grid), the total deformation distance and the slope of the force deformation curve for this second force were recorded.

3.13.2.4 Warner Bratzler Shear Force Test

A 7 cm length of sausage was placed in the single cell OTMS Warner Bratzler Shear Test apparatus as shown in Plate 3.9.

In this test the sausage was cut in a transverse plane. The resistance to shear force was recorded along with the deformation distance and the slope of the force deformation curve.

3.13.2.5 Relaxation time

A 2 cm length of the test sausage was compressed as in 3.13.2.1 until a 1.0 kg force was applied whereupon the crosshead was stopped. The time from that moment until

this force reduced to 66.6% of the maximum was recorded. This is called the relaxation time.

3.14 Foaming capacity and foaming stability

A horizontal pneumatic shaker (built at the Meat Industry Research Institute of New Zealand, Hamilton) was used to evaluate the foaming properties of the test proteins and dried egg albumin. This shaker, shown in Plate 3.10 is based on the design of Yatsumatsu et al. (1972). Two percent (m/m) protein dispersions of GL750, MassMPC, MyoMPC, SarcoMPC, SV07, SV02, CasN and Egg White Albumin were prepared in 100 ml measuring cylinders. The weighed protein material was transferred into the cylinder via a wide neck glass filter funnel with the aid of a dry brush for proteins in a powdered form. Distilled water was added to bring the mass to 50 g, the cylinders were stoppered and wetting of the protein material was initiated by gently inverting the mixtures. To complete dispersion of the proteins the stoppered cylinders were placed in an ultrasonic bath (Varian Aerograph) so that the water level of the bath was above the dispersion level in the cylinders. After one minute in the bath the cylinders were removed, inverted several times and replaced in the ultrasonic bath for a further minute. (This passive dispersion method was used to minimize foaming and thus, avoid interference with the true foaming values). After dispersion, the cylinders were placed in a 20^{o}C water bath for 30 minutes, during which time they were occasionally inverted to ensure thorough mixing of the dispersions. To measure foaming capacity each cylinder was secured in the reciprocating pneumatic shaker (driven at a constant air pressure of 300 kpa gauge) and shaken for either 1, 2, 4 or 6 minutes. After shaking for the required time, each cylinder was removed then un-stoppered and had a filter paper disc gently pressed down onto the foam head until resistance was encountered, as shown in Plate 3.11.The volume of foam, measured (ml) from the base of the foam to the filter paper disc by using the graduations on the cylinder, was recorded as the foaming capacity. Once this measurement was taken the

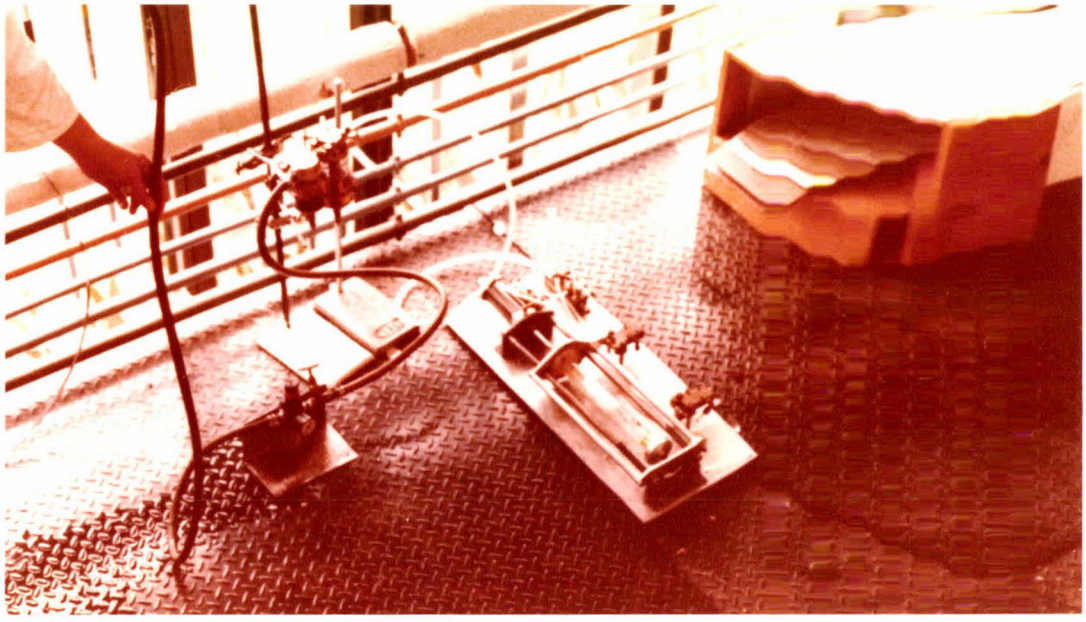
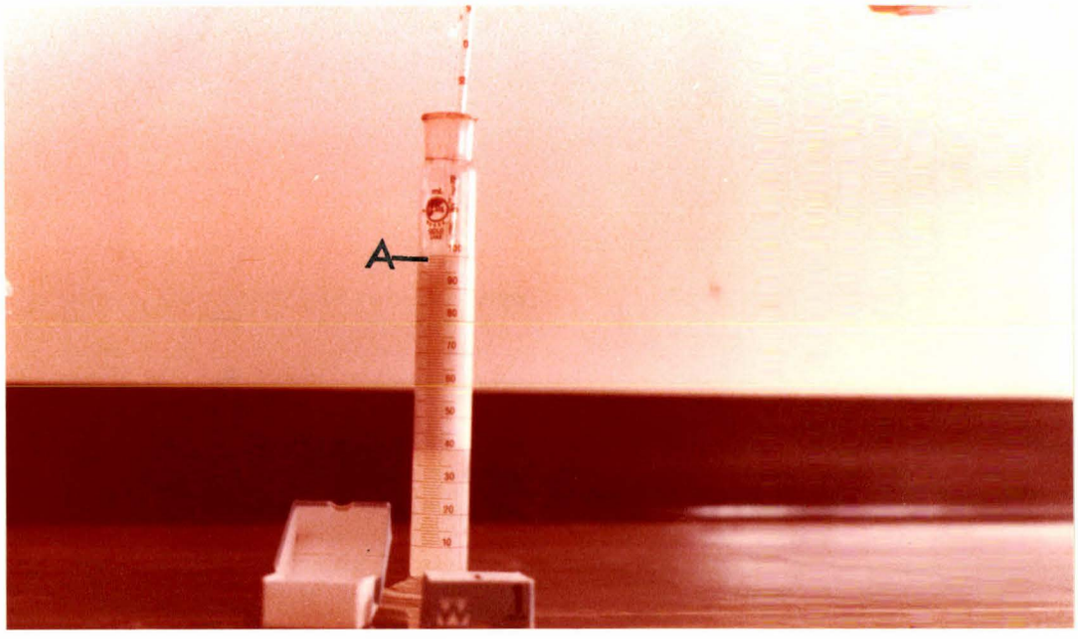


Plate 3.10. (above) Pneumatic shaker used for foaming capacity determinations.

Plate 3.11. (below) Determination of foam volume achieved by gently pressing a filter paper disc onto the foam head (A) until resistance is encountered.



cylinder was restoppered and placed back into the 20°C water bath. The foam height was measured after 30 seconds and 5, 10, 15, 20, 25, 30, 60 and 90 minutes post shaking. The decaying foam height over this period was recorded as an indication of the foam stability. All tests were done in triplicate and the average values reported.

Foaming properties of proteins are not important in the production of sausage systems. Therefore, the physical and chemical conditions used in these tests were not chosen to resemble those found in the sausage systems. Rather, they were chosen to resemble the conditions which food foaming agents would be subjected to under practical processing conditions. Furthermore, it was considered that the foaming capacity and stability results would be more meaningful if they were compared with the properties of a commercial food foaming agent. Dried egg albumin was chosen for this purpose and its foaming properties were determined under all the test conditions.

The foaming capacity test on CasN had a relative standard deviation of 4.39% and the test for foaming stability on this material 90 minutes post shaking had a relative standard deviation of 78.8% (Calculations shown in Appendix II).

3.15 Statistical analysis

Standard computer packages were used to carry out Analysis of variance, Bartlett's test and the New Duncan's multiple range test.

3.15.1 Analysis of variance

Analysis of variance (Steele and Torrie, 1960) was performed on the data for each objective test, performed on the cooked sausages, emulsion stability moisture loss data; sausage moisture loss data; and the deviation between the observed and predicted sausage water binding values. Analysis of variance was not performed on the other data

because the number of observations was too small, or this analysis was not required to interpret the data (I. Gravatt, personal communication).

3.15.2 Bartlett's test and New Duncan's multiple range test

Bartlett's test (Steele and Torrie, 1960) for homogeneity of variance was performed on the data subjected to the analysis of variance. If the variances of the data were found to be homogeneous a New Duncan's multiple range test (Steele and Torrie, 1960) was used to determine which proteins were different. Under these conditions between-treatment differences were determined by comparing individual mean values and the pooled standard error of the mean ($S\bar{x}$).

If, however variances were not homogeneous protein differences and treatment differences were assessed by comparing the individual means and standard errors of the mean.

3.15.3 Pearson's correlation

Two-sided Pearson correlations (Snedecor & Cochran, 1967) were performed for each protein type to identify relationships between:

i) the functional properties measured using model systems, and

ii) the properties of the sausage systems determined instrumentally and by taste panel evaluation

iii) the functional properties measured using model systems and the properties measured on the emulsion sausage systems

Mean values for each property of each protein type investigated were used in this analysis.

CHAPTER 4

RESULTS

4.1 Postal survey on the use of non-meat protein additives

Results of the postal survey of New Zealand smallgoods manufacturers are summarized in Table 4.1. Seventeen meat smallgoods companies were contacted of whom 11 (65%) replied with information concerning the use of non-meat protein additives in their company.

A major finding of this survey, which provided the basis for selection of test proteins, was the predominant use of soy concentrate GL750 as an additive. On the other hand soy isolate did not appear to be generally used in the production of sausages. Sodium caseinate was used less frequently than soy concentrate but more often than soy isolate. However, no preference for brand was exhibited.

4.2 Compositional analyses

Relevant chemical compositional analyses, determined in this study for all test materials are presented in Table 4.2.

4.3 Functional properties determined using model systems

4.3.1 Percent soluble protein

SarcoMPC contained the highest percentage of soluble protein at temperatures less than 50^{o}C, however a very marked decrease in solubility resulted after heating from 50^{o}C to 70^{o}C. MassMPC and MyoMPC exhibited the poorest solubilities and were almost completely insoluble over the temperature range investigated. The caseinate proteins were approximately 75% soluble and increasing the temperature from 10^{o}C to 70^{o}C had little or no effect on this property.

TABLE 4.1

The use of non-meat protein additives by New Zealand meat smallgoods manufacturers

Additive	Number of Respondents* using each additive	Product	Amount used**
Soy concentrates			
GL750 (Griffith Laboratories (N.Z.)Ltd.)	4	luncheon sausage	1.4-2.85%
		saveloys/polonies	0.5% dry wt.
		continental sausages	1-1.5%
		fresh sausages	1.4%
600 (Griffith Laboratories (N.Z.) Ltd.)	1	meat patties	4.8% dry wt.
Soy isolates			
IPSO (Globus Arlow (N.Z.))	2	ham products	1-3.5%
		fresh sausages	0.75%
		luncheon sausage	1%
Sodium caseinates			
N.Z.C.D.C. Ltd.	2	cooked sausages	0.5% dry wt.
		luncheon	0.5% dry wt.
Premi Premate	1	saveloys/polonies	0.5% dry wt.
No additives	3		

* some respondents used more than one additive

** % wet weight basis unless otherwise stated

TABLE 4.2

Compositional analyses of raw materials

Raw Material	Fat	Moisture (Percent)	Protein	Salt
Test Proteins				
SV07	< 1.0	6.59	91.20	ND
SV02	< 1.0	3.94	87.88	ND
CasN	< 1.0	6.84	86.95	ND
GL750	2.03	5.33	70.80	ND
MassMPC	< 0.5	82.00	18.12	ND
MyoMPC	< 0.5	81.82	18.15	ND
SarcoMPC	nil	76.10	20.00	3.87
Egg white albumin	ND*	3.40	77.50	ND
Sausage Ingredients				
Pork heart	6.71	76.95	15.71	ND
Pork trim	33.11	51.41	15.06	ND
Beef chuck	8.80	69.52	20.89	ND
Pork back fat	84.23	11.90	3.87	ND
Cornflour	< 1.0	5.0	2.0	ND

* ND = not determined

Limited solubility was exhibited by GL750 (approximately 12%) but this property appeared to be enhanced slightly when the material was heated from 10 to 70°C.

Salt addition did not appear to affect the solubilities of the GL750, MassMPC or MyoMPC proteins but seemed to cause the solubility of SV02 and CasN to increase while causing a decrease in the solubility of SV07. (Figures 4.1 and 4.2) (Raw data are presented in Appendix IV, Table IV.1.

4.3.2 Heat gel strength

Heat gel strength was affected by protein type and salt addition. Proteins could be grouped according to the type of gel they formed: very strong gel (formed by MassMPC and MyoMPC in both the presence and absence of salt); medium strength gel (formed by SV07 and GL750 in both the presence and absence of salt and SV02 in the presence of salt); weak gel (formed by SV02 and SarcoMPC in the absence of salt); and non-gel (formed by CasN in both the presence and absence of salt) (Table 4.3 and 4.4). The addition of 2% salt resulted in a decrease in the heat gel strength for all protein types except SV02 where an increase was observed.

4.3.3 Water binding capacity

4.3.3.1 Water binding capacity of ingredients used in sausage formulations

The water binding capacities of the five sausage ingredients are markedly different, the best binding capacities being exhibited by cornflour, pork heart and beef chuck proteins (Table 4.5).

Figure 4.1 The effect of temperature on the solubility of 1% protein solutions of SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲), MassMPC (□—□) and SarcoMPC (△—△).

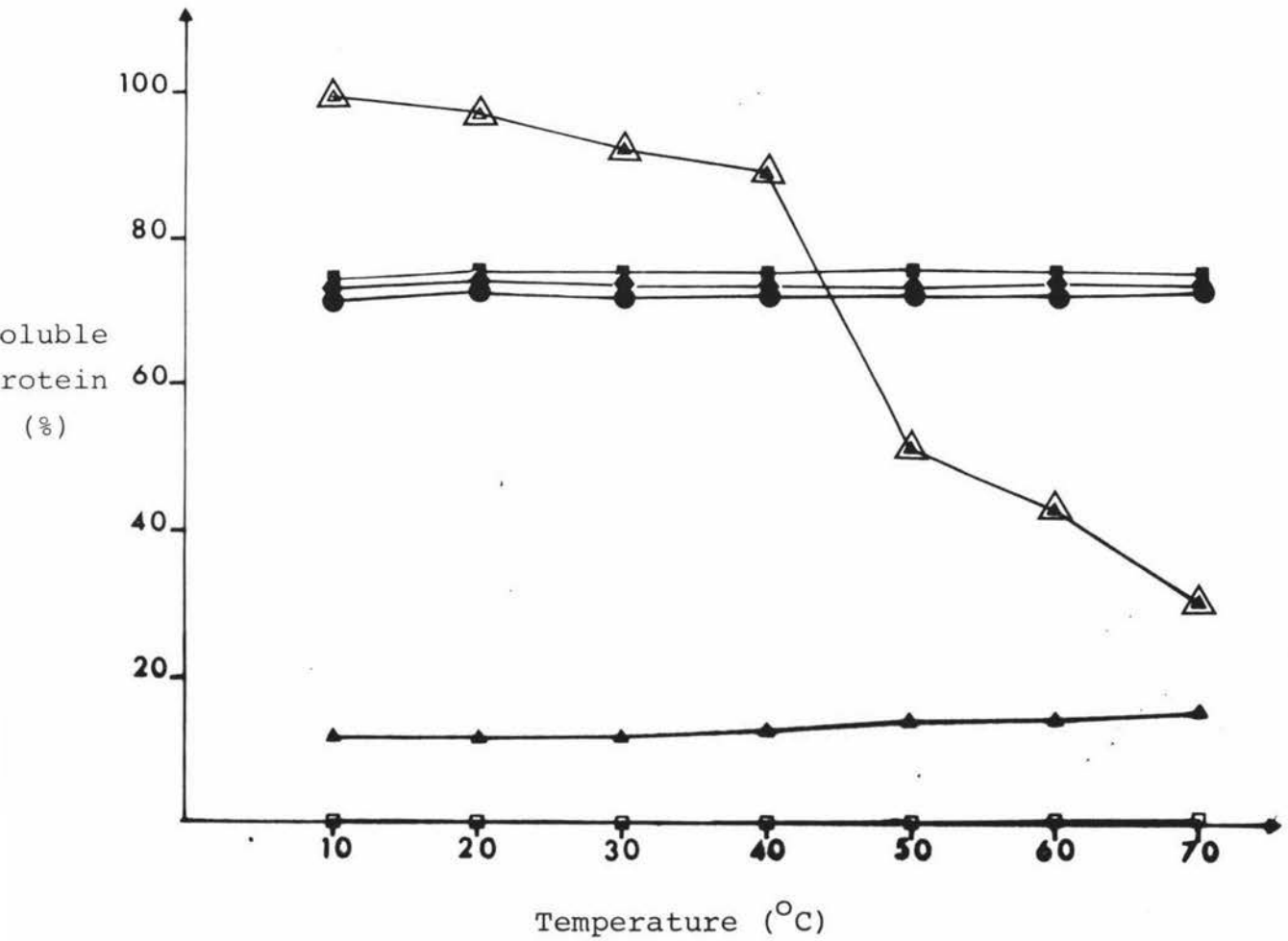


Figure 4.2 The effect of temperature on the solubility of 1% protein solutions of SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲) and MassMPC (□—□) in the presence of 2% NaCl.

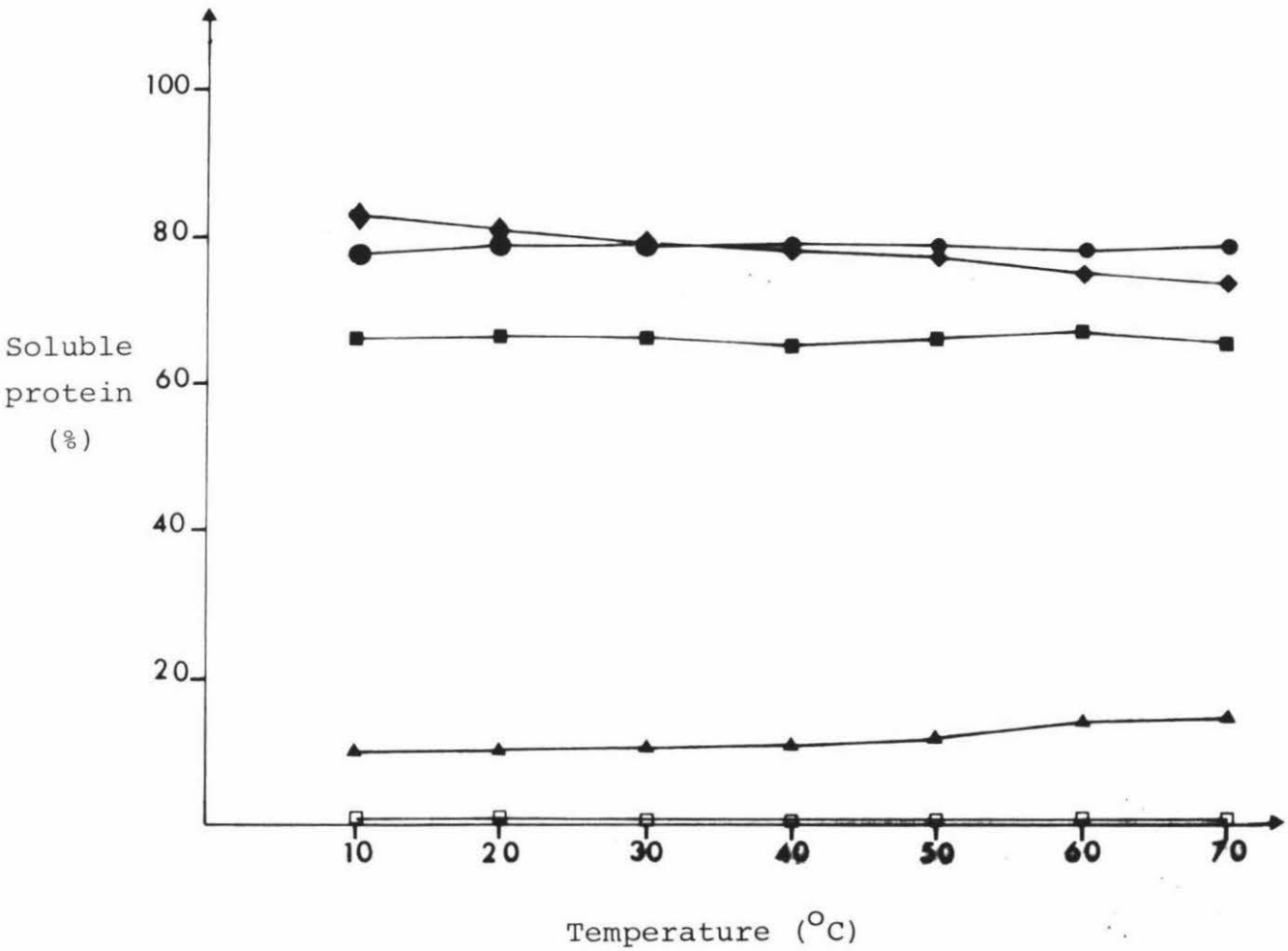


TABLE 4.3

Heat gel strength of SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC gels

Protein	Heat gel strength (Rupture force g)				$\bar{x}$	Observations
MassMPC	11,337	11,370	11,343	11,359	11,352	solid meat gel with clear exudate
MyoMPC	7,084	7,120			7,102	solid meat gel with less exudate than MassMPC
SV07	434.2	446.8	454.0	450.3	446.3	solid gel
GL750	341.8	337.1	325.0	349.5	338.4	swollen particulate solid (not a true gel)
SV02	56.7	61.0	54.3	60.1	58.0	gel
SarcoMPC	35.2				35.2	very weak solid gel
CasN	23.3	24.0	25.7	23.9	24.2	exhibited flow properties

TABLE 4.4

Heat gel strength of SV07, SV02, CasN, GL750, MassMPC and MyoMPC gels with 2% (m/m) salt*

Protein	Heat gel strength (Rupture force g)					Observations
MyoMPC	6,461	6,490			6,476	solid mass gel with clear exudate (less than in the absence of salt)
MassMPC	2,839	2,794	2,788	2,801	2,806	same as MyoMPC
SV07	398.0	392.7	395.9	386.4	393.3	solid gel
GL750	249.0	254.3	248.8	257.4	252.4	swollen particulate solid (not a true gel)
SV02	105.5	110.4	106.6	109.0	107.9	gel
CasN	14.2	16.7	14.1	15.8	15.2	exhibited flow properties

* Heat gel strength in the presence of salt was not determined for SarcoMPC

TABLE 4.5

Water binding capacities of sausage ingredients (70°C, 2% salt)

Ingredients	Water binding capacity (g water bound/g protein)		
protein to water ratio	1:5	1:10	1:20
Pork heart	5.00		
Beef chuck	5.00		
Pork trim	3.67		
Pork backfat	3.12		
Cornflour	5.00	10.00	16.00

4.3.3.2 Water binding capacity of test proteins

Temperature, protein to water ratio and the addition of 2% salt were all factors which affected the water binding capacity of the proteins examined (Figures 4.3 to 4.6, data are summarized in Appendix V, Table V.1.

SarcoMPC exhibited no capacity to bind water under the test conditions.

At a protein to water ratio of 1:5 (m/m) and in the absence of salt, water binding capacity decreased for all proteins above 60°C, with GL750 being the least affected. In the presence of salt, temperature had no effect on the water binding capacity of SV07 but the other proteins exhibited similar but lessened effects than shown in the absence of salt. Generally the meat proteins exhibited the poorest ability to bind water.

At a protein to water ratio of 1:10 and in the presence of salt all proteins showed increased water binding capacities at temperatures less than 50°C. With increasing temperature (to 70°C) the water binding

Figure 4.3 The effect of temperature on the water binding capacity of SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲) MassMPC (□—□) and MyoMPC (○—○) at a protein to water ratio of 1:5

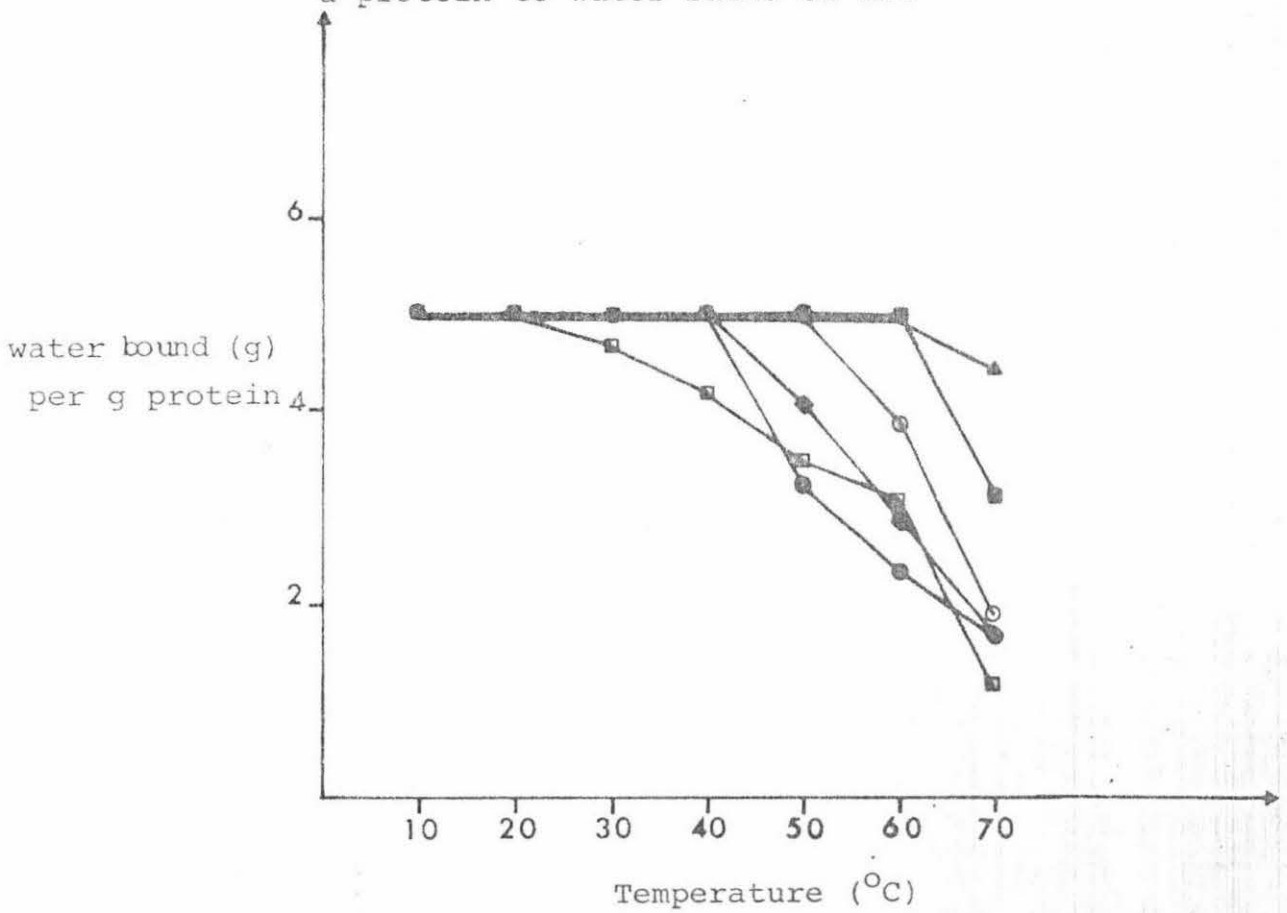


Figure 4.4 The effect of temperature on the water binding capacity of SV07 (■——■), SV02 (●——●), CasN (◆——◆), GL750 (▲——▲), MassMPC (□——□) and MyoMPC (○——○) in the presence of 2% salt, at a protein to water ratio of 1:5

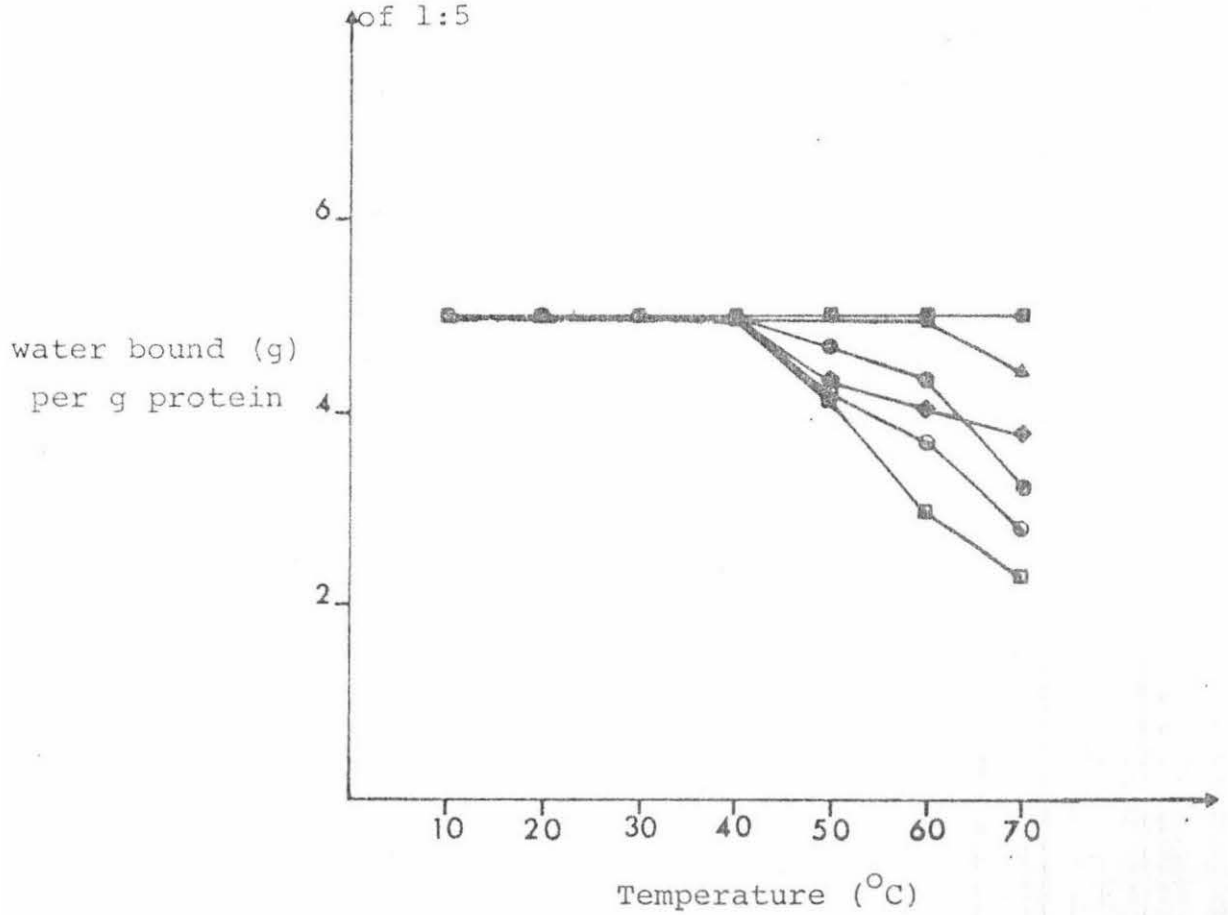


Figure 4.5 The effect of temperature on the water binding capacity of SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲), MassMPC (□—□), and MyoMPC (○—○) at a protein to water ratio of 1:10

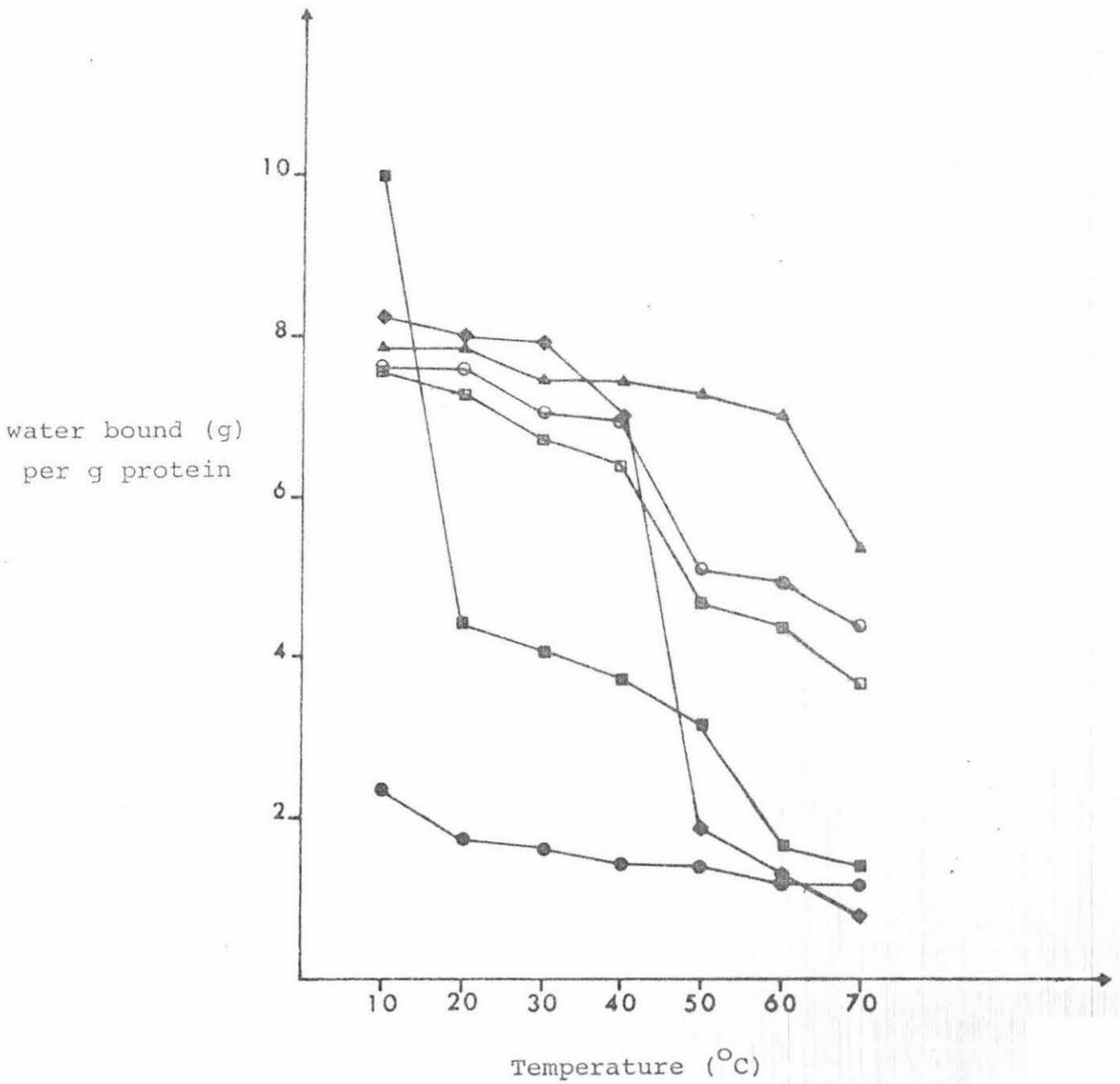
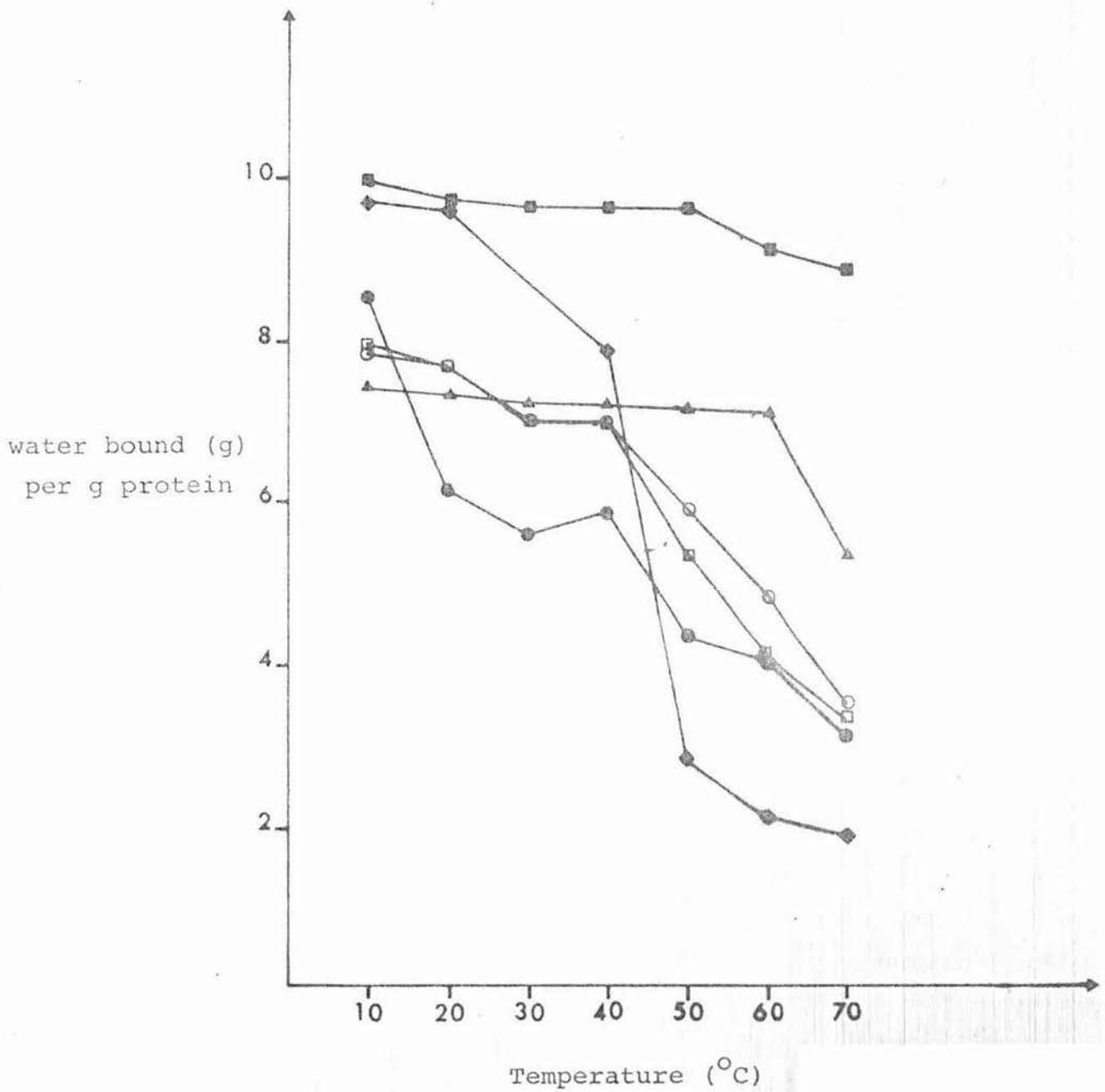


Figure 4.6 The effect of temperature on the water binding capacity of SV07 (■——■), SV02 (●——●), CasN (◆——◆), GL750 (▲——▲), MassMPC (□——□) and MyoMPC (○——○) in the presence of 2% salt at a protein to water ratio of 1:10



capacities fell to approach the values recorded at the 1:5 ratio, except for SV07 which was almost unaffected by temperature. A similar reaction occurred in the absence of salt except for SV07 and SV02. At temperatures above 10°C the water binding capacities of both SV07 and SV02 decreased with increases in temperature and at 70°C gave the lowest values for any of the proteins, under any of the test conditions. The binding abilities of MassMPC and MyoMPC were generally enhanced at this protein to water ratio and were superior to those of SV02 and CasN.

4.3.4 Emulsion capacity

Of the proteins tested SarcoMPC had the highest emulsion capacity while the other two meat protein concentrates (MassMPC and MyoMPC) exhibited the most inferior properties. The non-meat proteins exhibited emulsion capacities intermediate between these two extremes (Table 4.6). Addition of 2% salt generally enhanced emulsion capacity properties although this effect did not apply to all the casein proteins.

TABLE 4.6

Mean (n = 4) emulsion capacities of SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC in the presence and absence of salt (2% (m/m))

Protein *	Emulsion capacity ($\bar{x}$) ml oil/g protein	
	without salt	with salt*
SV07	17.78 B	18.02 c
SV02	18.02 B	17.40 c
CasN	15.88 C	20.89 b
GL750	30.18 A	36.83 a
MassMPC	12.83 D	14.25 d
MyoMPC	10.80 E	12.86 e
SarcoMPC**	41.59	-
Pooled $S\bar{x}$ = 0.42		

* Significant main effect differences ($P < 0.001$) Interaction (protein x salt addition) significant ($P < 0.001$)

** SarcoMPC was not tested with salt

4.3.5 Emulsion stability

4.3.5.1 Visual assessment of emulsion stability

All three meat proteins appeared to form relatively unstable emulsions, especially SarcoMPC, while a high degree of stability was observed for the non-meat protein emulsions (Table 4.7). The addition of 2% salt did not affect the visible differences between these emulsions.

TABLE 4.7

Visual observations of the stability of emulsions formed by SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC (protein to water to fat ratio 1:5:1.67)

Protein	Observations
SV07	creamy stable emulsion
SV02	creamy stable emulsion
CasN	creamy stable emulsion
GL750	non creamy but stable matrix
MassMPC	emulsion appears to be breaking but fat migration prevented by high viscosity of mix
MyoMPC	same as MassMPC
SarcoMPC	rapid fat coalescence and emulsion breakdown occurring

4.3.5.2 Moisture loss

Over the temperature range studied SV07 formed the most stable emulsion with respect to moisture loss. Greater losses were exhibited by SV02, CasN and GL750 with the largest losses occurring in the cases of the three meat protein concentrates. Increasing the temperature from 10°C to 70°C generally caused the moisture loss to increase (Figures 4.7 and 4.8) (mean (n = 3) data are summarized in Appendix VI, Table VI.1 Salt reduced the moisture loss from the emulsions especially at 10°C.

4.3.5.3 Fat loss

Fat was not lost from the caseinate emulsions at any of the temperatures studied and only occurred for emulsions prepared with the remaining test proteins at temperatures greater than 40°C. The greatest losses occurred for emulsions prepared with the meat proteins. Increasing the

Figure 4.7 The effect of temperature on the moisture lost from emulsions (protein to fat to water ratio = 1:5:1.67) prepared with SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲), MassMPC (□—□), MyoMPC (○—○), and SarcoMPC (△—△)

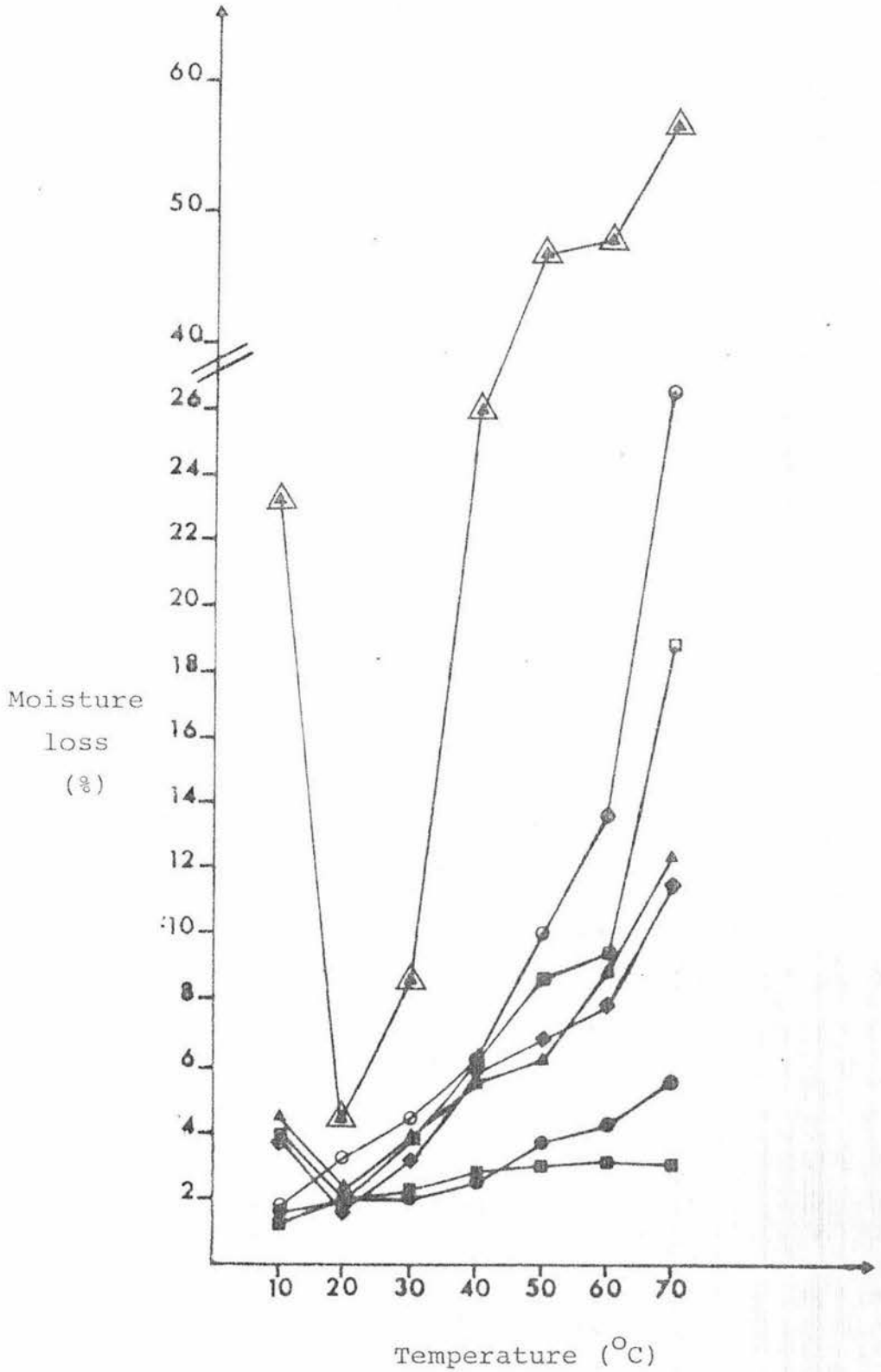


Figure 4.8 The effect of temperature on the moisture lost from emulsions (protein to fat to water ratio = 1:5:1.67) prepared with SV07 (■—■), SV02 (●—●), CasN (◆—◆), GL750 (▲—▲), MassMPC (□—□) and MyoMPC (○—○) in the presence of 2% salt

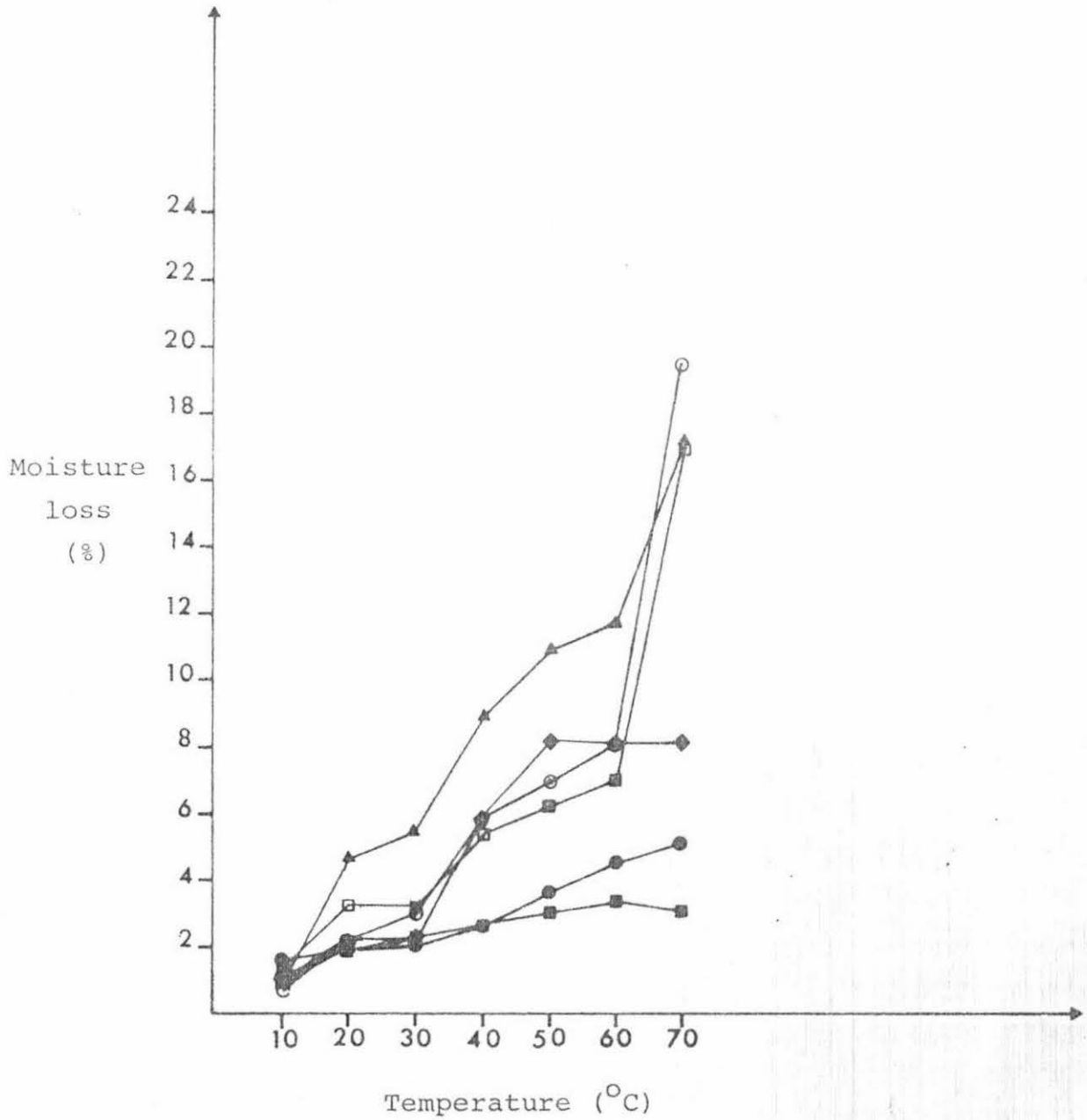


TABLE 4.8

The effect of temperature and 2% salt addition on the fat lost from emulsions (protein to fat to water ratio = 1:5:1.67) prepared with SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC (mean results, n = 3)

Temperature (°C)	Salt present or absent	Fat loss (%)						
		SV07	SV02	CasN	GL750	MassMPC	MyoMPC	SarcoMPC
10	absent	0	0	0	0	0	0	0
20	absent	0	0	0	0	0	0	0
30	absent	0	0	0	0	0	0	0
40	absent	0	0	0	0	0	0	44.00
50	absent	0	0	0	3.70	0	0	59.52
60	absent	0	0	0	3.73	3.08	3.90	76.48
70	absent	0	0	0	4.65	13.11	18.85	86.79
10	present	0	0	0	0	0	0	*
20	present	0	0	0	0	0	0	
30	present	0	0	0	0	0	0	
40	present	0	0	0	0	0	0	
50	present	0	0	0	1.58	0	0	
60	present	0	0	0	2.68	1.55	3.31	
70	present	0	0	0	3.65	5.87	18.48	

* SarcoMPC was not examined in the presence of salt

temperature from 40°C to 70°C increased fat loss. The addition of 2% salt caused slight to moderate decreases in fat loss for the proteins concerned (Table 4.8).

4.4 Sausage formulations

The sausage formulations (derived using goal programming) used to manufacture the test sausages are presented in Table 4.9. The functional values, percentage deviations contributing to these values, constraint values and predicted water binding capacities (WBC) of each formulation are summarized in Table 4.10. Salt was added so that the final concentration in all formulations was 2% (m/m) (wet loss).

4.5 Functional properties assessed using an emulsion-type sausage system

4.5.1 Cooking losses

4.5.1.1 Moisture loss

At the 3% protein replacement level the greatest moisture loss was exhibited by the sausage containing GL750. Mass(3) and Myo(3) had the smallest moisture losses along with SV02(3). Sausages containing SV07, CasN and SarcoMPC had moisture losses between those exhibited by the above groups of sausages.

Increasing the protein replacement level from 3% to 30% increased the moisture lost from all sausages. Sausages containing SarcoMPC showed the greatest increase (Table 4.11).

TABLE 4.9

Test sausage formulations

Sausage	Ingredients (Percentage of batch)						
	Beef chuck	Pork heart	Pork trim	Pork backfat	Cornflour	Water	Protein
WBC60	46.15	10.84	-	18.01	4.00	17.23	-
WBC56	32.08	11.79	19.31	11.81	4.00	17.09	-
WBC40	-	18.46	56.54	-	4.00	16.53	-
SV07(30)	-	40.25	8.88	16.95	4.00	21.98	3.95
SV02(30)	-	40.27	8.86	16.96	4.00	22.08	4.10
CasN(30)	-	40.21	8.96	16.92	4.00	21.95	4.14
GL750(30)	-	40.14	9.07	16.81	4.00	21.97	5.08
Mass(30)	31.51	-	6.99	17.54	4.00	15.89	19.90
Myo(30)	31.33	-	7.34	17.41	4.00	15.97	19.80
Sarco(30)	28.80	3.36	7.26	17.56	4.00	17.66	18.00
SV07(3)	31.63	18.79	9.38	15.21	4.00	16.70	0.395
SV02(3)	31.63	18.79	9.37	15.21	4.00	16.71	0.410
CasN(3)	31.63	18.78	9.38	15.20	4.00	16.70	0.414
GL750(3)	31.61	18.80	9.40	15.19	4.00	16.69	0.508
Mass(3)	37.89	10.78	9.01	15.32	4.00	17.07	1.990
Myo(3)	37.86	10.80	9.04	15.31	4.00	17.08	1.980
Sarco(3)	37.32	11.50	9.05	15.32	4.00	17.16	1.800

TABLE 4.10

Functional values, percentage deviations contributing to functional values and constraint values for test sausage formulations

Sausage	Functional value	% Deviation	Constraint values						Predicted WBC
			Protein	Moisture	Fat	Meat	Batch	WBC	
WBC60	4.77	1.0 protein 3.77 batch	12.12	60	20	75.0	96.23	60	60
WBC56	3.91	3.91 batch	12	60	20	75.0	96.09	56	56
WBC40	26.08	4.47 batch 17.40 WBC 4.25 protein	11.49	60	20	75.0	95.53	40	46.96
SV07(30)	15.83	3.98 batch 11.88 meat	12	60	20	66.09	96.02	40	58.00
SV02(30)	15.59	3.73 batch 11.88 meat	12	60	20	66.09	96.27	40	51.50
CasN(30)	15.67	3.81 batch 11.88 meat	12	60	20	66.09	96.19	40	53.20
GL750(30)	14.86	11.96 meat 2.92 batch	12	60	20	63.04	97.08	40	55.80
Mass(30)	5.42	1.25 meat 4.17 batch	12	60	20	76.25	95.83	40	48.20
Myo(30)	5.33	1.20 meat 4.13 batch	12	60	20	76.20	95.87	40	49.50
Sarco(30)	3.34	3.34 batch	12	60	20	75.0	96.66	40	46.96

TABLE 4.10 ctd.

Sausage	Functional value	% Deviation	Constraint values						Predicted WBC
			Protein	Moisture	Fat	Meat	Batch	WBC	
SV07(3)	3.90	3.90 batch	12	60	20	75.0	96.10	56	57.80
SV02(3)	3.88	3.88 batch	12	60	20	75.0	96.12	56	57.15
CasN(3)	3.89	3.89 batch	12	60	20	75.0	96.11	56	57.32
GL750(3)	3.80	3.80 batch	12	60	20	75.0	96.20	56	57.58
Mass(3)	3.93	3.93 batch	12	60	20	75.0	96.07	56	56.82
Myo(3)	3.92	3.92 batch	12	60	20	75.0	96.08	56	56.95
Sarco(3)	3.84	3.84 batch	12	60	20	75.0	91.16	56	56.00

TABLE 4.11

Mean (n = 4) moisture losses from emulsion-type sausages with 3% and 30% of their protein replaced by SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC

Protein*	Moisture loss %	
	3% protein replacement	30% protein replacement*
SV07	11.12 b	18.15 c
SV02	7.56 d	10.53 e
CasN	9.32 c	12.44 d
GL750	15.57 a	19.57 b
MassMPC	5.37 e	10.50 e
MyoMPC	7.60 d	8.15 f
SarcoMPC	9.48 c	26.27 a
Pooled $S\bar{x}$ = 0.45		

* Significant main effect differences ($P < 0.001$) Interaction (protein type x protein replacement level) significant ($P < 0.001$)

4.5.1.2 The deviation of the predicted water binding capacity from the observed water binding capacity for the test sausages

The WBC60, WBC56 and WBC40 control sausages had mean deviations between their predicted and observed water binding capacities of 1.83, 0.22 and 2.28 respectively.

At the 3% protein replacement levels the minimum deviation between predicted and observed water binding capacities occurred for sausages containing the meat proteins and SV02. Maximum deviation was exhibited by GL750(3) and SV07(3).

Increasing the replacement level from 3% to 30% generally resulted in an increased deviation although a decrease was observed for sausages containing CasN.

At the higher replacement level those sausages containing meat proteins exhibited deviation values exceeded only by the values for SV07(30) and GL750(30 (Table 4.12).

TABLE 4.12

Mean (n = 4) values for the deviation of the predicted water binding capacity (WBC) from the observed WBC for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at the 3% and 30% protein replacement levels

Protein*	|WBC predicted - WBC observed|	
	3% replacement	30% replacement*
SV07	4.47 B (-)[x]	8.90 A (-)
SV02	1.69 D (-)	2.17 D (+)[y]
CasN	2.94 C (-)	0.86 E (+,-)[z]
GL750	6.93 A (-)	7.59 B (-)
MassMPC	0.31 E (+,-)	5.64 C (+)
MyoMPC	1.51 D (-)	5.59 C (+)
SarcoMPC	1.69 D (-)	2.72 D (-)
Pooled $S\bar{x}$ = 0.26		

* Significant main effect differences ($P < 0.001$) Interaction (protein x protein replacement level) significant ($P < 0.001$)

x observed values < predicted values

y observed values > predicted values

z observed values < and > predicted values

4.5.1.3 Fat losses

Small fat losses (0.10% and 0.18%) were observed for the WBC56 and WBC40 control sausages, while no loss was observed for the WBC60 sausage.

At the 3% protein replacement level fat was not lost by any sausages except Sarco(3) where a 0.37% loss was recorded.

Fat losses at the 30% replacement level occurred for sausages containing the meat proteins and GL750, Greatest losses occurred for Mass(30), and Myo(30) (Table 4.13).

TABLE 4.13

Mean (n = 4) fat losses from emulsion-type sausages with 3% and 30% of their protein replaced by SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC

Protein	Fat loss (%)	
	3% protein replacement	30% protein replacement
SV07	0	0
SV02	0	0
CasN	0	0
GL750	0	0.73
MassMPC	0	2.16
MyoMPC	0	1.10
SarcoMPC	0.37	0.55

4.5.2 Taste panel assessment of sensory properties

In this section only the group discussion means are considered. These values are summarized in Appendix VII, Table VIII.1 No units are associated with these values as they represent a score between 0 and 10 on an equal interval scale.

4.5.2.1 Firmness

Firmness scores were affected by the type of protein added and the level of addition.

At the 3% protein replacement level the firmest sausages contained SV02 and SarcoMPC followed in decreasing order by those containing GL750, MassMPC, SV07, CasN and MyoMPC. These sausages were generally firmer than the control sausages and their 30% replacement counterparts.

At the 30% replacement level sausages were generally scored less firm than the sausages containing no protein additives with the firmest sausages containing SV07 or the meat protein concentrates.

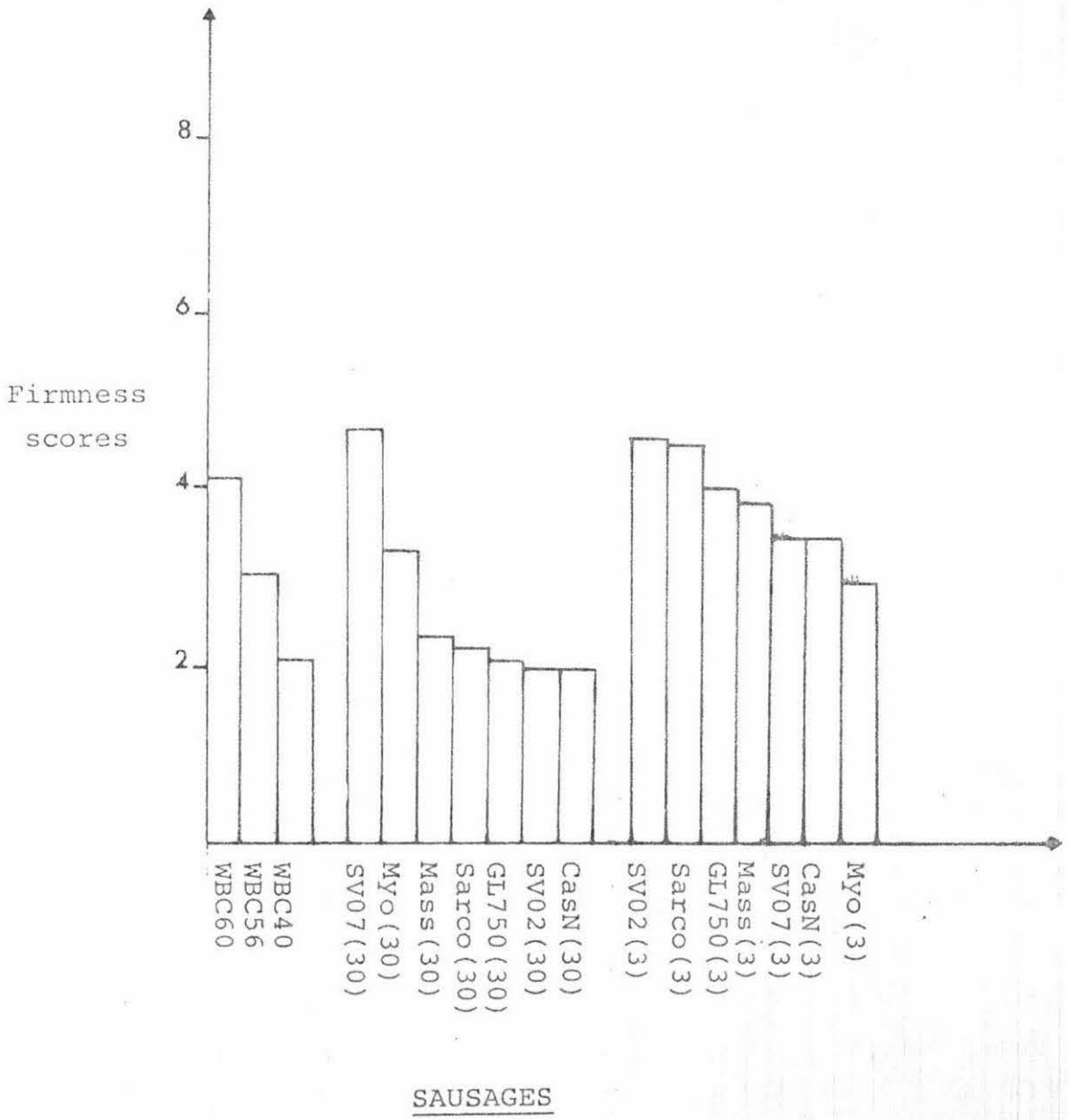
The firmness values for the three control sausages decreased as the water binding of the mix decreased from 60 g water/100 g mix to 40 g water/100 g mix (Figure 4.9).

4.5.2.2 Cleanness of first bite

Protein additive type and level of addition affected the cleanness of first bite scores for sausages containing additives were similar to those not containing additives.

At the 3% protein replacement level the addition of GL750 resulted in a sausage with the cleanest first bite followed in order of increasing resistance to break by sausages containing SarcoMPC, CasN, SV07, SV02. Addition of the fibrous meat proteins (MassMPC and MyoMPC) resulted in sausages with the greatest resistance to break.

Figure 4.9 Firmness scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at the 3% and 30% protein replacement levels



Increasing the protein replacement value from 3% to 30% reduced resistance to break scores for sausages containing SV07, SV02, MyoMPC and SarcoMPC in contrast with sausages containing GL750, CasN and MassMPC.

The WBC56 sausage exhibited more resistance to break than either of the other two control sausages (Figure 4.10).

4.5.2.3 Rate of breakdown

The type of protein additive and the level at which it was added to a sausage affected the rate of breakdown.

At the 3% level sausages containing CasN and MyoMPC broke down fastest followed in decreasing rate by those containing SV07, SV02, GL750, MassMPC and SarcoMPC. These sausages broke down at a similar rate to those without any protein additives.

Increasing the protein replacement level from 3% to 30% resulted in a faster rate of breakdown for all sausages (especially for those containing MassMPC, SarcoMPC and GL750) except those containing MyoMPC where a decrease was observed. Sausages prepared at the 30% protein replacement level generally broke down faster than sausages with no protein additives.

The control sausage with a water binding capacity of 56 g water/100 g mix broke down faster than the other control products (Figure 4.11).

4.5.2.4 Chewiness

Sausage chewiness was affected by the presence of different protein types and the level of addition of non-meat protein additives.

At the 3% replacement level the caseinates formed the most chewy sausages followed in decreasing order by Mass(3), GL750(3), Sarco(3) and Myo(3). These sausages were

Figure 4.10 Cleanness of first bite scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at the 3% and 30% protein replacement levels

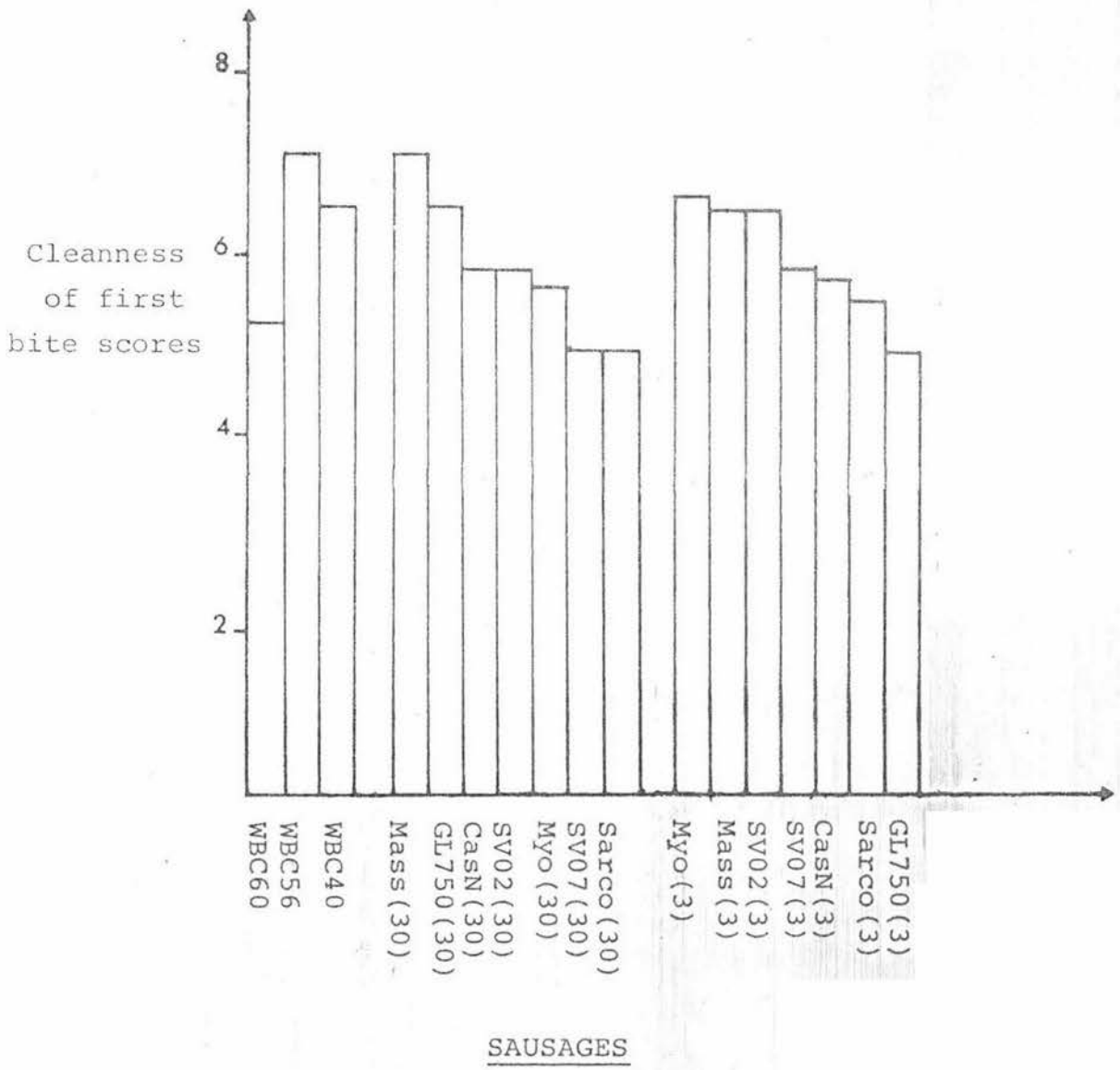
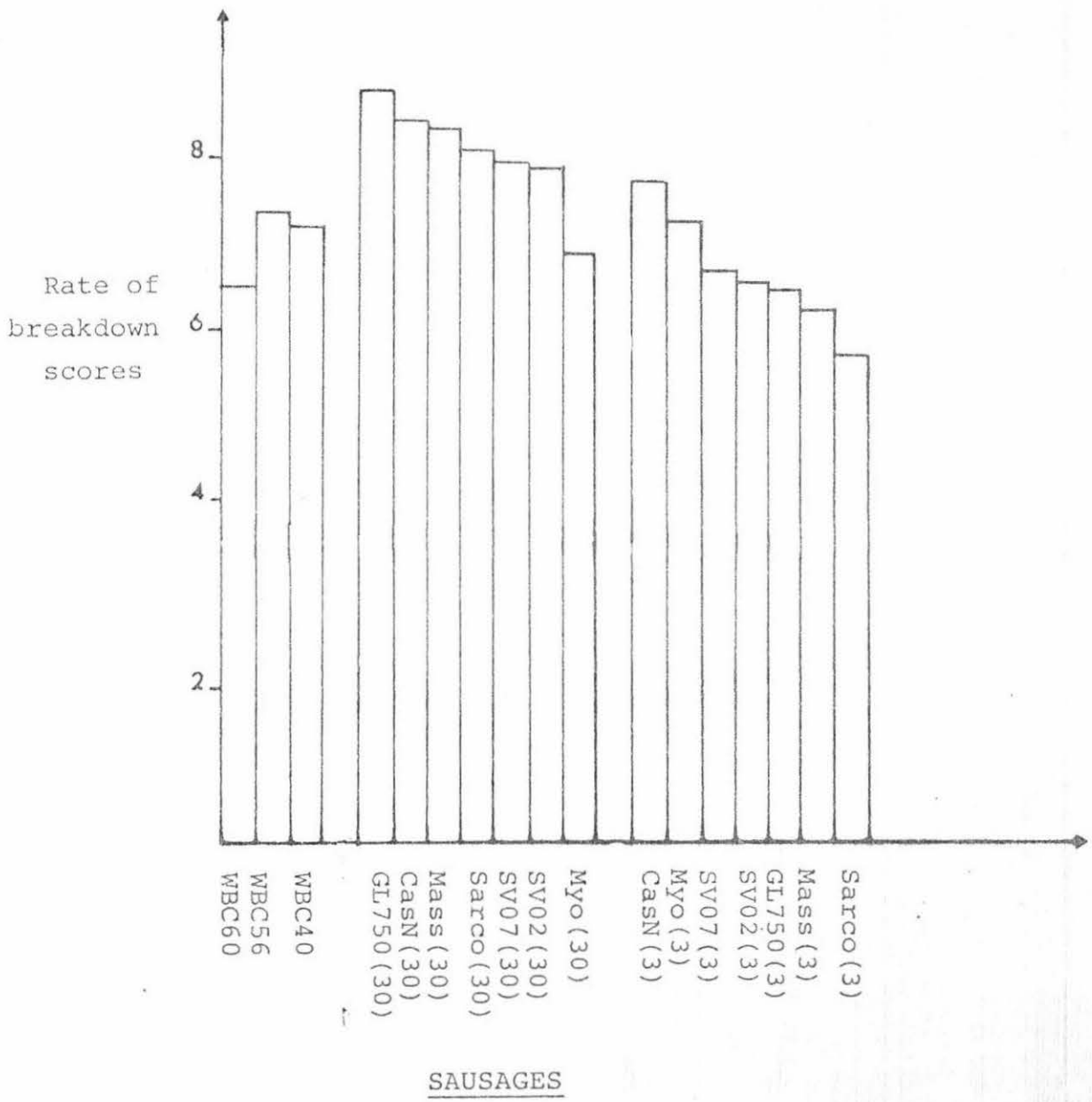


Figure 4.11 Rate of breakdown scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at the 3% and 30% protein replacement levels



generally slightly more chewy than the control sausages.

Increasing the protein replacement level from 3% to 30% resulted in less chewy sausages for all protein types, except MyoMPC where a more chewy sausage resulted. All these sausages had chewiness properties slightly less than controls. At this higher replacement level CasN and SV02 no longer resulted in the most chewy sausage and the meat proteins and GL750 produced the more chewy sausages.

Very little difference in chewiness was detected between the control sausages (Figure 4.12).

4.5.2.5 Stickiness

The type of protein additive and the replacement level affected the stickiness of the sausages examined.

At the 3% protein replacement level sausages containing SV02 and MyoMPC were the most sticky followed in decreasing order by sausages containing GL750, CasN, SarcoMPC, MassMPC and SV07. On the other hand at the 30% protein replacement level the most sticky sausage contained MassMPC followed in decreasing order by CasN(30), GL750(30), Sarco(30), SV02(30), Myo(30) and SV07(30).

For individual proteins an increase in stickiness score generally resulted as the replacement level was increased from 3% to 30%.

The WBC56 and WBC40 control sausages had similar stickiness scores and were more sticky than the WBC60 sausage. Sausages containing protein additives at both replacement levels, had stickiness values similar to these sausages which did not contain any additives (Figure 4.13).

4.5.2.6 Juiciness

The type of protein additive and level of protein replacement affected the juiciness of the sausages studied.

Figure 4.12 Chewiness scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at the 3% and 30% protein replacement levels

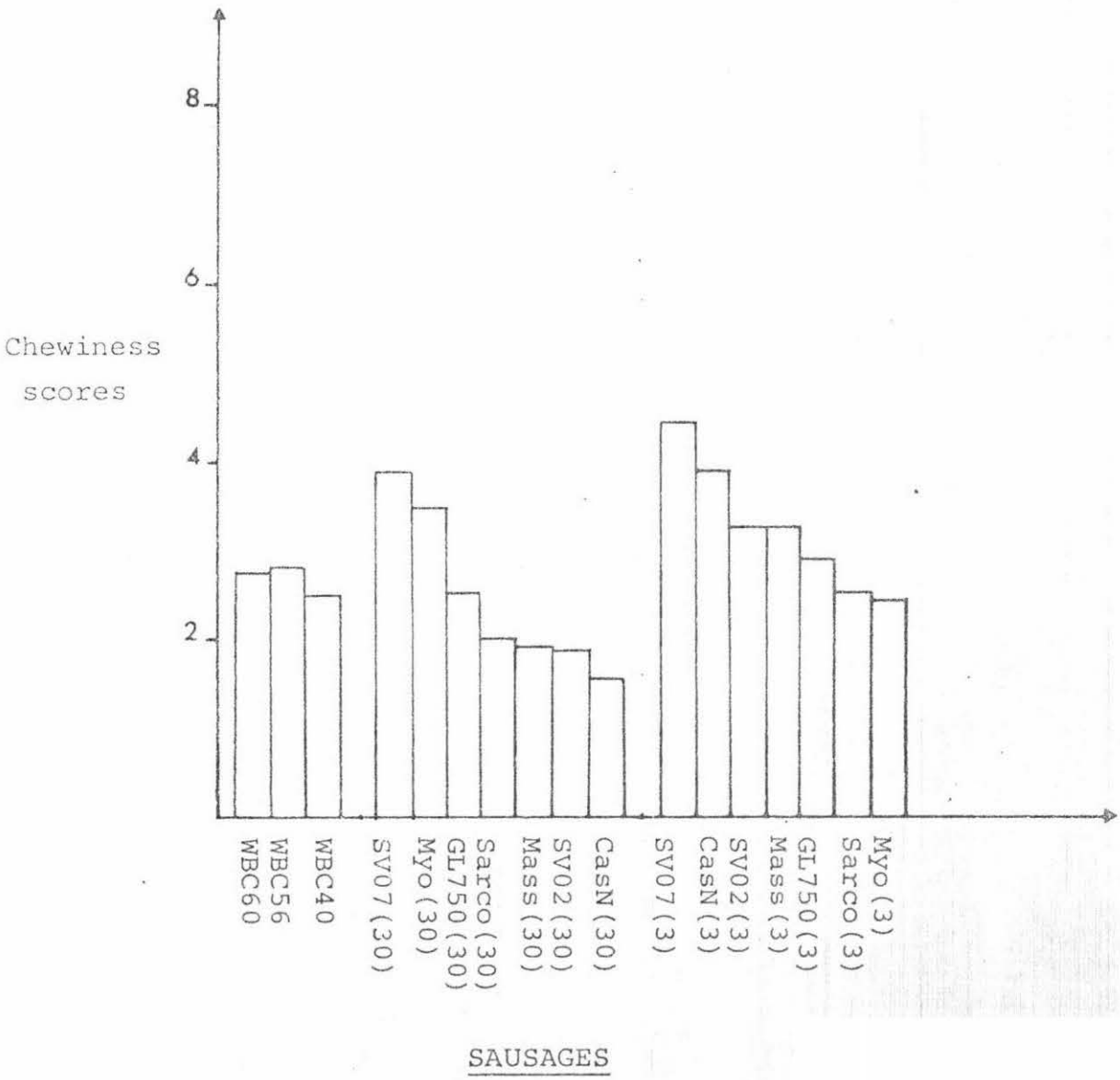
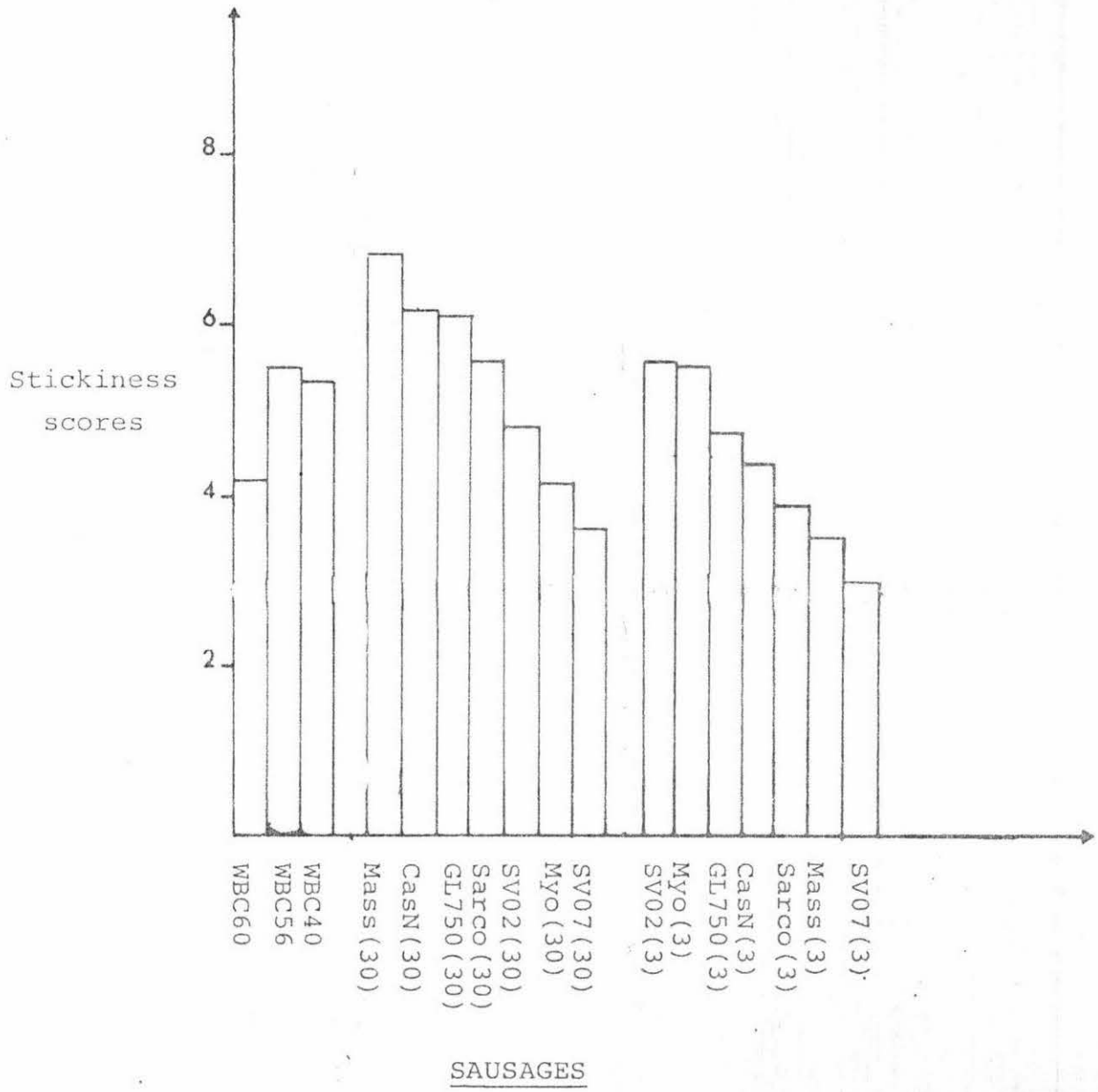


Figure 4.13 Stickiness scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels



At the 3% protein replacement level the meat proteins formed the most juicy sausages followed in decreasing order by CasN(3), GL750(3), SV07(3) and SV02(3). However at the 30% replacement level sausages containing the fibrous meat proteins were least juicy with the most juicy sausage containing SarcoMPC.

Sausages with 30% of the protein replaced by test protein were generally more juicy than their counterparts with only 3% replacement. The exceptions being MassMPC and MyoMPC where big decreases were observed between these two treatments.

The WBC56 and WBC40 sausages were given the same juiciness score and were considered to be more juicy than the WBC60 sausage. Juiciness scores for the sausages containing protein additives were similar to those of the control sausages (Figure 4.14).

4.5.2.7 Fattiness

SV02 and the meat proteins produced the most fatty sausages at the 3% protein replacement level followed in decreasing order by CasN, SV07 and GL750.

Increasing the replacement level generally caused an increase in fatiness, although a decrease was observed for sausages containing MassMPC and SV02. At the 30% replacement level the meat proteins once again produced the most fatty sausages followed in decreasing order by CasN, SV02, GL750 and SV07.

The most fatty control sausage was WBC56 and very little difference existed between WBC60 and WBC40. These sausages containing no protein additives had similar fattiness scores to sausages which had 30% of the protein replaced by additives, but were considered to be more fatty than most of the sausages with only 3% replacement (Figure 4.15).

Figure 4.14 Juiciness scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

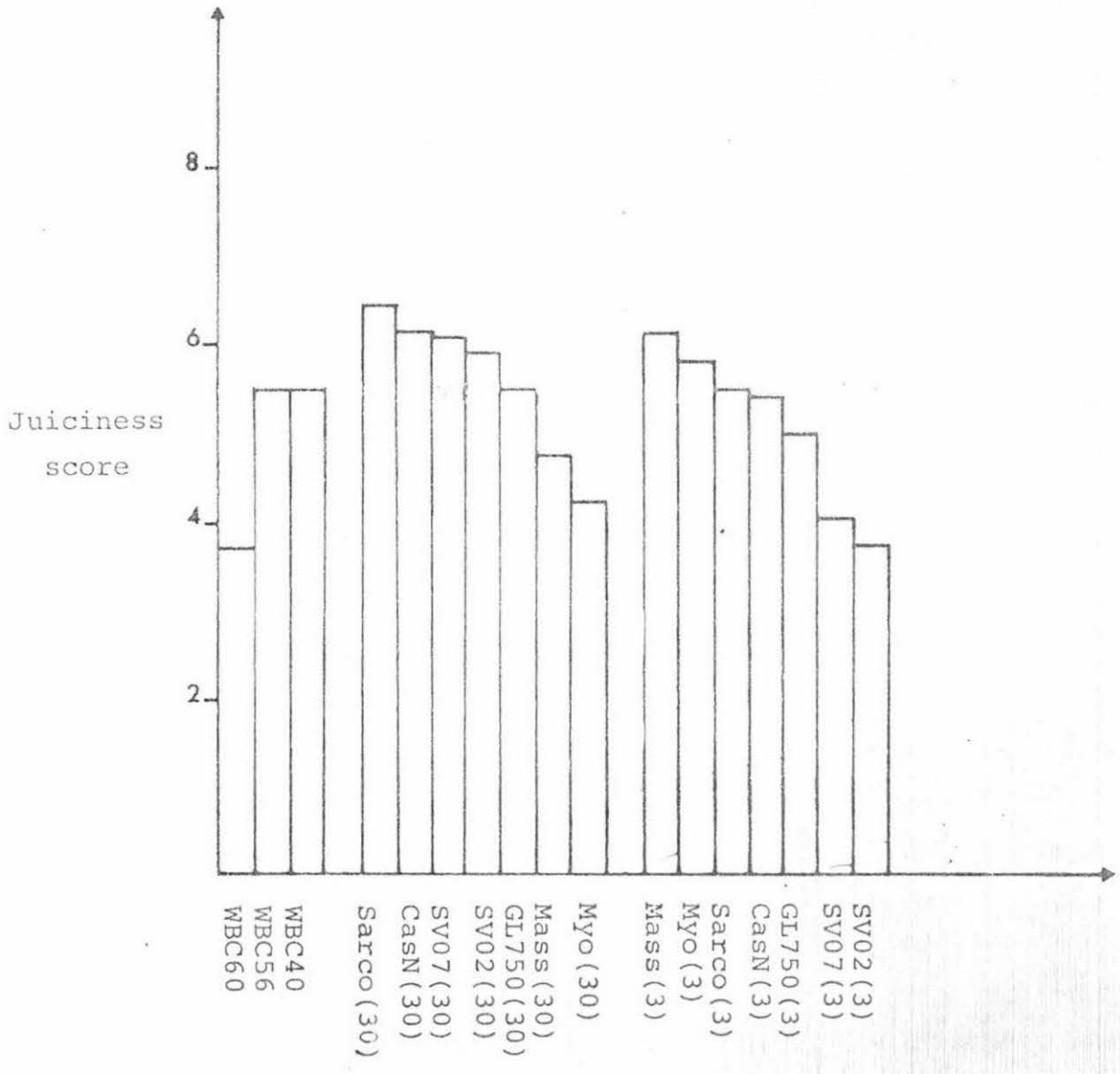
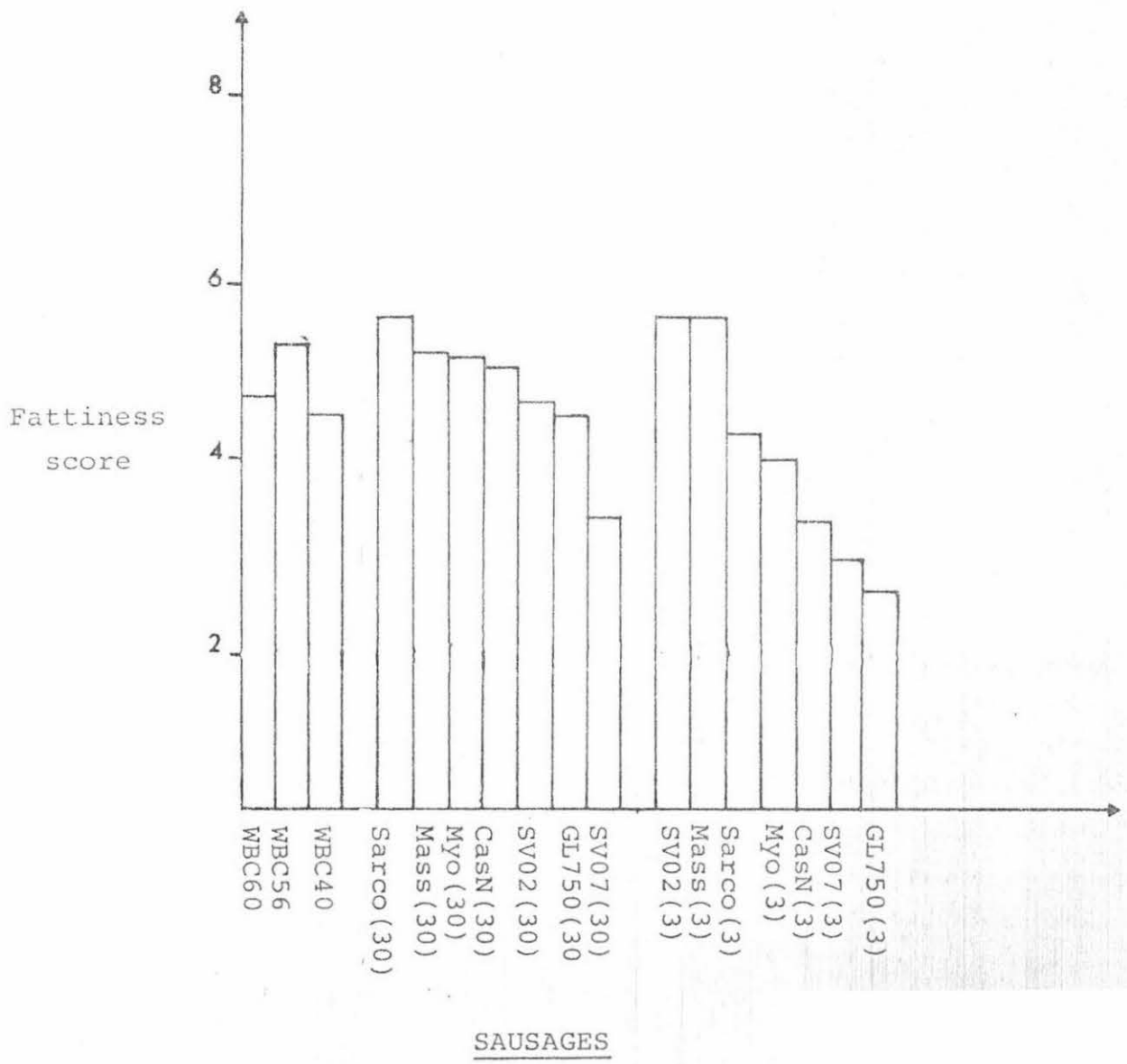


Figure 4.15 Fattiness scores for control sausages and sausages containing SV07, SV02, CasN, GL750, MassMCP, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

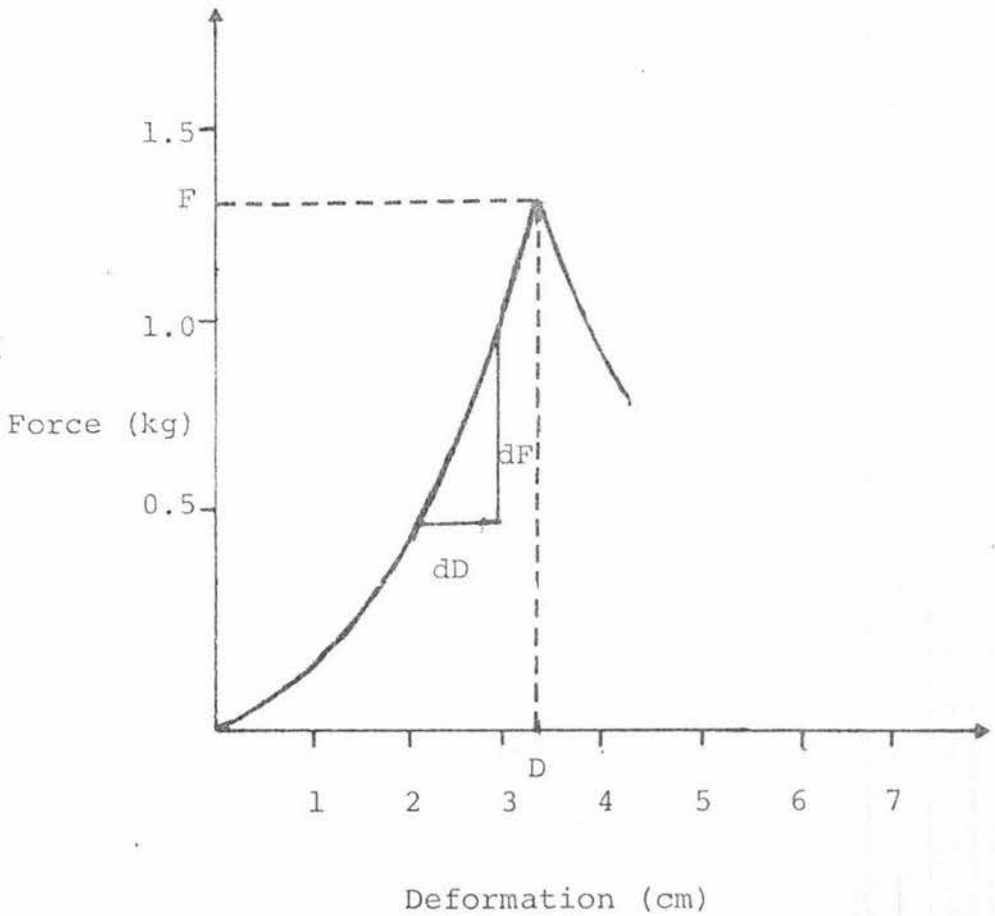


4.5.3 Instrumental measurement of textural properties

4.5.3.1 Compression test

In the compression test the force built up non-linearly to a maximum which coincided with an abrupt failure as the sausage fractured (Figure 4.16).

Figure 4.16 Typical compression test force-deformation curve showing rupture force (F), deformation (D), and slope (dF, dD) measurements



The type of protein additive and the protein replacement level had a marked effect on the rupture force and the slope of the force deformation curve in this test. However, the deformation value was not greatly affected (Tables 4.14, 4.15 and 4.16).

i) Rupture force

At the 3% protein replacement level, the greatest rupture forces occurred for sausages containing MassMPC, followed by CasN(3) and Sarco(3) and sausages containing the other two caseinates, GL750(3) and Myo(3) required the smallest rupture forces.

Increasing the replacement level to 30% generally resulted in a decrease in rupture force. At this replacement level the sausages containing the myofibrillar meat protein concentrates required the least rupture force and the greatest forces were required for Sarco(30) and GL750(30).

TABLE 4.14

Mean (n = 6) compression test rupture force (kg) values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Rupture force (kg)	
	3% protein replacement	30% protein replacement*
SV07	3.35 d	2.72 B
SV02	3.31 d	1.82 D
CasN	4.22 b	1.94 C
GL750	1.55 f	3.00 A
MassMPC	4.31 a	1.63 E
MyoMPC	2.32 e	1.69 E
SarcoMPC	3.44 c	2.71 B
Pooled $S\bar{x}$ = 0.03		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.15

Mean (n = 6) compression test deformation (cm) values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Deformation (cm)	
	3% protein replacement	30% protein replacement*
SV07	1.81 B	2.20 A
SV02	2.00 A	1.84 C
CasN	1.51 C	1.81 C
GL750	2.02 A	2.02 B
MassMPC	1.82 B	1.78 C
MyoMPC	2.02 A	1.42 D
SarcoMPC	2.04 A	2.01 B
Pooled $S\bar{x}$ = 0.03		

* Significant main effect difference ($P < 0.001$)
Interaction (protein x protein replacement level) significant ($P < 0.001$)

TABLE 4.16

Mean (n = 6) compression test slope (g/mm) values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Slope (g/mm)	
	3% protein replacement	30% protein replacement*
SV07	185.2 C	123.8 c
SV02	165.7 D	98.4 e
CasN	279.8 A	107.3 d
GL750	76.8 F	148.7 a
MassMPC	237.1 B	90.6 e
MyoMPC	115.0 E	119.5 c
SarcoMPC	169.1 D	134.9 b
Pooled $S\bar{x}$ = 2.91		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

Mean (n = 6) rupture force values of 1.21, 3.00 and 2.03 kg were observed for the WBC60, 56 and 40 control sausages respectively. Tnese values were generally similar to those of sausages with 30% of the protein replaced by additive but less than those for sausages with only 3% replacement.

ii) Deformation

Deformation values were similar for all sausages including the controls which had values of 2.01 (WBC60), 2.03 (WBC56) and 1.22 (cm) (WBC40).

iii) Slope

At the 3% protein replacement level the steepest slope was observed for sausages containing CasN followed in decreasing order by MassMPC, SV07, SarcoMPC, MyoMPC, SV02 and GL750.

Increasing the replacement level generally reduced the slope value (although increases were observed for sausages containing GL750 and MyoMPC). The sausage containing GL750 had the steepest slope followed in decreasing order by those containing SarcoMPC, SV02, MyoMPC, CasN, SV02 and MassMPC. Control sausages had slope values of 60.2 (WBC60), 14.50 (WBC56) and 166.9 (g/mm) (WBC40) which were similar to those of the sausages with 30% protein replacement but generally less than those with only 3% replacement.

4.5.3.2 Multiple compression test

In the multiple compression test the force that was applied to rupture the sausage caused the same changes to occur as have already been described for the single compression test.

Rupture force and the slope of the force-deformation curve were significantly affected by the type of protein additive and level of protein replacement by these additives. These factors however did not cause pronounced changes in deformation value (Tables 4.17, 4.18 and 4.19).

i) Rupture force

At the 3% protein replacement level the rupture force was greatest for sausages containing MassMPC followed in decreasing order by CasN(3), SV07(3), Sarco(3), SV02(3), GL750(3) and Myo(3).

TABLE 4.17

Mean (n = 6) multiple compression test rupture force values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Rupture force (kg)	
	3% protein replacement	30% protein replacement*
SV07	3.10 C	3.61 A
SV02	2.42 E	2.11 D
CasN	3.45 B	1.60 E
GL750	2.51 E	2.53 B
MassMPC	3.95 A	1.02 G
MyoMPC	1.90 F	1.25 F
SarcoMPC	2.65 D	2.41 C
Pooled $S\bar{x}$ = 0.03		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.18

Mean (n = 6) multiple compression test deformation values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Deformation (cm)	
	3% protein replacement	30% protein replacement*
SV07	2.00 ab	1.85 b
SV02	2.03 a	1.64 d
CasN	1.66 d	1.63 d
GL750	1.78 c	1.75 c
MassMPC	1.73 cd	1.02 f
MyoMPC	1.53 e	1.45 e
SarcoMPC	1.91 b	2.01 a
Pooled $S\bar{x}$ =0.03		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.19

Mean (n = 6) multiple compression slope values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Slope (g/mm)			
	3% protein replacement		30% protein replacement*	
		$S\bar{x}$		$S\bar{x}$
SV07	155.1	1.82	195.5	4.58
SV02	119.4	2.17	128.7	1.77
CasN	207.9	1.31	98.2	1.50
GL750	141.6	4.38	144.8	2.79
MassMPC	228.7	4.90	99.4	3.88
MyoMPC	124.7	5.11	86.3	2.27
SarcoMPC	138.9	2.00	120.0	2.24
Pooled $S\bar{x}$ = 3.19				

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

Generally, increasing the protein replacement level from 3% to 30% resulted in a decrease in sausage rupture force with sausages containing the two fibrous meat protein additives requiring the lowest rupture forces.

The control sausages had rupture force values of 1.56 (WBC60), 3.24 (WBC56) and 2.98 (kg) (WBC40). These values were similar to those of sausages containing protein additives at the 30% replacement level but slightly lower than those of the 3% level.

ii) Deformation values

Very little difference existed between the different sausages prepared at the 3% protein replacement level. At the 30% level the greatest deformation value occurred for Sarco(30) with sausages containing MassMPC and MyoMPC resulting in the smallest values. Slight to moderate decreases in deformation generally resulted as the replacement level was increased from 3% to 30%.

The WBC60 and WBC56 had the same deformation values (1.30 and 1.32, respectively) while WBC40 had the greatest value of 2.01 cm.

iii) Slope

The steepest force-deformation curve at the 3% protein replacement level occurred for sausages containing MassMPC, followed by CasN(3), SV07(3), GL750(3), Sarco(3), Myo(3) and SV02(3). At the 30% replacement level this order changed with the steepest slope resulting for the force-deformation curve of SV07(30) followed in decreasing order by GL750(30), SV02(30), SarcoMPC(30), CasN(30) and the two sausages containing the fibrous meat proteins.

The control sausages had slope values of 120.1 (WBC60), 245.7 (WBC56) and 149.3 (WBC40) which were similar to those of sausages with 3% of the protein replaced by a protein additive but generally steeper than those for sausages prepared at the 30% replacement level.

4.5.3.3 Extrusion through a wire grid

In this test the force applied to the sausage built up non-linearly whereupon the sausage fractured. The force then increased non-linearly to a second peak and quickly decreased as the sausage was extruded through the wire grid (Figure 4.17).

All parameters measured in this test were affected by protein type and protein replacement level although the effect wasn't as pronounced for rupture deformation (Tables 4.20-4.25).

i) Rupture force

Of the sausages with 3% protein replacement the greatest rupture force was required for SV07(3) followed in decreasing order by Mass(3), Sarco(3), CasN(3), SV02(3), Myo(3) and GL750(3).

TABLE 4.20

Mean (n = 6) extrusion test rupture force values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Rupture force (kg)	
	3% protein replacement	30% protein replacement*
SV07	3.03 A	1.57 E
SV02	1.54 E	1.81 C
CasN	2.01 D	2.22 A
GL750	1.13 F	1.70 D
MassMPC	2.62 B	1.01 G
MyoMPC	1.53 E	1.13 F
SarcoMPC	2.30 C	2.01 B
Pooled $S\bar{x}$ = 0.03		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level significant ($P < 0.001$)

Figure 4.17 Typical force-deformation curve for the extrusion test showing measurements for rupture force (F), rupture deformation (D), rupture slope (dF, dD), extrusion force (EF), extrusion deformation (ED) and extrusion slope (dEF, dED)

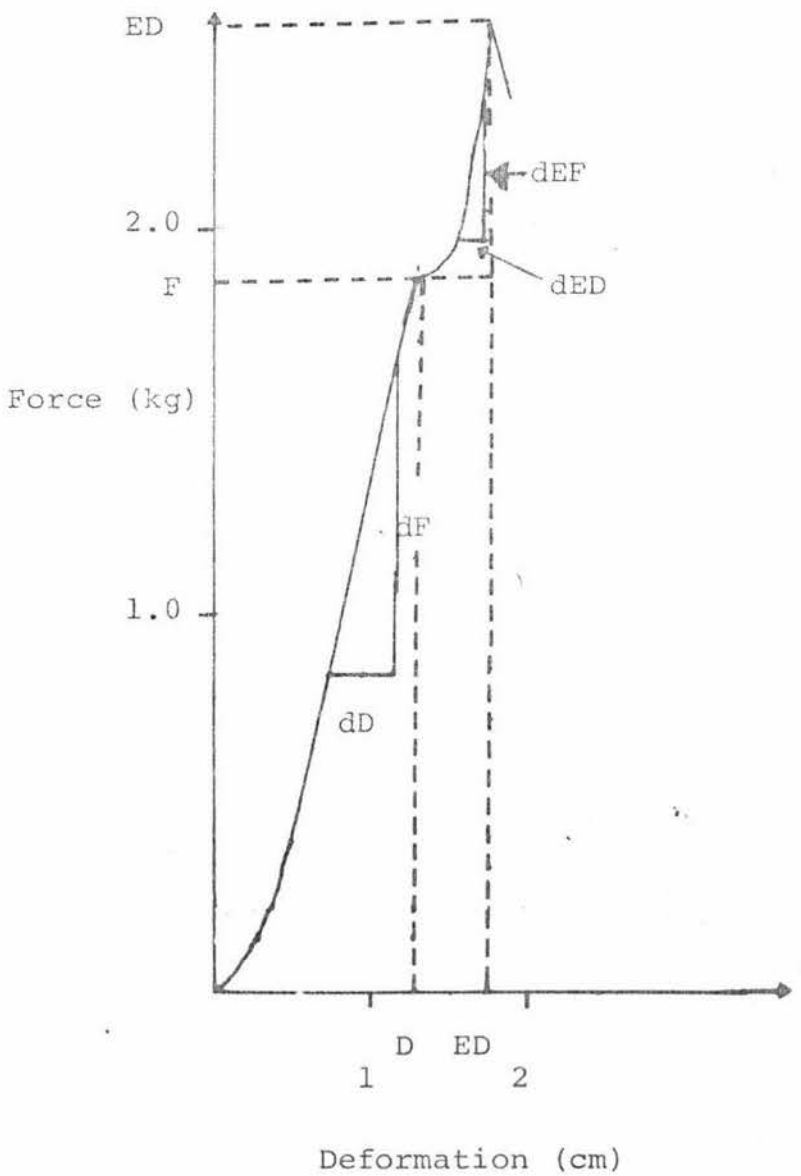


TABLE 4.21

Mean (n = 6) extrusion test rupture deformation values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Deformation (cm)	
	3% protein replacement	30% protein replacement**
SV07	2.31 b	1.54 d
SV02	1.46 e	2.04 e
CasN	1.52 e	2.45 a
GL750	1.90 d	2.02 b
MassMPC	2.47 a	2.01 b
MyoMPC	2.01 c	1.86 c
SarcoMPC	2.03 c	2.01 b
Pooled $S\bar{x}$ = 0.13		

* Significant main effect difference ($P < 0.001$)

** Significant main effect different ($P < 0.05$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.22

Mean (n = 6) extrusion test rupture slope values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Slope (g/mm)	
	3% protein replacement	30% protein replacement*
SV07	132.0 a	102.2 a
SV02	105.7 c	89.7 bc
CasN	132.4 a	90.7 b
GL750	61.2 e	84.3 c
MassMPC	106.1 c	50.3 e
MyoMPC	76.3 d	60.7 d
SarcoMPC	113.4 b	100.2 a
$S\bar{x}$ = 2.07		

* Significant main effect differences ($P < 0.001$) Interaction (protein type x protein replacement level) significant ($P < 0.001$)

Increasing the replacement level to 30% caused a reduction in rupture force for sausages including the meat proteins and SV07 with the sausages containing the fibrous meat proteins having the lowest rupture force.

The control sausages had rupture forces of 0.90 (WBC60), 2.51 (WBC56) and 2.33 kg (WBC40) which were similar to the range of values for the sausages containing the protein additives at both replacement levels.

ii) Rupture deformation

At the 3% protein replacement level sausages containing the meat proteins and SV07 had the greater deformation

TABLE 4.23

Mean (n = 6) extrusion test extrusion force values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Force (kg)	
	3% protein replacement	30% protein replacement*
SV07	3.11 ab	2.53 b
SV02	2.90 c	2.52 b
CasN	2.34 d	2.57 b
GL750	2.33 d	1.82 c
MassMPC	3.15 a	1.20 e
MyoMPC	1.65 e	1.70 d
SarcoMPC	3.04 b	3.24 a
Pooled $S\bar{x}$ = 0.03		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.24

Mean (n = 6) extrusion test extrusion deformation values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Deformation (cm)	
	3% protein replacement	30% protein replacement*
SV07	2.62 e	2.42 e
SV02	3.20 a	3.02 a
CasN	2.50 f	3.01 ab
GL750	2.84 c	2.43 d
MassMPC	3.03 b	2.71 c
MyoMPC	2.74 d	2.92 b
SarcoMPC	3.00 b	2.72 c
Pooled $S\bar{x}$ = 0.02		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

distances in comparison with the other sausages in this treatment. A similar ranking occurred at the 30% replacement level.

Deformation values of 1.80 (WBC60), 2.00 (WBC56) and 2.13 cm (WBC40) were observed for the control sausages which were similar to those values observed for sausages containing protein additives at both replacement levels.

iii) Rupture slope

At both the 3% and 30% protein replacement levels sausages incorporating GL750 and the two fibrous meat protein concentrates had the smallest rupture slope values. Increasing the protein replacement level from 3% to 30% generally caused the rupture slope values of sausages to decrease.

WBC60, WBC56 and WBC40 control sausages had rupture slope values of 50.06, 125.56 and 109.47 g/mm respectively. These values were similar for sausages with 3% of the protein replaced by additive but were generally higher than those at the 30% replacement level.

iv) Extrusion force

At the 3% protein replacement level sausages containing MassMPC and SV07 required the greatest force to extrude them through the wire bars followed in decreasing order by Sarco(3), SV02(3), CasN(3), GL750(3) and Myo(3).

When 30% of the protein was replaced with test protein, sausages containing SarcoMPC and caseinates required the greater extrusion forces while sausages containing the fibrous meat protein required the lowest extrusion forces.

The control sausages had extrusion force values which decreased with increased water binding ability giving values of 1.61 , 3.10 and 3.12 kg for WBC60, WBC56, WBC40, respectively. These values were similar to the range of values found for all other test sausages.

TABLE 4.25

Mean (n = 6) extrusion test extrusion slope values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Slope (g/mm)			
	3% protein replacement		30% protein replacement**	
		$S\bar{x}$		$S\bar{x}$
SV07	33.3	1.17	105.6	2.33
SV02	77.8	1.37	70.0	1.09
CasN	60.0	1.65	58.3	1.49
GL750	133.0	1.83	25.0	1.27
MassMPC	91.7	1.68	28.6	1.99
MyoMPC	21.4	0.97	54.5	1.22
SarcoMPC	70.0	2.35	171.4	2.08

* Significant main effect difference ($P < 0.001$)

** Significant main effect difference ($P < 0.01$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

v) Extrusion deformation

Sausages with 3% protein replacement by additives can be ranked SV02(3), Mass(3), Sarco(3), GL750(3), Myo(3), SV07(3) and CasN(3) with respect to decreasing deformation value.

At the 30% level sausages containing SV07 and CasN produced the greatest deformation values followed by sausages containing the meat proteins with the smallest deformation values being recorded for GL750(30) and SV07(30).

All sausages (except for those containing MyoMPC and CasN) had smaller deformation values at the 30% replacement level in contrast to the 3% level.

Values of 3.04 (WBC60), 3.41 (WBC56) and 3.00 cm (WBC40) were recorded for the control sausages.

vi) Slope

At the 3% protein replacement level sausages containing GL750 produced the steepest extrusion-force-deformation curve followed in decreasing order by Mass(3), SV02(3), Sarco(3), CasN(3), SV07(3) and Myo(3). Changes in this rank order occurred when the replacement level was increased to 30% with the steepest slope being recorded for Sarco(30) followed in decreasing order by SV07(30), SV02(30), CasN(30), Myo(30), GL750(30) and Mass(30).

Values of 53.30, 42.90 and 88.90 g/mm were associated with the three control sausages WBC60, WBC56 and WBC40. these were within the range of values observed for the two groups of test sausages.

4.5.3.4 Warner Bratzler test

In the Warner Bratzler test the force applied to the sausages typically built up non-linearly until rupture was initiated. The force gradually reduced as successive areas of the sample ruptured (Figure 4.18).

The type of protein additive and the level of incorporation both had significant effects on the textural parameters of sausages measured using the Warner-Bratzler Shear test (Tables 4.26, 4.27 and 4.28).

i) Shear force

Sausages containing the fibrous meat proteins at the 3% level required the lowest force to shear them. However, at the 30% replacement level sausages containing these fibrous meat proteins required the greatest shear forces followed by sausages containing the caseinates and

Figure 4.18 Typical force deformation curve for the Warner Bratzler test showing shear force (F), rupture deformation (D) and rupture slope (dF, dD) measurements

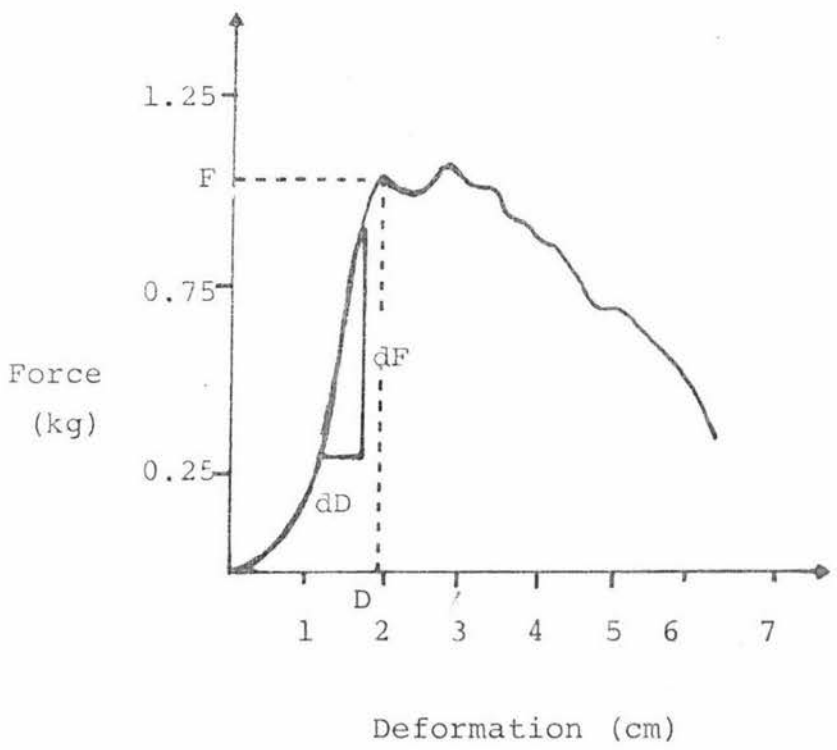


TABLE 4.26

Mean (n = 6) Warner Bratzler rupture force values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Rupture force (kg)	
	3% replacement level	30% replacement level*
SV07	1.14 D	1.10 C
SV02	1.50 B	0.73 D
CasN	1.05 E	0.71 D
GL750	3.38 A	0.17 F
MassMPC	0.72 F	1.21 B
MyoMPC	0.50 G	9.16 A
SarcoMPC	1.25 C	0.42 E
Pooled $S\bar{x}$ = 0.013		

* Significant main effect differences ($P<0.001$)
Interaction (protein type x protein replacement level) significant ($P<0.001$)

TABLE 4.27

Mean (n = 6) Warner Bratzler deformation values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Deformation (cm)	
	3% replacement level	30% replacement level*
SV07	2.46 B	1.54 D
SV02	3.23 A	2.21 B
CasN	1.67 E	2.30 A
GL750	2.11 D	0.48 G
MassMPC	2.34 C	2.03 C
MyoMPC	0.57 F	0.79 E
SarcoMPC	2.12 D	0.68 F
Pooled $S\bar{x}$ = 0.02		

* Significant main effect differences ($P < 0.001$) Interaction (protein type x protein replacement level) significant ($P < 0.001$)

TABLE 4.28

Mean (n = 6) Warner Bratzler slope values for sausages containing SV07, SV02, CasN, GL750, MassMPC, MyoMPC and SarcoMPC at 3% and 30% protein replacement levels

Protein*	Slope (g/mm)	
	3% protein replacement	30% protein replacement*
SV07	25.0 E	37.5 c
SV02	27.5 E	18.2 e
CasN	65.6 C	26.9 d
GL750	172.7 A	25.0 d
MassMPC	26.9 E	57.1 b
MyoMPC	150.0 D	120.0 a
SarcoMPC	36.4 B	20.0 e
Pooled $S\bar{x}$ = 1.41		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

SarcoMPC, with the lowest value being recorded for the sausages containing GL750.

Increasing the protein replacement level from 3% to 30% resulted in a very marked increase in shear force for sausages containing MyoMPC while a small increase was recorded when MassMPC was added. For all other proteins a decrease in rupture force occurred.

For the control sausages the largest shear force (3.02 kg) was required for WBC60 with very little difference occurring for WBC56 (0.35 kg) and WBC40 (0.45 kg).

ii) Deformation

At the 3% protein replacement level sausages containing the caseinates, SV02 and SV07, gave the highest deformation values followed in decreasing order by Mass(3), GL750(3), Sarco(3), CasN(3) and Myo(3). At the higher replacement level (30%) the largest deformation values occurred for sausages containing CasN and SV02 followed in decreasing order by those containing MassMPC, SV07, MyoMPC, SarcoMPC and GL750. Increasing the protein replacement level from 3% to 30% generally caused deformation values to decrease.

Deformation values for the control sausages increased with decreasing water binding ability giving values of 1.00 (WBC60), 1.42 (WBC56) and 2.01 cm (WBC40). The range of these results was similar to that of the test sausages prepared at the 3% protein replacement level but was generally slightly higher than sausages prepared at the 30% level.

iii) Slope

The slope value for the force deformation curve at the 3% protein replacement level was greatest for the sausage containing GL750 followed in decreasing order by Sarco(3), CasN(3), Myo(3) with SV07(3), SV02(3) and Mass(3) sharing the lowest slope value.

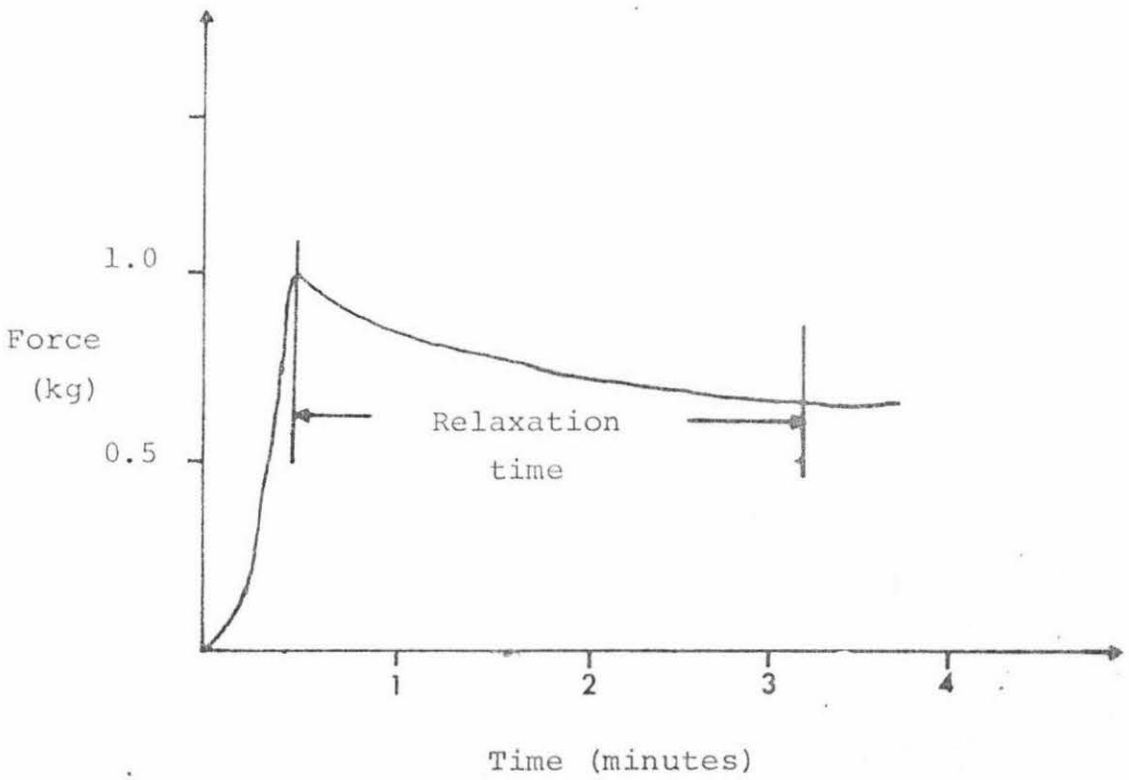
At the 30% replacement level this order was changed with sausages containing the fibrous meat proteins producing the steepest curves. Increasing the protein replacement level from 3% to 30% generally caused slope values to decrease.

Slope values of 200.0, 25.0 and 22.5 g/mm were recorded for the control sausages WBC60, WBC56 and WBC40, respectively. This range of values was similar to that of the test sausages at both protein replacement levels.

4.5.3.5 Relaxation

In the relaxation test the force built up non-linearly until the crosshead was stopped, whereupon it started to relax exponentially (Fig. 4.19).

Figure 4.19 Typical relaxation curve showing relaxation time measurement



The greatest relaxation times for sausages with 3% protein replaced with test protein were recorded for sausages containing SV07.

There was no difference in relaxation values of Myo(3), Sarco(3), Mass(3), GL750(3) and CasN(3). The shortest time was recorded for SV02(3). At the 30% level the sausage containing GL750 had the longest relaxation time followed in decreasing order by sausages containing SarcoMPC, MyoMPC, SV07, SV02, CasN and MassMPC.

Increasing the protein replacement level from 3% to 30% resulted in: no change in relaxation time for sausages containing SarcoMPC; an increase for those containing GL750; and a decrease for sausages containing all other test proteins (Table 4.29). The control sausages had relaxation times of 27.0 (WBC60), 16.2 (WBC56) and 37.2 (WBC 40) which were similar to sausages with 30% replacement of proteins by additive proteins. These times were generally less than those of sausages with only 3% replacement.

TABLE 4.29

Mean (n = 6) relaxation values for sausages with 3% and 30% of the protein replaced by test protein

Protein*	Relaxation time (seconds)	
	3% protein replacement	30% protein replacement*
SV07	47.4 a	27.0 d
SV02	32.1 c	21.9 e
CasN	33.6 bc	19.2 f
GL750	36.0 b	48.7 a
MassMPC	33.9 bc	14.4 g
MyoMPC	45.0 a	30.3 c
SarcoMPC	36.0 b	33.9 b
Pooled $S\bar{x}$ = 0.95		

* Significant main effect differences ($P < 0.001$)
Interaction (protein type x protein replacement level) significant ($P < 0.001$)

4.5.4 The correlation between sensory measurements and instrumental measurements

Significant ($P < 0.05$) correlation coefficients found between sensory and instrumental measurements on emulsion-type sausages are summarized in Table 4.30.

For each sensory property correlations with instrumental measurements were affected by the replacement level in the test sausages. In no case did the same correlation exist at both replacement levels.

4.6 The correlation between functional properties measured using model systems

Significant ($P < 0.05$) correlation coefficients found between functional properties measured using model systems are summarized in Table 4.31.

A greater number of linear relationships existed between functional properties measured in the absence of salt than in the presence of 2% salt ($P < 0.05$).

4.7 Properties of a cooked emulsion-type sausage correlated to functional properties of added proteins

Significant ($P < 0.05$) correlation coefficients found between the properties of cooked emulsion-type sausages and the functional properties of added proteins are summarized in Table 4.32.

Generally more linear relationships, between functional properties of added proteins and the properties of cooked sausages in which these proteins were incorporated, were determined when 30% of the protein in the sausage was replaced with additive protein than at a 3% replacement level

TABLE 4.30

Significant ($P < 0.05$) correlation coefficients between sensory and instrumental measurements of textural properties* for emulsion-type sausages with 3% and 30% of the protein replaced by additive protein (n = 7)

Sensory Measurement	Instrumental measurement	
	3% replacement level	30% replacement level
Firmness	vs WBD 0.77 vs GRIDED 0.80	vs GRIDD -0.84
Cleanness of first bite	vs WBF -0.79	vs MCD -0.79 vs GRIDS -0.73 vs GRIDEF -0.82 vs GRIDES -0.85
Rate of breakdown	Nil	vs WBF -0.87 vs WBS -0.76
Chewiness	Nil	vs GRIDD -0.85
Stickiness	vs GRIDF -0.86	Nil
Juiciness	vs MCD -0.80	vs MCD 0.76 vs WBS -0.87 vs GRIDF 0.87 vs GRIDS 0.92 vs GRIDEF 0.87
Fattiness	vs GRIDED 0.81	vs MCF -0.76 vs MCS -0.88

*Key

WBF - Warner Bratzler Shear Force
WBD - Warner Bratzler Deformation
WBS - Warner Bratzler Slope
MCF - Multiple compression force
MCD - Multiple compression deformation
MCS - Multiple compression slope
GRIDF - Extrusion test rupture force
GRIDD - Extrusion test rupture deformation
GRIDS - Extrusion test rupture slope
GRIDEF - Extrusion test extrusion force
GRIDED - Extrusion test extrusion deformation
GRIDES - Extrusion test extrusion slope

Table: 4.31 Significant ($P<0.05$) correlation coefficients between functional properties determined using model systems in both the presence and absence of salt*.(n=7).

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Heat Gel Strength	SOL 20	-.76	ESF 70	0.97
	SOL 30	-.76		
	SOL 40	-.77		
	SOL 50	-.77		
	SOL 60	-.77		
Emulsion Capacity	ESW 10	0.85	ESF 50	0.95
	ESW 40	0.79	WBC 510	-0.95
	ESW 50	0.77	WBC 520	-0.95
	ESW 60	0.77	WBC 530	-0.95
	ESF 40	0.82	WBC 540	-0.95
	ESF 50	0.86		
	ESF 60	0.83		
	WBC 510	-0.83		
	WBC 520	-0.83		
	WBC 530	-0.81		
	WBC 540	-0.79		
Emulsion Stability moisture loss at 10°C	ESW 20	0.82		
	ESW 30	0.94		
	ESW 40	0.99		
	ESW 50	0.98		
	ESW 60	0.97		
	ESW 70	0.91	NIL	
	ESF 40	0.99		
	ESF 50	0.99		
	ESF 60	0.99		
	ESF 70	0.96		
	WBC 510	-0.99		
	WBC 520	-0.99		
	WBC 530	-0.99		
	WBC 540	-0.98		
	WBC 550	-0.90		
	WBC 560	-0.79		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Emulsion Stability moisture loss at 20°C	ESW 30	0.93	ESW 30	0.96
	ESW 40	0.88	ESW 40	0.83
	ESW 50	0.89	ESW 60	0.83
	ESW 60	0.92	ESF 50	0.89
	ESW 70	0.93	WBC 510	-0.89
	ESF 40	0.85	WBC 520	-0.89
	ESF 50	0.86	WBC 530	-0.89
	ESF 60	0.88	WBC 540	-0.89
	ESF 70	0.93		
	WBC 510	-0.86		
	WBC 520	-0.86		
	WBC 530	-0.85		
	WBC 540	-0.83		
Emulsion Stability moisture loss at 30°C	ESW 40	0.98	ESW 40	0.85
	ESW 50	0.97	ESW 60	0.83
	ESW 60	0.98	ESF 50	0.93
	ESW 70	0.98	WBC 510	-0.93
	ESF 40	0.93	WBC 520	-0.93
	ESF 50	0.93	WBC 530	-0.93
	ESF 60	0.95	WBC 540	-0.93
	ESF 70	0.97		
	WBC 510	-0.93		
	WBC 520	-0.93		
	WBC 530	-0.93		
	WBC 540	-0.93		
	WBC 550	-0.78		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Emulsion Stability moisture loss at 40 °C	ESW 50	1.00	ESW 50	0.99
	ESW 60	0.99	ESW 60	0.99
	ESW 70	0.96		
	ESF 40	0.98		
	ESF 50	0.98		
	ESF 60	0.99		
	ESF 70	0.98		
	WBC 510	-0.98		
	WBC 520	-0.98		
	WBC 530	-0.98		
	WBC 540	-0.98		
	WBC 550	-0.88		
	WBC 560	-0.79		
Emulsion Stability moisture loss at 50 °C	ESW 60	1.00	ESW 60	0.98
	ESW 70	0.96		
	ESF 40	0.99		
	ESF 50	0.98		
	ESF 60	0.99		
	ESF 70	0.99		
	WBC 510	-0.99		
	WBC 520	-0.99		
	WBC 530	-0.99		
	WBC 540	-0.98		
	WBC 550	-0.89		
	WBC 560	-0.81		
Emulsion Stability moisture loss at 60°C	ESW 70	0.97		
	ESF 40	0.97		
	ESF 50	0.97		
	ESF 60	0.98		
	ESF 70	0.99		
	WBC 510	-0.97		
	WBC 520	-0.97	NIL	
	WBC 530	-0.98		
	WBC 540	-0.97		
	WBC 550	-0.86		
	WBC 560	-0.79		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Emulsion Stability moisture loss at 70°C	ESF 40	0.90	ESF 60	0.93
	ESF 50	0.90	SOL 10	-0.93
	ESF 60	0.92	SOL 20	-0.94
	ESF 70	0.97	SOL 30	-0.94
	WBC 510	-0.90	SOL 40	-0.94
	WBC 520	-0.90	SOL 50	-0.94
	WBC 530	-0.91	SOL 60	-0.95
	WBC 540	-0.91	SOL 70	-0.95
	WBC 550	-0.79		
Emulsion Stability fat loss at 40°C	ESF 50	1.00		
	ESF 60	1.00		
	ESF 70	0.97		
	WBC 510	-1.00		
	WBC 520	-1.00		
	WBC 530	-1.00		
	WBC 540	-0.99	NIL	
	WBC 550	-0.91		
	WBC 560	-0.81		
	WBC1010	-0.76		
Emulsion Stability fat loss at 50°C	ESF 60	1.00	WBC 510	-1.00
	ESF 70	0.97	WBC 520	-1.00
	WBC 510	-1.00	WBC 530	-1.00
	WBC 520	-1.00	WBC 540	-1.00
	WBC 530	-1.00		
	WBC 540	-0.98		
	WBC 550	-0.90		
	WBC 560	-0.79		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Emulsion Stability fat loss at 60°C	ESF 70	0.98	ESF 70	0.84
	WBC 510	-1.00	SOL 10	-0.91
	WBC 520	-1.00	SOL 20	-0.91
	WBC 530	-1.00	SOL 30	-0.91
	WBC 540	-0.99	SOL 40	-0.91
	WBC 550	-0.90	SOL 50	-0.91
	WBC 560	-0.79	SOL 60	-0.91
			SOL 70	-0.90
Emulsion Stability fat loss at 70°C	WBC 510	-0.97		
	WBC 520	-0.97		
	WBC 530	-0.98		
	WBC 540	-0.98	NIL	
	WBC 550	-0.87		
	WBC 560	-0.78		
Solubility at 10°C	SOL 20	1.00	SOL 20	1.00
	SOL 30	1.00	SOL 30	1.00
	SOL 40	1.00	SOL 40	1.00
	SOL 50	0.90	SOL 50	1.00
	SOL 60	0.86	SOL 60	1.00
	SOL 70	0.77	SOL 70	1.00
	WBC1040	-0.76	WBC1010	0.82
	WBC1050	-0.85		
	WBC1060	-0.91		
	WBC1070	-0.95		
Solubility at 20°C	SOL 30	1.00	SOL 30	1.00
	SOL 40	1.00	SOL 40	1.00
	SOL 50	0.91	SOL 50	1.00
	SOL 60	0.87	SOL 60	1.00
	SOL 70	0.79	SOL 70	1.00
	WBC1050	-0.87		
	WBC1060	-0.91		
	WBC1070	-0.94		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Solubility at 30°C	SOL 40	1.00	SOL 40	1.00
	SOL 50	0.92	SOL 50	1.00
	SOL 60	0.89	SOL 60	1.00
	SOL 70	0.81	SOL 70	1.00
	WBC1050	-0.87		
	WBC1060	-0.91		
	WBC1070	-0.94		
Solubility at 40°C	SOL 50	0.93	SOL 50	1.00
	SOL 60	0.90	SOL 60	1.00
	SOL 70	0.83	SOL 70	1.00
	WBC1050	-0.86		
	WBC1060	-0.91		
	WBC1070	-0.94		
Solubility at 50°C	SOL 60	1.00	SOL 50	1.00
	SOL 70	0.97	SOL 70	1.00
	WBC1060	-0.81		
	WBC1070	-0.84		
Solubility at 60°C	SOL 70	0.99	SOL 70	1.00
	WBC1060	-0.77		
	WBC1070	-0.80		
Water binding capacity (protein to water ratio 1: 5, at 10°C)	WBC 520	1.00	WBC 520	1.00
	WBC 530	1.00	WBC 530	1.00
	WBC 540	0.99	WBC 540	1.00
	WBC 550	0.91		
	WBC 560	0.81		
	WBC1010	0.76		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Water binding capacity (proten to water ratio 1:5, at 20°C).	WBC 530	1.00	WBC 530	1.00
	WBC 540	0.99	WBC 540	1.00
	WBC 550	0.91		
	WBC 560	0.81		
	WBC1010	0.76		
Water binding capacity (protein to water ratio 1:5, at 30°C).	WBC 540	1.00	WBC 540	1.00
	WBC 550	0.92		
	WBC 560	0.81		
Water binding capacity (protein to water ratio 1:5 at 40°C	WBC 550	0.93	WBC 560	0.97
	WBC 560	0.82	WBC 570	0.91
Water binding capacity (protein to water ratio 1:5, at 50°C)	WBC 560	0.95	WBC 560	0.97
	WBC 570	0.79	WBC 570	0.91
	WBC1010	0.88		
	WBC1020	0.76		
	WBC1040	0.76		
Water binding capacity (protein to water ratio 1:5, at 60°C).	WBC 570	0.90	WBC 570	0.93
	WBC1010	0.88		
	WBC1050	0.79		
Water binding capacity (protein to water ratio 1:10, at 10°C)	WBC1020	0.82		
	WBC1030	0.82	NIL	
	WBC1040	0.81		

Table: 4.31 Continued

Functional Property	Correlated Functional Property			
	Without Salt		With 2% Salt	
Water binding capacity (protein to water ratio 1:10 at 20°C).	WBC1030	1.00	WBC1030	0.98
	WBC1040	1.00	WBC1040	0.90
	WBC1050	0.76		
Water binding capacity (protein to water ratio 1:10 at 30°C).	WBC1040	1.00	WBC1040	0.97
Water binding capacity (protein to water ratio 1:10 at 40°C).	WBC1050	0.79	NIL	
	WBC1060	0.77		
Water binding capacity (protein to water ratio 1:10 at 50°C).	WBC1060	0.98	WBC1060	0.99
	WBC1070	0.97	WBC1070	0.97
Water binding capacity (protein to water ratio 1:10 at 60°C).	WBC1070	0.99	WBC1070	0.97

Table: 4.31 Continued

*KEY:

HGS	-Heat gel strength.
EC	-Emulsion capacity.
ESW_i	-Emulsion stability moisture loss at temperature $(^{\circ}C)_i$
EFF_i	-Emulsion stability fat loss at temperature $(^{\circ}C)_i$
SOI_i	-Solubility at temperature $(^{\circ}C)_i$
$WBC5_i$	-Water binding capacity, protein to water ratio 1:5 temperature $(^{\circ}C)_i$
$WBC10_i$	-Water binding capacity, protein to water ratio 1:10, temperature $(^{\circ}C)_i$

Table 4.32: Significant ($P<0.05$) correlation coefficients between functional properties determined using model systems (in the presence and absence of 2% salt) and properties of a cooked emulsion type sausage (with 3% and 30% of the protein replaced by additive protein) (n=7)*.

Functional Property	Correlated Sausage Property					
	3% Replacement Level			30% Replacement Level		
	Without Salt		With Salt	Without Salt		With Salt	
Heat Gel Strength	Nil		Nil	Juiciness	-.84	Juiciness	-.93
				Fat Loss	.92	ComD	.90
				MCD	-.90	WBF	.93
				GRIDF	-.88	WBS	.98
				GRIDS	-.94		
				GRIDEF	-.81		
Emulsion Capacity	Fat Loss	.83	Cleanness	-.94	Mois Loss	.93	Nil
			WBC	.89	ComF	.76	
			Mois Loss	.91			
			WBF	.97			
Emulsion stability moisture loss at 10°C	Fat Loss	.99	WBD	.91	Mois Loss	.78	Nil
			WBS	-.84	GRIDES	.76	
			GRIDEF	.94			
Emulsion stability moisture loss at 20°C	Fat Loss	.85	GRIDES	.85	Nil		Nil
Emulsion stability moisture loss at 30°C	Fat Loss	.93	Nil		Nil		Nil
Emulsion stability moisture loss at 40°C	Fat Loss	.99	WBS	.86	Nil		Nil

Table: 4.32 Continued

Functional Property	Correlated Sausage Property							
	3% Replacement Level				30% Replacement Level			
	Without Salt		With Salt		Without Salt		With Salt	
Emulsion stability moisture loss at 50°C	Fat Loss	.99	Nil		GRIDES	.78	Nil	
Emulsion stability moisture loss at 60°C	Fat Loss	.97	WBS	.84	Nil		Nil	
Emulsion stability moisture loss at 70°C	Fat Loss	.90	Nil		Nil		Juiciness GRIDS GRIDEF GRIDES	-.87 -.82 -.87 -.82
Emulsion stability fat loss at 10°C	Fat Loss	1.00	Nil		Nil		Nil	
Emulsion stability fat loss at 20°C	Fat Loss	1.00	Nil		Nil		Nil	
Emulsion stability fat loss at 30°C	Fat Loss	1.00	Nil		Nil		Nil	
Emulsion stability fat loss at 40°C	Fat Loss	.97	Nil		Mois Loss GRIDES	.76 .84	Nil	
Emulsion stability fat loss at 50°C	Nil		Cleanness Mois Loss WBF	-.83 .84 .94	Mois Loss GRIDES	.79 .83	Relax	.88

Table: 4.32 Continued

Functional Property	Correlated Sausage Property					
	3% Replacement Level			30% Replacement Level		
	Without Salt	With Salt		Without Salt		With Salt	
Emulsion stability fat loss at 60°C	Nil	Cleanness WBS GRIDS	-.85 .83 -.88	Mois Loss GRIDES	.76 .82	Juiciness WBD	-.84 -.83
Emulsion stability fat loss at 70°C	Nil	Nil		GRIDES	.77	Juiciness WBF WBS	-.92 .94 .96
Solubility at 10°C	Nil	Nil		Juiciness Fat Loss GRIDF GRIDS GRIDEF GRIDES	.92 -.76 .80 .88 .96 .80	Juiciness Fat Loss GRIDS GRIDEF	.89 -.86 .81 .94
Solubility at 20°C	Nil	Nil		Juiciness Fat Loss GRIDF GRIDS GRIDEF GRIDES	.93 -.77 .80 .88 .95 .79	Juiciness Fat Loss GRIDS GRIDEF	.89 -.86 .81 .94

Table: 4.32 Continued

Functional Property	Correlated Sausage Property					
	3% Replacement Level		30% Replacement Level			
	Without Salt	With Salt	Without Salt		With Salt	
Solubility at 30° C	Nil	Nil	Juiciness	.93	Juiciness	.89
			Fat Loss	-.78	Fat Loss	-.86
			GRIDF	.80	GRIDS	.81
			GRIDS	.88	GRIDEF	.94
			GRIDEF	.95		
			GRIDES	.77		
Solubility at 40° C	Nil	Nil	Juiciness	.93	Juiciness	.89
			Fat Loss	-.80	Fat Loss	-.86
			GRIDF	.80	GRIDS	.81
			GRIDS	.89	GRIDEF	,94
			GRIDEF	.94		
			GRIDES	.75		
Solubility at 50° C	Nil	Nil	Juiciness	.87	Juiciness	.89
			Fat Loss	-.88	Fat Loss	-.87
			GRIDF	.75	GRIDS	.82
			GRIDS	.84	GRIDEF	.94
			GRIDEF	.80		
Solubility at 60° C	Nil	Nil	Juiciness	.84	Juiciness	.90
			Fat Loss	-.88	Fat Loss	-.88
			GRIDS	.82	GRIDS	.84
					GRIDEF	.95

Table: 4.32 Continued

Functional Property	Correlated Sausage Property							
	3% Replacement Level				30% Replacement Level			
	Without Salt		With Salt		Without Salt		With Salt	
Solubility at 70°C	Nil		Nil		Juiciness Fat Loss GRIDS	.77 -.87 .76	Juiciness Fat Loss GRIDS GRIDEF	.90 -.88 .84 .95
Water binding capacity (protein to water ratio 1:5 10°C)	Fat Loss	-1.00	Cleanness Mois Loss WBF	.83 -.84 -.94	Mois Loss GRIDES	-.76 -.85	Relax	-.88
Water binding capacity (protein to water ratio 1:5 20°C)	Fat loss	-1.00	Cleanness Mois Loss WBF	.83 -.84 -.94	Mois Loss GRIDES	-.77 -.85	Relax	-.88
Water binding capacity (protein to water ratio 1:5 30°C)	Fat Loss	-1.00	Cleanness Mois Loss WBF	.83 -.84 -.94	GRIDES	-.83	Relax	-.88
Water binding capacity (protein to water ratio 1:5 40°C)	Fat Loss	- .99	Cleanness Mois Loss WBF	.83 -.84 -.94	GRIDES	-.79	Relax	-.88
Water binding capacity (protein to water ratio 1:5 50°C)	Fat Loss	- .91	WBC	.84	Nil		Fattiness Fat Loss Mois Loss ComF ComS MCF MCD	-.86 .87 .90 .81 .94 .85 .90

Table: 4.32 Continued

Functional Property	Correlated Sausage Property							
	3% Replacement Level				30% Replacement Level			
	Without Salt		With Salt		Without Salt		With Salt	
Water binding capacity (protein to water ratio 1:5 60°C)	Fat Loss	-.81	WBC	.86	Nil		Fattiness Fat Loss Mois Loss ComS MCF GRIDS	-.80 .82 .87 .91 .95 .87
Water binding capacity (protein to water ratio 1:5 70°C)	WBC Mois Loss	.87 .76	WBC	.86	Nil		Fattiness Fat Loss Mois Loss ComS MCF MCD GRIDS	-.86 .89 .89 .92 .89 .85 .87
Water binding capacity (protein to water ratio 1:10 10°C)	Fat Loss	-.76	Chewiness GRIDS	.88 .92	Nil		GRIDES	.82
Water binding capacity (protein to water ratio 1:10 20°C)	MCD	-.77	Nil		GRIDES	-.83	Nil	
Water binding capacity (protein to water ratio 1:10 30°C)	MCD	-.77	Stickiness	-.81	GRIDES	-.82	Nil	

Table: 4.33 Continued

	Correlated Sausage Property							
	3% Replacement Salt				30% Replacement Salt			
	Without Salt		With Salt		Without Salt		With Salt	
Water binding capacity (protein to water ratio 1:10 40°C)	MCD	-.77	Stickiness GRIDED	-.86 -.82	GRIDES	-.83	Nil	
Water binding capacity (protein to water ratio 1:10 50°C)	Nil		Nil		GRIDEF GRIDES	-.81 -.77	Chewiness WBC GRIDD GRIDED	.84 .95 -.90 -.87
Water binding capacity (protein to water ratio 1:10 60°C)	WBS GRIDS	.79 -.84	Nil		GRIDEF GRIDES	-.83 -.77	Fattiness WBC MCF MCS GRIDED	-.85 .91 .82 .86 -.88
Water binding capacity (protein to water ratio 1:10 70°C)	WBS GRIDS	.77 -.83	Nil		GRIDEF GRIDES	-.86 -.76	Fattiness WBC MCF MCS GRIDD GRIDED	-.92 .85 .87 .91 -.85 -.84

Table: 4.32 Continued

*KEY:

ComF	- Compression test force
ComD	- Compression test deformation
ComS	- Compression test slope
WBF	- Warner Bratzler force
WBD	- Warner Bratzler deformation
WBS	- Warner Bratzler slope
GRIDF	- Extrusion test rupture force
GRIDD	- Extrusion test rupture deformation
GRIDS	- Extrusion test rupture slope
GRIDEF	- Extrusion test extrusion force
GRIDED	- Extrusion test extrusion deformation
GRIDES	- Extrusion test extrusion slope
Relax	- Relaxation time
MCF	- Multiple compression test force
MCD	- Multiple compression test deformation
MCS	- Multiple compression test slope
Mois loss	- Moisture loss from cooked sausage
WBC	- $\left\lvert WBC_{predicted} - WBC_{observed} \right\rvert$
Cleanness	- Cleanness of first bite property

4.8 Foaming capacity and stability

No foaming properties were exhibited by MyoMPC and MassMPC under the conditions used in this study.

4.8.1 Foaming capacity

Foaming capacity generally increased with increasing shaking time. SarcoMPC exhibited superior properties to egg white albumin at all shaking times studied and better properties than the other proteins at shaking times of 1, 2 and 4 minutes. At 6 minutes shaking time the caseinates exhibited superior foaming capacities (Figure 4.20).

4.8.2 Foaming stability

Foam volume of all protein types decayed with time (Figures 4.21 to 4.26). Decay patterns were non linear, generally faster immediately post shaking, and did not appear to be markedly affected by shaking time. The most stable foams were formed by GL750 and SarcoMPC, these being comparable with egg white albumin.

Figure 4.20 The effect of shaking time on the foaming capacity of 2% protein dispersions of SV07 (■——■), SV02 (●——●), CasN (◆——◆), GL750 (▲——▲), SarcoMPC (△——△) and egg white albumin (◇——◇)

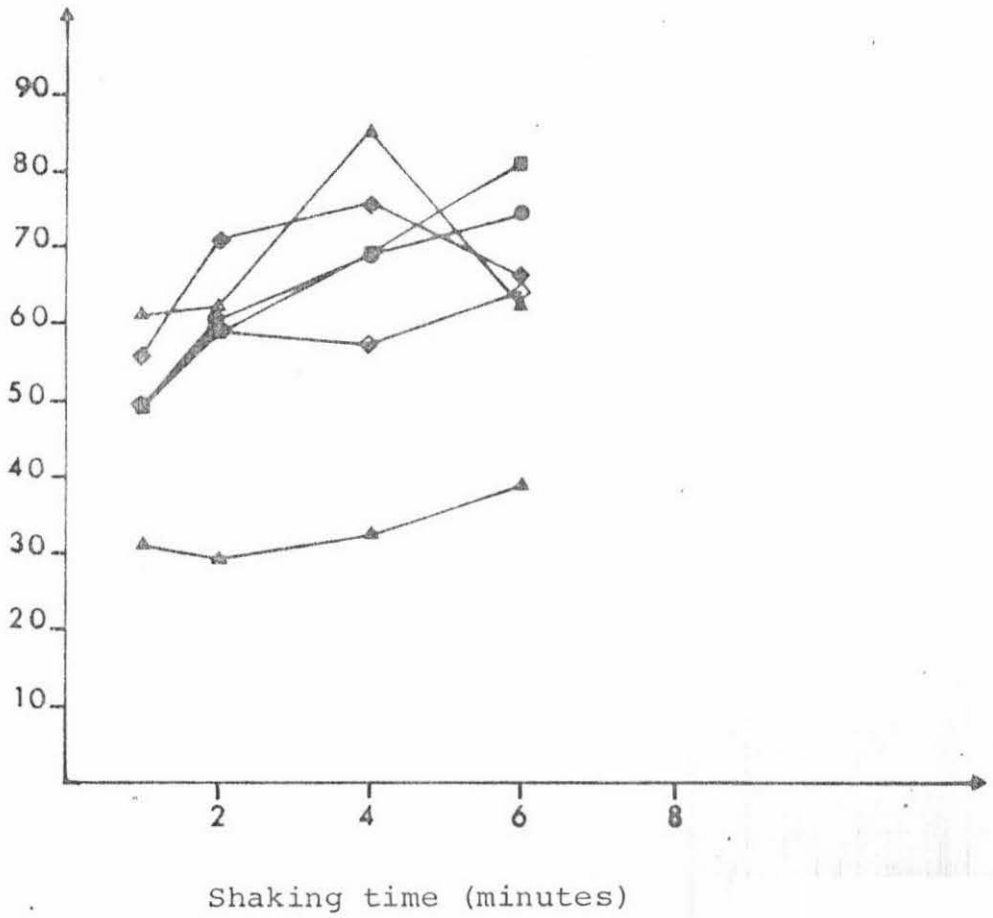


Figure 4.21 The effect of shake time on foam volume decay for a 2% protein dispersion of SV07

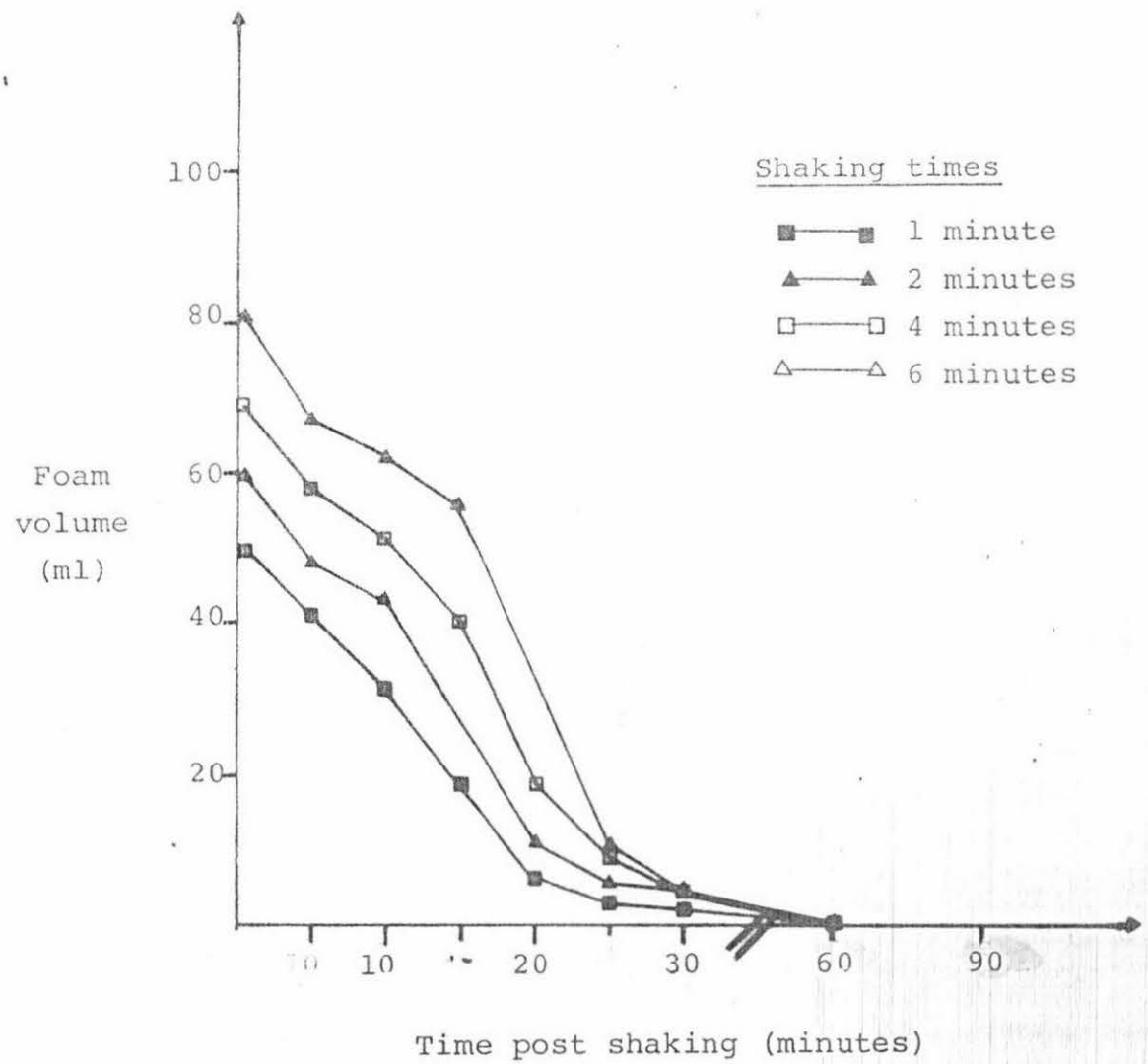


Figure 4.22 The effect of shaking time on foam volume decay for a 2% protein dispersion of SV02

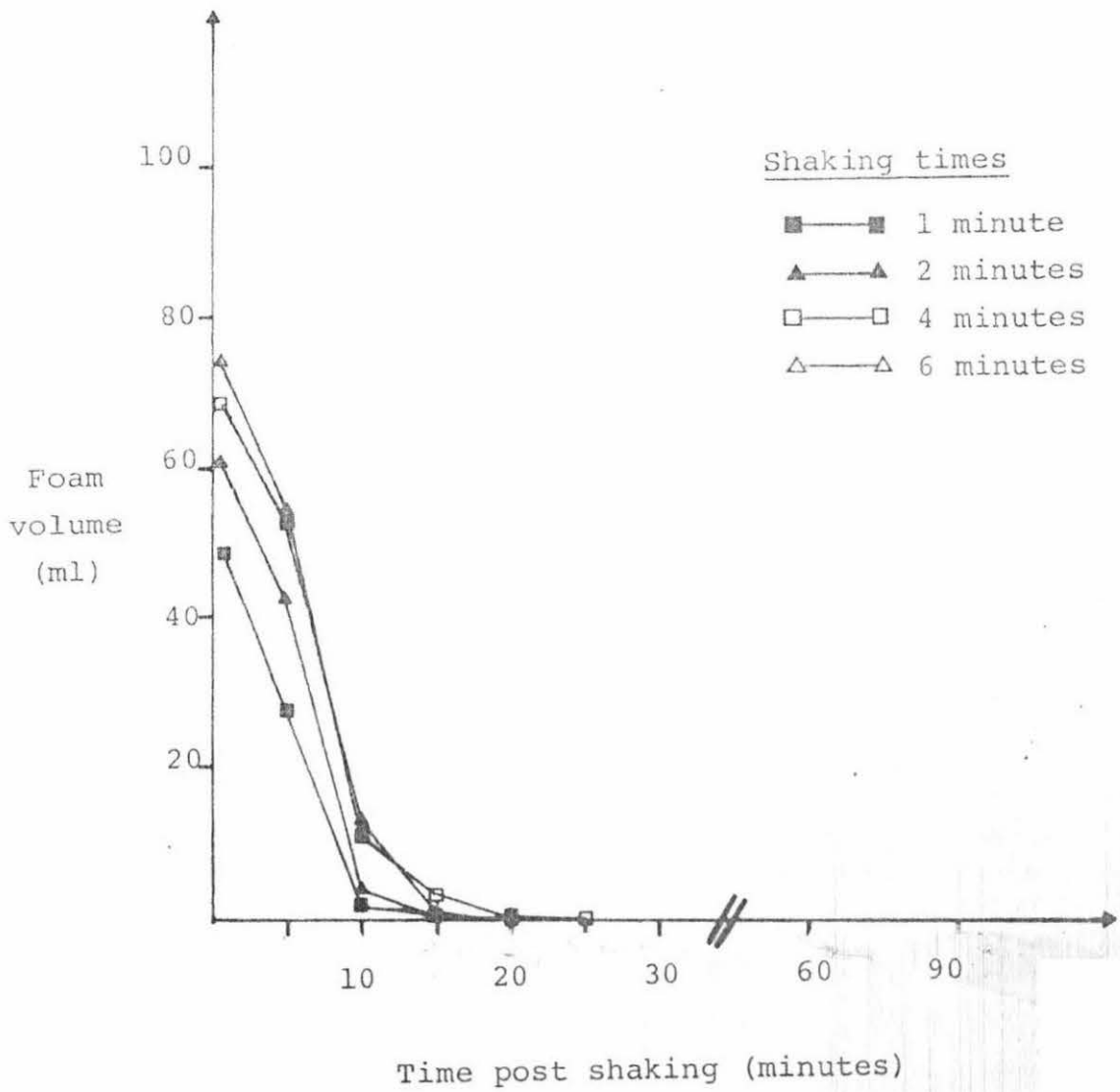


Figure 4.23 The effect of shaking time on foam volume decay for a 2% protein dispersion of CasN

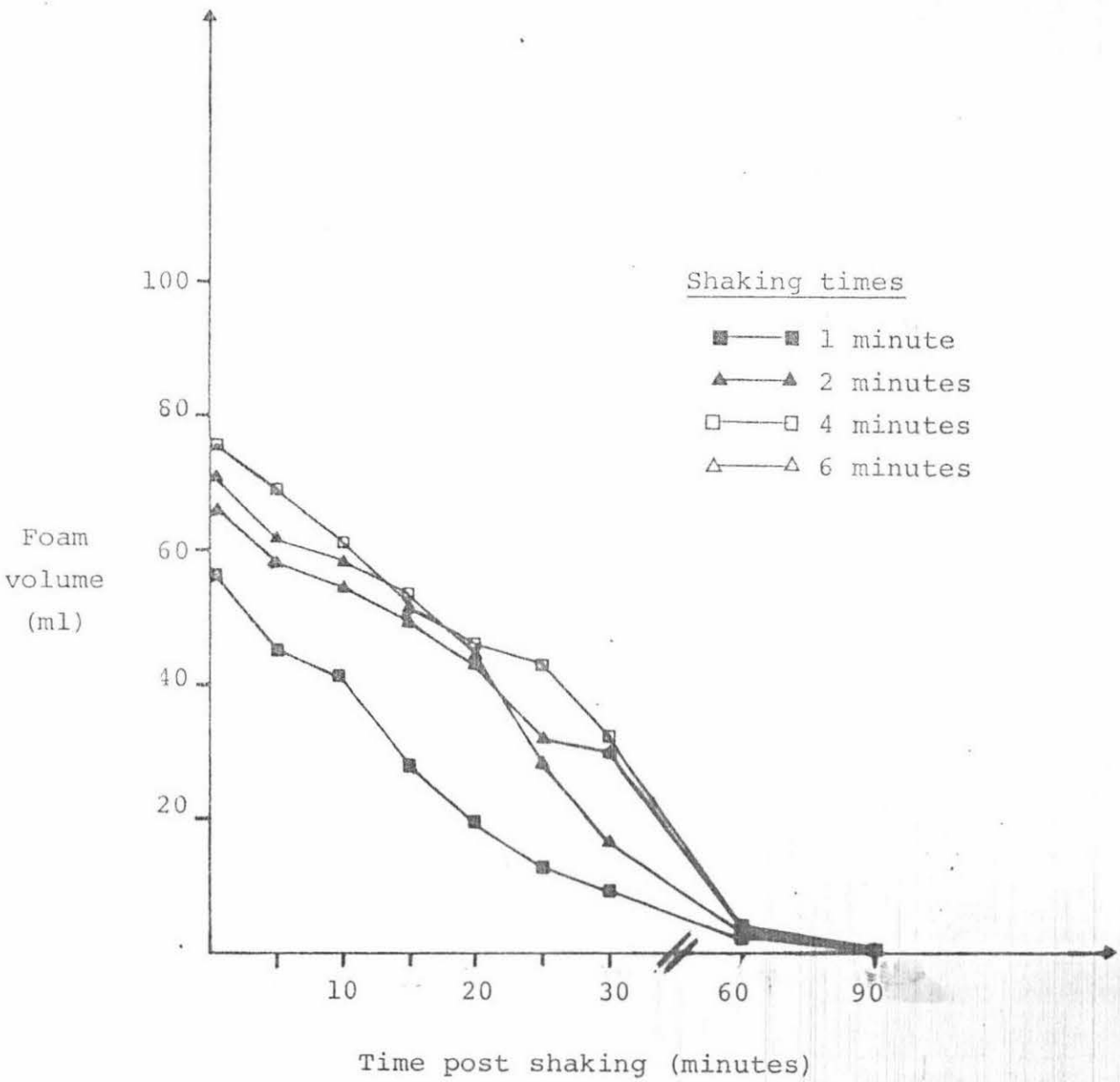


Figure 4.24 The effect of shaking time on foam volume decay for 2% protein dispersions of GL750

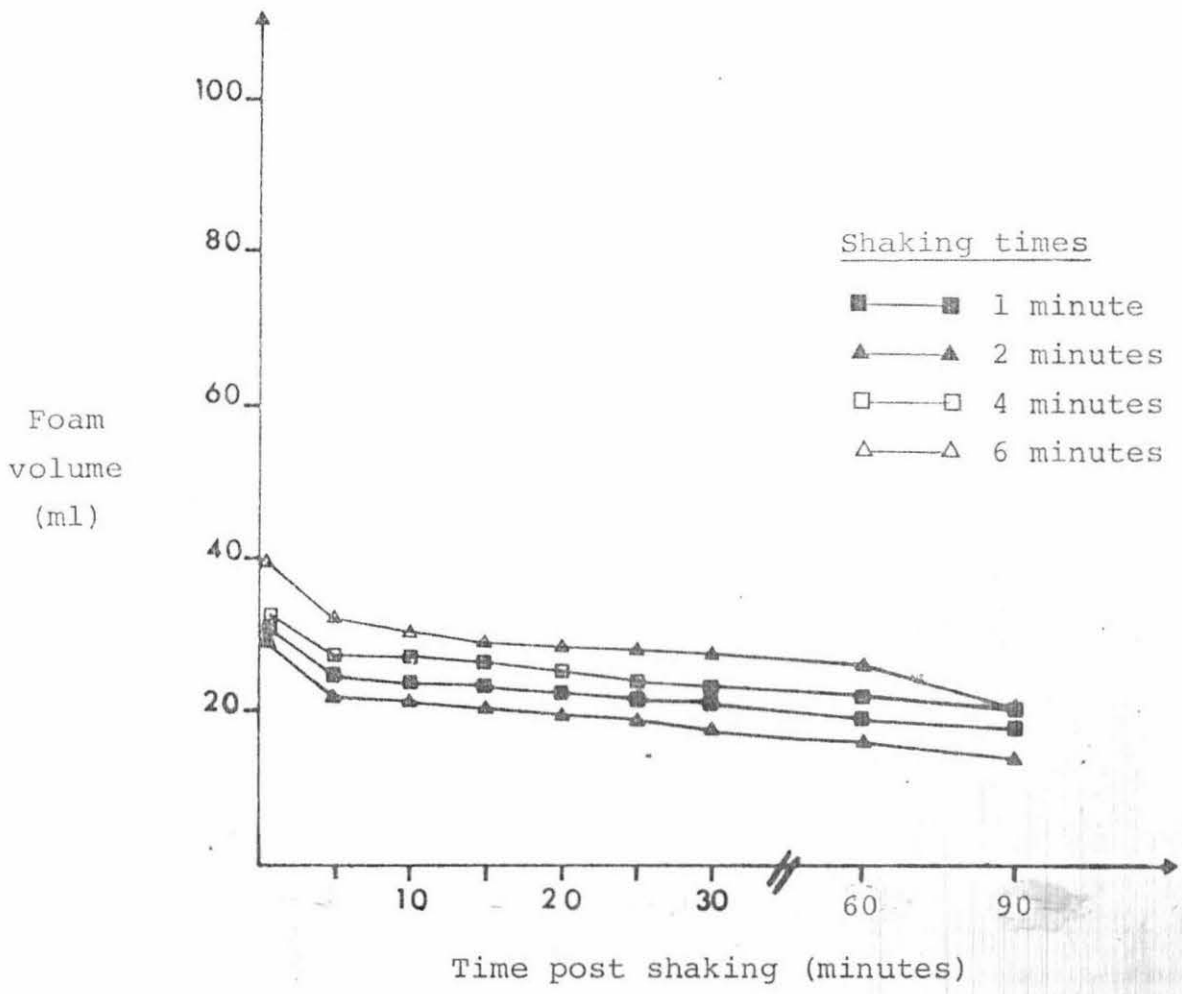


Figure 4.25 The effect of shaking time on foam volume decay for a 2% protein dispersion of SarcoMPC

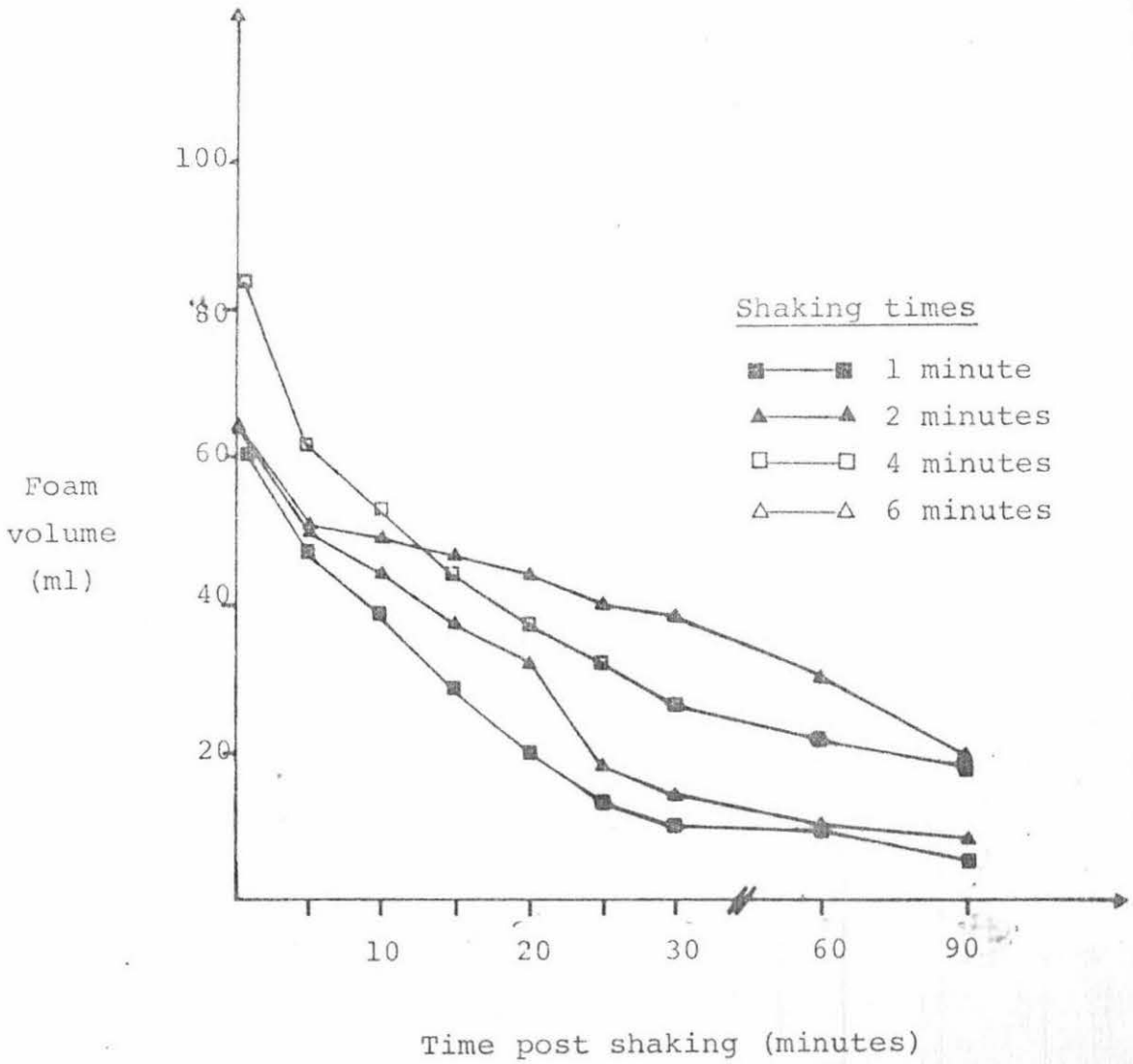
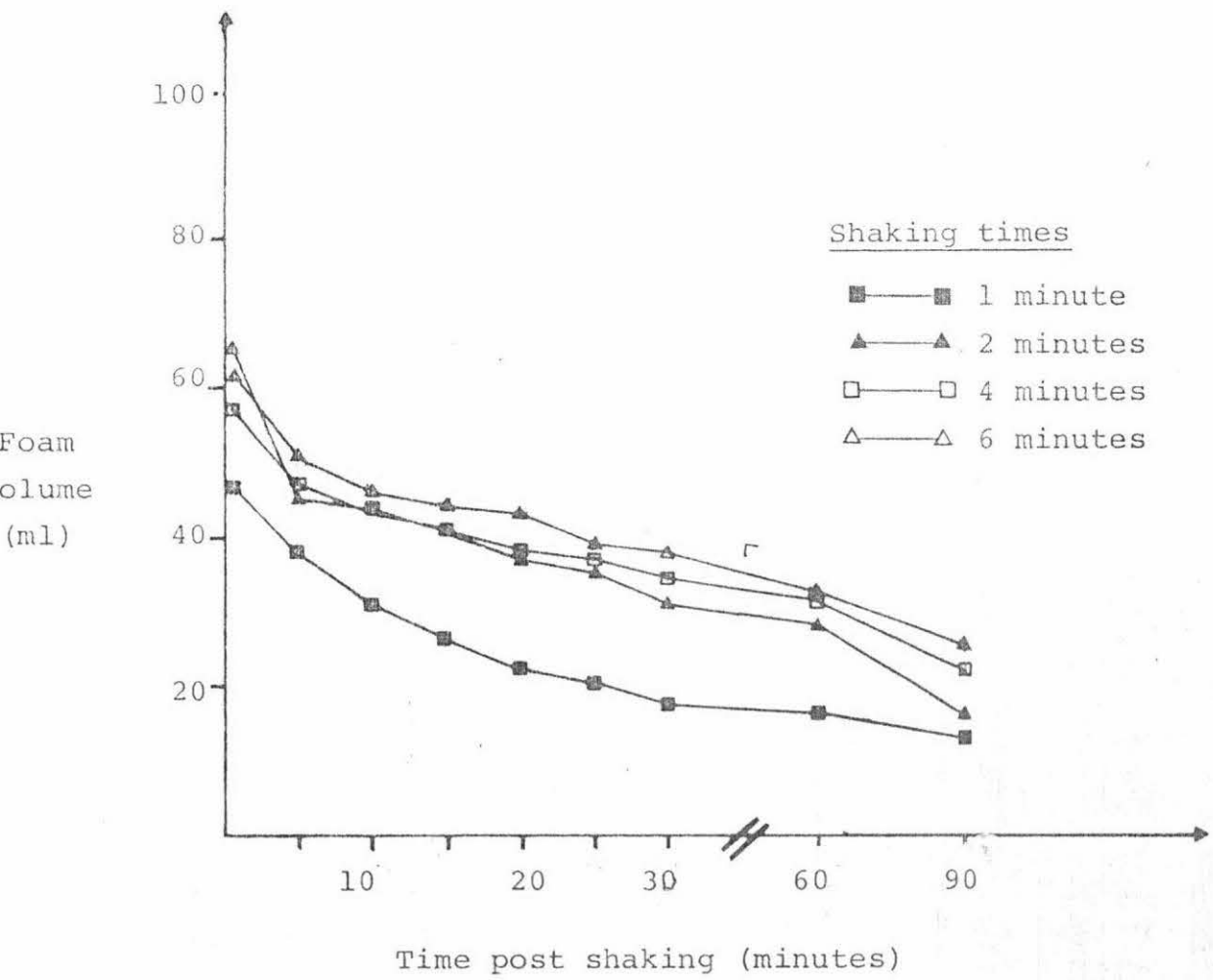


Figure 4.26 The effect of shaking time on foam volume decay for a 2% protein dispersion of egg white albumin



CHAPTER 5

DISCUSSION

5.1 Introduction

In this chapter the functional properties exhibited by the meat protein concentrates (MassMPC, MyoMPC and SarcoMPC) measured using model systems are discussed in relation to the properties of the soy and caseinate proteins. The usefulness of the interrelationships found between these functional properties in future studies is discussed. Functional properties of the meat proteins are also assessed by comparing their performance in emulsion-type sausages with the performance of soy and caseinates in similar products. Also, a comparison is made between the properties of sausages containing the meat protein additives and sausages containing no additives.

The usefulness of relationships determined between sensory and instrumental measurements of textural properties is discussed in terms of assessing the textural properties important in commercial sausages. Finally the relationships found between functional properties of proteins measured using model systems and properties of sausages containing these proteins are evaluated. The approach to measuring functional properties of proteins is discussed and an alternative approach suggested.

5.2 Selection of non-meat protein additives

Because of their empirical nature, the results of functional property determinations for new protein products using model systems are only meaningful if they are compared to values for accepted commercial products measured using the same system. The commercial products are usually chosen because: they could be potential competitors for the new product should the latter ever reach the market; and/or they possess functional properties which would provide useful bench marks for determining the significance of results obtained for the protein under study.

Four non-meat protein additives were chosen for comparative functional property evaluation with the three new meat protein concentrates. It was envisaged that the three meat protein products could have application in the meat smallgoods industry. This led to the choice of GL750 for this study because the results of the postal survey indicated that it had widespread use in this industry in New Zealand and it was thus a potential competitor.

Although not used extensively in production of smallgoods, three sodium caseinates were chosen because it was anticipated that they would possess different functional properties than GL750, thereby providing bench marks for assessing the properties of MassMPC, MyoMPC and SarcoMPC. Also the two higher viscosity caseinates (SV07 and SV02) were reported to be used extensively by the European processed meat industry and could thus be potential competitors for the meat proteins if they were to be launched onto this overseas market.

5.3 Functional characteristics of MassMPC, MyoMPC and SarcoMPC assessed using model systems

From a careful review of the literature it is obvious that functional properties of proteins are very dependent on the environment and processing conditions to which they are subjected.

Methods employed in this study to measure functional properties using model systems had chemical and physical conditions as close as possible to those used in the production and cooking of an emulsion-type sausage. This approach was taken in an attempt to identify relationships between functional properties measured using model systems and the properties of food systems containing the protein under study.

5.3.1 Percent soluble protein

The decision to use 1% protein dispersions in the percent soluble protein determinations was made after preliminary investigations showed that at the 1:5 protein to water ratio there was insufficient water to solvate the caseinates and soy proteins. One percent dispersions were used as this was the most frequently used concentration reported in the literature. The data from this study is useful as an indicator of the relative solubility of the three meat protein concentrates in comparison with the commercial protein products but it cannot be considered conclusive because of insufficient replication during data collection (Figures 4.1 and 4.2).

SarcoMPC was approximately 95% soluble at temperatures less than 50^{o}C and was the most soluble of the protein additives examined over this temperature range. This high solubility is probably due to the presence of the water soluble albumins plus the globulins which have been solubilized by the salt naturally present in this concentrate.

At temperatures above 50^{o}C a sharp decrease in the solubility of SarcoMPC resulted giving it a value below that of the three caseinates but still superior to the

solubility data for GL750 and the other two meat proteins. This decrease in solubility can be attributed to thermal coagulation of the protein molecules and is consistent with the range of temperatures reported by Hamm (1966) and Schut (1976) for this to occur.

MassMPC and MyoMPC were almost completely insoluble in the presence and absence of salt and exhibited solubility properties inferior to all other proteins studied. Based on the discussion of Hermansson (1972,1973a) the insoluble nature of these proteins is indicative of strong attractive forces between the molecules which causes them to associate.

It is not surprising that these concentrates are insoluble under all the conditions examined because in their native state they depend on an ionic strength of 0.5 to 0.6 to go into solution (Schut, 1976),

Furthermore these data suggest that the alkali and freezing conditions to which MyoMPC and MassMPC are subjected to during extraction do not enhance their intrinsic solubility properties. Detrimental effects caused by this process cannot be discounted but data is insufficient to draw any conclusions to this effect. In view of the poor solubility of these materials it would be expected that they would also exhibit insignificant emulsion capacity, emulsion stability and foaming properties.

The solubility of GL750 seemed to be slightly enhanced following heating. This may be due to partial thermal denaturation which causes unfolding of the molecule revealing hydration sites but is not sufficient enough to cause the molecules to aggregate. Heat coagulation of the caseinates did not occur which is a characteristic of this type of protein and in some instances it would give this material an advantage over heat coaguable SarcoMPC.

According to the theory of Hamm (1975) the slight increase in solubility observed for SV02 and CasN in the presence of salt would indicate that the pH of these

materials is greater than their isoelectric point in contrast to SV07 which is expected to be below this value. Soy concentrate would seem to be near its isoelectric point or otherwise the ionic strength is not great enough for any change in solubility to be observed as is also the case for MassMPC and MyoMPC.

5.3.2 Heat gel strength

The heat gel strength test used in this study was chosen because the same apparatus could be used to measure the gel strength of a cooked sausage.

Although SarcoMPC formed the weakest "true" gel it still had an advantage over CasN which formed a viscous solution which exhibited flow properties. CasN probably did not gel because of the paucity of tri- or higher functional units on its peptide chain (Tables 4.3 and 4.4).

The strongest heat set gels were formed by the myofibrillar protein concentrates, these being approximately 1,700 x and 2,200 x stronger than the medium strength gels formed by SV07, SV02 and GL750 in the presence and absence of salt, respectively. MassMPC was stronger than MyoMPC in the absence of salt but the reverse occurred when salt was added. Fluid was released from the MyoMPC and MassMPC gels following cooling which indicates that these protein structures may result from protein floculation and heat coagulation (as described by Bull, 1947) rather than true gelation. Disulphide linkages between the peptides may be important which has been discussed by Chou and Morr (1979). The fact that the myofibrillar protein concentrates exhibited superior gelling properties when compared to SarcoMPC coincides with the findings of Tsai *et al*. (1972).

The addition of salt caused the heat gel strength of the myofibrillar protein concentrates to decrease (as was the case for SV07, CasN and GL750). It is proposed that this may be due to the chloride ions binding to sites

on the protein molecules which would otherwise participate in crosslink formations during heat gelation. This does not depend on the chlorine ions being present at a concentration which results in solubilization of these protein molecules.

5.3.3 Water binding capacity

Water binding capacity was measured using the filtration method because it was able to handle the variety of materials presented for testing. Also it was thought to measure fluid release which would be released in a food product with the same physical and chemical conditions. Centrifugal and pressure methods were surmised to release more fluid than under normal cooking conditions and results would therefore underpredict the water binding capacities of the test materials.

SarcoMPC did not possess any water binding ability which is explained on the basis that it was almost 100% soluble at preparation temperatures. Therefore, when it was placed in the filter apparatus it immediately flowed through the filter funnel (Figures 4.3 to 4.6).

These results, however, are not consistent with those of the heat gelation study. SarcoMPC was observed to form a solid gel when prepared with a protein to water ratio of 1:5, which indicates that SarcoMPC is in fact able to physically hold water after a heat treatment to $70^{\circ}C$. Therefore if the results of the water binding study are considered independently unexpected water binding properties may be observed in products in which SarcoMPC is incorporated. This will depend on the food product being able to physically retain SarcoMPC until the latter heat coagulates and participates in water binding. This could happen in a stable emulsion-type sausage.

The water binding capacity of MassMPC and MyoMPC in relation to the other test proteins depended on the test conditions. In all cases, however, MassMPC exhibited inferior water binding capacity than MyoMPC. At a protein to water ratio of 1:5 in the absence of salt both of these meat concentrates had water binding capacities inferior to those of the soy and caseinate proteins but in the presence of salt MyoMPC had better binding ability than SV02 and CasN.

MassMPC and MyoMPC generally both had superior properties to the three caseinates when the protein to water ratio was lowered to 1:10.

The greater water binding ability exhibited by the myofibrillar meat protein concentrates in contrast to the sarcoplasmic protein concentrate supports the observations of Hamm (1963) and Pedersen (1971).

Soy generally had the best water binding ability. This may not be entirely due to the properties of the protein fraction but may also be attributable to the water imbibing and swelling properties of the carbohydrate component of this material.

All proteins showed decreases in water binding capacity with increasing temperature although this was also dependent on the salt and protein concentration in the environment. The most significant decreases for MassMPC and MyoMPC generally occurred at temperatures greater than 40^{o}C although in some cases marked decreases were observed after 20^{o}C. These decreases are likely to be due to thermal denaturation and coagulation of the proteins causing the myofibrillar network to tighten and squeeze out some of the water held in the matrix as described in Section 2.5.3.1.

The remaining water is stabilized in the heat coagulated gel matrix. Hamm (1966) described the changes in meat proteins on heating and concluded that coagulation of the fibrous proteins occurs from 35^{o}C to 50^{o}C which is slightly

higher than that observed in this study. The reason for this discrepancy is not known but can probably be attributed to changes in the thermal coagulation temperatures of these materials brought about by the severe alkali and freezing conditions used in the extraction process.

Soy protein water binding capacity generally decreased slightly between 60°C and 70°C. This is probably due to thermal denaturation of the molecule. For the caseinates the decline in water binding capacity is accompanied by a simultaneous decline in viscosity. The latter is probably directly responsible for the changes in water binding capacity because caseinates do not undergo heat coagulation at the temperatures studied.

Decreasing the protein to water ratio generally increased the water binding capacity of the proteins studied which is in line with the findings of Grabowska and Hamm (1978). Further reduction of this parameter would not necessarily result in a continuation of this trend according to the findings of these researchers.

Addition of salt caused the water binding capacity of MassMPC and MyoMPC to increase. This is probably due to the chloride ion binding to the positively charged groups on the peptide chains. As a result the net repulsion between the molecules increases and the peptide chain network opens up enabling it to imbibe more water. The ionic strength however was not strong enough to solubilize the molecules which would have resulted in a decrease in this property. The water binding capacity of SV07 increased with salt addition which conflicts with the results of the solubility study. The reason for this discrepancy is not understood. Salt effects on the other caseinates and GL750 can all be explained according to the Chloride ion binding theory and are consistent with the results of the solubility study.

The results of this study on the water binding properties of various proteins demonstrates the complex interactions that exist between environmental conditions. This emphasises the importance of choosing environmental conditions in model systems which approximate those of a food system in which the test material may be used.

5.3.4 Emulsion capacity

The value of data on emulsion capacity from model systems has been questioned because of the marked difference between protein and fat concentrations in model systems versus food products (Hegarty et al., 1963; Tsai et al., 1972; Randall, 1978) and because this property is unrelated to the emulsion stability of proteins. This former criticism may be justified if the emulsion capacity is being determined for a protein which is to be added to an emulsion-type sausage made with animal fat. In this case the protein to water ratio does not approach the limits of those found in an emulsion capacity test. Also the difference between the fat types used in this product and an emulsion capacity test system would produce different emulsion capacity results (Christian and Saffle, 1967).

It was considered worthwhile to study this simple property because it gave an indication of the acceptability of these additives for use in products, other than emulsion-type sausages, with high oil concentrations (such as a savourysalad dressing). It was also important to study this property in the presence of salt because of the widespread use of this seasoning in food products. This information would be useful when combined with the emulsion stability data.

Monitoring changes in electrical resistance as an indication of emulsion collapse was chosen in favour of visual observations of changes in viscosity because it removed some of the error brought about by the subjective nature of the latter method. This method could be further improved if the resistance meter was connected to an electronic device which would immediately cut off the oil flow when the resistance changed.

SarcoMPC had the greatest emulsion capacity of all the proteins studied which is probably related to the high percentage of salt soluble protein in this material. The emulsion capacity of the other proteins cannot be explained as simply in terms of salt solubility (Table 4.6).

MassMPC and MyoMPC exhibited the poorest ability to emulsify oil although MassMPC was able to emulsify slightly more oil than MyoMPC.

It is surprising that these two meat proteins were able to emulsify any oil because in the presence and absence of salt these materials were almost insoluble and they did not exhibit any foaming properties (discussed later). This suggests that a classical emulsion was not being formed for these materials but rather oil was bound in the system by being physically entrapped in the protein matrix. When the matrix became saturated with oil the resistance would also change as would be the case when an emulsion inverts. This could be verified by carrying out microscopic studies on the freshly prepared emulsions at various levels of oil addition. This may also explain the unexpectedly high emulsion capacity results reported for GL750 which contrasts previous reports describing a poor emulsifying ability for soy proteins (Schut, 1976). The results observed in this study cannot be explained in terms of percentage soluble protein because although GL750 had

a superior emulsifying capacity compared to the three sodium caseinates the latter all contained a greater percentage of salt soluble protein. The ranking of the myofibrillar and sarcoplasmic meat proteins with respect to emulsion capacity is in conflict with the findings of Hegarthy et al. (1963). This can probably be attributed to the different methods used to prepare the proteins in this study.

Salt generally enhanced the ability of proteins to emulsify fat although this is probably not due to increases in salt soluble proteins because the results conflict with the solubility test findings.

5.3.5 Emulsion stability

Pork back fat was used in favour of vegetable oil for this emulsion stability study in an attempt to find a relationship between these results and the stability of sausage emulsions containing this fat. The Polytron laboratory mixer was used because of its availability. Fat and moisture loss following preparation and during cooking were studied because both these parameters affect the stability of a sausage emulsion (Schut, 1976) and are thought to give more meaningful results than the previous fundamental measurement of creaming.

The stability results for emulsions prepared with SarcoMPC readily demonstrate that emulsion stability is not related to emulsion capacity (Figures 4.7 and 4.8, Table 4.8).

5.3.5.1 Moisture loss

Moisture loss was greatest for the emulsion containing SarcoMPC which supports the results from the water binding capacity investigation (SarcoMPC was unable to bind water). However, in the presence of fat, some water binding capacity is exhibited by the simple emulsion system containing SarcoMPC. This is indicative of the complex relationships between water binding properties and the

presence of additional food components.

MassMPC and MyoMPC formed emulsions that were more stable than that formed by SarcoMPC but lost more water than emulsions formed with the non-meat protein additives. This is probably because these proteins had water binding properties that were generally inferior to the non-meat proteins at a protein to water ratio of 1:5. MassMPC emulsions generally lost less water than those formed by MyoMPC. The emulsion containing GL750 lost more water than the emulsions stabilized by the caseinates although GL750 exhibited superior water binding capacity than these materials. This indicates that the addition of fat tissue interferes with the complex protein-water relationships that exist in a simple protein-water environment.

Of the caseinates, CasN exhibited the least ability to hold water in the emulsion which reflects the findings of the water binding capacity study.

The increase in water loss with increasing emulsion temperature is probably due to: decreasing viscosity of the casein emulsions; thermal denaturation of the soy proteins; and thermal coagulation of the meat proteins.

Salt generally enhanced the water binding capacity of MassMPC, MyoMPC, SV02 and CasN emulsions but resulted in no change for those formed with GL750. These results are consistent with those of the water binding study. A slight decrease in water binding ability resulted for emulsions containing SV07 which is not in line with the findings of the water binding study.

5.3.5.2 Fat loss

The emulsion formed with SarcoMPC appeared to be breaking down immediately following preparation although no fat loss was recorded until the material was heated to temperatures above 30°C. Animal fat was unable to permeate the collagen casings at temperatures below

this. Fat released at higher temperatures from this emulsion may result from the interfacial protein films surrounding some of the free fat undergoing thermal coagulation. The unstable emulsion formed by SarcoMPC may result because: the concentration of soluble protein is too great; or the film formed does not possess the fundamental rheological properties required for film stability (these have been described by Graham and Phillips (1976a)); or the method used to form this emulsion was too vigorous resulting in excessive surface denaturation of the proteins leading to aggregation.

Less fat was released from the emulsions formed with MassMPC, and MyoMPC than those formed with SarcoMPC although MassMPC seemed to hold the fat better than MyoMPC. Emulsions formed with GL750 were slightly more stable with respect to fat loss than those formed by the meat proteins but were inferior to those formed by the caseinates. Heating caused the fat to be lost from MassMPC, MyoMPC and GL750 emulsions and, according to the theory advanced in Section 5.3.4, this is likely to be due to thermal denaturation and coagulation of the protein matrixes. This results in the formation of holes and pores by which some of the liquid fat escapes. The remaining fat is immobilized in the matrix but is not emulsified.

The caseinates formed the most stable emulsions which released no fat. This is consistent with the reports of Kutscher and Pfaff (1961), Pearson et al. (1965), Pfaff (1968) and Schut (1968).

The decrease in fat lost from MassMPC emulsions following the addition of 2% salt may be due to increased water binding and stability of the protein matrix. However it cannot be explained why salt did not have this effect on MyoMPC emulsions. In the case of the latter emulsion no change in fat loss resulted when salt was added.

5.3.6 Foaming properties

Preliminary investigations showed that at a 2% protein concentration the foaming properties of all test materials could be measured.

No foaming properties were exhibited by MassMPC and MyoMPC which is to be expected because of the insoluble nature of these proteins. The foaming capacity of SarcoMPC was better than that of egg white albumin and comparable with the values recorded for the caseinates. GL750 exhibited the most inferior foaming properties. These results are consistent with foaming theory. The foam volume of SarcoMPC appeared to be maximized after 4 minutes shaking which was faster than observed for the other protein types. This indicates that the SarcoMPC proteins are more flexible and are able to absorb at the air-liquid interface more quickly than the other proteins. Shaking the SarcoMPC solution for 6 minutes probably resulted in surface coagulation and precipitation of the proteins making them unavailable for film formation.

GL750 and SarcoMPC formed very stable foams which were comparable with egg white albumin. Thus SarcoMPC has the properties which could make it an acceptable foaming agent although its application in foods may be limited by its distinct colour and flavour properties (Figures 4.20 to 4.26).

5.3.7 Summary of functional properties of MassMPC, MyoMPC and SarcoMPC measured using model systems

MassMPC and MyoMPC form a heat gel that is stronger than the gels formed by the non-meat protein additives used in this study and this may give them a commercial advantage over these materials. However, the emulsion properties, foaming properties and solubility characteristics of these materials are inferior to the non-meat proteins. Water binding capacity of the myofibrillar proteins is almost compatible with those of the sodium caseinates although this was not the case for all conditions studied. MyoMPC and MassMPC had similar solubilities, and foaming properties but MassMPC exihbited superior emulsion capacity and stability characteristics. MyoMPC possessed superior water binding properties under all conditions studied and produced a stronger heat set gel in the presence of salt when compared to MassMPC. For MassMPC and MyoMPC all functional properties, except solubility, were affected by the salt and temperature conditions investigated.

SarcoMPC had different functional properties than those exhibited by the myofibrillar meat protein concentrates. It exhibited solubility and emulsion capacity properties that were very much superior to those of the non-meat protein additives. The emulsion capacity results, however, are of little consequence because this protein formed the least stable emulsion of all the proteins studied. Foaming properties comparable with those of a commercial food foaming agent were exhibited by SarcoMPC but its potential application as a food foaming agent may be limited by its colour and flavour. The weak gel and lack of water binding capacity of this material also decrease the commercial acceptability of this meat protein concentrate in contrast to the non-meat protein additives currently being used. Temperature affected the functional properties of SarcoMPC.

After considering these results it would seem that the myofibrillar proteins could be marketed for their

heat gelling properties while the solubility properties of the SarcoMPC should be emphasized. Another consideration would be to recombine the myofibrillar concentrate with the sarcoplasmic concentrate so that the functional advantages of each material are complemented. Further studies on the functional properties of various combinations of these extracts would be needed to validate this suggestion.

The fact that MassMPC and MyoMPC possessed different functional properties suggests that the choice of processing conditions (pH, temperature etc.) are important and it may be possible to select conditions to improve the functional properties in the end products.

5.3.8 The relationships between functional properties measured using model systems

The functional properties assessed using model systems were examined in an attempt to identify useful relationships between these measurements that would hold for all proteins. These results could lead to a new approach to measuring the functional properties of new proteins using model systems, and an improved understanding of the interrelationships between functional properties of proteins.

A greater number of significant linear relationships ($P < 0.05$) were found to exist between functional properties measured in the absence of salt than in the presence of salt (Table 4.31). This can be attributed to the fact that the effect of salt addition on functional properties was protein type dependent. For a given protein type, the addition of salt often caused some of the functional properties to increase, other properties decreased, while some showed no change. The functional properties that fell into each of these groups would differ from another protein type.

The most important relationships that were identified in this study were:

i) the negative correlation between emulsion stability moisture losses at 10°C to 70°C and water binding capacity (protein to water ratio 1:5) results at 10°C to 60°C in the absence of salt

ii) the negative correlation between solubility measured at temperatures ranging from 10°C to 60°C and water binding capacity (protein to water ratio 1:10) at 40°C to 70°C in the absence of salt

The positive relationships between values for a particular functional property and values for the same property measured at higher temperatures, although unimportant, were to be expected.

The negative relationship between emulsion stability moisture loss and water binding occurred which means that proteins with lower water binding abilities (measured using a protein to water ratio of 1:5) would release more water from a protein, water and fat emulsion (prepared with the same protein to water ratio) than proteins with higher water binding abilities.

The fact that this relationship is significant means that the measurement of bound water is similar in both the filtration water binding capacity test and the emulsion stability moisture loss determination. Thus, in future studies of the functional properties of new protein products it is not necessary to measure both the water binding capacity and emulsion stability moisture loss since one of these parameters can be used to predict the other. This is provided that the value of both of these properties measured under the same physical and chemical conditions is of interest.

The negative correlation between solubility at 10°C to 60°C and the water binding capacity of proteins from 40°C and 70°C (in the absence of salt, protein to water ratio of 1:10) supports the findings of Hermansson and

Akesson (1975). The reason no negative relationship existed for all proteins between water binding capacity and solubility at 70°C is probably because at this temperature a very pronounced positive relationship occurred for one of the proteins included in the correlation calculation (i.e. at 70°C SarcoMPC exhibited poor solubility (Figure 4.1) in addition to zero water binding ability in this study). As discussed previously (Section 5.3.3) SarcoMPC did exhibit the ability to bind water in the heat gel study and if this could have been measured and used in this correlation calculation the solubility of all proteins at 70°C also may have shown a significant negative relationship with water binding ability. Solubility only showed significant correlations with water binding capacities that were measured using a protein to water ratio of 1:10 in contrast to water binding values determined using a ratio of 1:5. This illustrates the importance of considering environmental conditions when assessing functional properties and interpreting the results of these studies. Therefore this relationship could be assumed in future studies only if the same chemical and physical conditions were used to measure the solubility and water binding capacity properties.

The negative correlation between emulsion stability fat loss and the water binding capacity of proteins is probably an indirect relationship brought about by the dependence of the stability of the continuous phase of an emulsion on water binding capacity. In theory poor water binding ability can result in an unstable continuous phase which in turn decreases the overall stability of the matrix resulting in fat loss. This relationship, however, may be exaggerated in this study because for some of the proteins studied it is thought that a classical emulsion is not formed and fat release depended entirely on the stability of the protein water matrix. This relationship may not hold for proteins with similar water binding capacities but poor interfacial film forming and stabilizing properties.

The remaining relationships that were identified are unimportant and/or could be misleading because they would not hold up for protein types other than those used in this study. Therefore these relationships are of no value for assessing the functional properties of proteins in future studies. For example, the negative correlation between heat gel strength and solubility in the absence of salt existed from temperatures of 10°C to 60°C because under these conditions MassMPC and MyoMPC formed the strongest gels (Table 4.3) and were also insoluble. However the heat gel strength of MassMPC and MyoMPC is likely to be due to the fibrous nature of these proteins and their ability to undergo thermal coagulation
rather than their insoluble nature. This relationship between solubility and heat gel strength did not hold at 70°C and this is probably because at this temperature the solubility of SarcoMPC decreased to approximately 30% (Figure 4.1) while the gel formed by this protein was one of the weakest for all the proteins studied. Similarly the relationship between heat gel strength and the emulsion stability fat loss results at 70°C in the presence of salt is unimportant. This relationship is probably almost entirely due to the properties of MassMPC and MyoMPC (Tables 4.4 and 4.8) and would not occur if only the properties of a specific group of proteins such as the caseinates were being studied.

The relationships found between emulsion capacity and emulsion stability fat loss data and other functional properties are misleading and are peculiar to this set of data. In these emulsifying tests some of the proteins are emulsifying fat while others are binding it. These two properties are dependent on entirely different protein characteristics and the fact that they are related to other functional properties has no simple explanation. These correlations would probably not arise for another group of proteins.

At first sight the relationship between the emulsion stability moisture loss at 70°C and the solubility results

at 10°C to 70°C, in the presence of salt, seems valid. However, if the properties of a protein such as SarcoMPC were included in this calculation the correlation would no longer exist (Note the emulsion stability moisture loss and solubility of SarcoMPC were not determined in the presence of added salt).

The positive relationship between heat gel strength and moisture loss that had been identified by Hermansson and Akesson (1975) was not identified in this study. This can probably be attributed to two factors:

i) The water binding capacity of SarcoMPC at temperatures above approximately 40°C could not be measured using the filtration method (discussed in Section 5.3.3) However, it exhibited no water binding properties but a weak gel was formed. In contrast MassMPC and MyoMPC had relatively poor water binding capacity but produced the strongest gel.

ii) GL750 exhibited good water binding ability and produced a medium strength gel. However, the water binding ability of this protein was probably misleading in comparison to its gelling ability because the water binding was probably partly attributable to the carbohydrate component of this material. In contrast the gel strength was probably almost entirely dependent on the strength of the protein network.

These results show that relationships identified between functional properties measured using model systems are very dependent on the types of protein understudy. Therefore many of them have very limited application for further studies on the functional properties of another group of proteins. The exception to this seems to be the positive relationship between moisture loss and solubility which was identified by Hermansson and Akesson (1975) and confirmed in this study. In addition, the relationship between emulsion stability moisture loss and water binding capacity identified in this study may be useful. However, this relationship

could only be used if the water binding and emulsion stability moisture loss properties of interest in a future study are similar to those measured in this study.

Interrelationships between other functional properties would be more useful if they were established for a specific group of proteins (e.g. caseinates, fibrous proteins, cereal proteins, etc.). Then the appropriate relationships could be used when evaluating a new protein product depending on what group of proteins it belongs to.

5.4 Functional properties of MassMPC, MyoMPC and SarcoMPC assessed in a commercial sausage

Matill (1971) has emphasized the deficiencies and misleading information that can be associated with evaluating functional properties of new protein materials using only empirical model systems. An improved approach is to test the materials in commercial food systems under controlled conditions in addition to using model systems as was done in this study. Using this approach the possible effects of adding new proteins to a commercial food product can be predicted. Also relationships between the additive protein and food components can be identified and estimated by relating the empirically determined functional properties of the additives to properties of the food system in which they are incorporated (see Section 5.5).

The commercial viability of using the three meat protein concentrates as additives in the meat small goods industry was assessed by comparing their performance in an emulsion-type sausage with the properties of sausages containing no additives and sausages containing soy and caseinate proteins. All additives were incorporated into the sausage formulations so that either 3% or 30% of the total sausage protein was replaced by additive protein. The 3% replacement level was chosen as additives are often incorporated into commercial sausage formulations at this level. However, the 30% replacement level was chosen so that: the contribution of

the additives to the sausage properties might be amplified; the commercial viability of adding the meat protein additives at this level could be assessed; and to gain a better understanding of the relationships between the protein additives and food components (Section 5.5).

The performance of the various additives was assessed by measuring the fat loss, moisture loss and rheological properties of the cooked sausage in which they were incorporated. Sensory and instrumental measurement of rheological properties were performed on skinned sausages as the contribution of the skins to these properties was not important in this study.

A trained taste panel was used to assess the rheological properties that were considered important in commercial cooked sausages. Correlations between these properties and instrumental rheological measurements recorded for the sausages were determined so that in future studies of commercially important properties may be evaluated using only instrumental methods. The tedious task of training a taste panel and measuring properties using this method could thus be avoided.

In the discussion that follows explanations concerning the performance of specific proteins in sausages in relation to their functional properties will be limited as this will be established in Section 5.5.

5.4.1 Cooking losses

Formulations were designed so that the overall water binding capacity of all the ingredients, excluding the additive, was approximately the same for all sausages manufactured at a given protein replacement level. Therefore any differences in water loss between sausages prepared with different additives could be attributed to the water binding of the additive protein in the presence of the other food components. (This was verified by preparing three control sausages which did not contain additives.

The goal program predicted they would bind 60 g water/ 100 g mix, 56 g water/100 g mix and 46.96 g water/100 g mix. In practice these sausages were found to bind 58.17 g water/ 100 g mix, 55.88 g water/100 g mix and 44.68 g water/100 g mix respectively). Fat emulsification or binding ability of the formulations was not controlled in this manner. Thus, the observed differences in fat loss between sausages could have arisen from differences in total fat emulsification or binding ability of the ingredients other than the additives in the sausages; and/or differences in the fat emulsification or binding ability of the protein additive used in the sausage. Table 4.9 shows that the proportion of each ingredient varied between formulations.

5.4.1.1 Moisture loss

Increased moisture losses were observed for sausages containing additives at the 30% protein replacement level in contrast to those sausages produced at the 3% level (Table 4.10). This was expected although deviations from predicted moisture loss values did occur (Table 4.12).

MassMPC or MyoMPC would be the most desirable additives to incorporate into sausages at the 3% protein replacement level with respect to moisture loss (Table 4.11). Of these two protein concentrates MassMPC would be best. SarcoMPC would be used in favour of SV07, CasN and GL750.

SarcoMPC would not be suitable for use at a 30% water replacement level. MassMPC and MyoMPC produce better binding results at this replacement level than GL750, SV07 and CasN. MyoMPC exhibits slightly superior properties compared to MassMPC when included in sausages at the 30% replacement level.

The difference in moisture loss between sausages containing SarcoMPC at the 3% and 30% protein replacement levels suggests that the water binding capacity of sausages containing additives at the 3% level is still largely

dependent on the water binding ability of the non-additive ingredients. At the 30% level, however, the water binding ability of the additive is vitally important in stabilizing the matrix.

5.4.1.2 Fat loss

All proteins studied could be added to sausages (containing 20% fat and meat ingredients similar to those used in this study) at the 3% protein replacement level without causing detrimental effects on fat loss (Table 4.13). At the 30% replacement level, however, the caseinates should be used in preference to the other additives if minimal fat loss is desired. MassMPC and MyoMPC would be least acceptable while GL750 and SarcoMPC would be slightly more desirable.

No fat loss at the 3% replacement level suggests that the fat binding capacity of the non-additive ingredients is more than adequate to deal with the amount of fat present (20%). It may be useful to use a higher fat content in test sausage systems in future studies (e.g. 30%) which will show up fat binding differences attributable to different additives.

The fat binding ability of sausages containing SarcoMPC at the 30% protein replacement level is unexpectedly high. This indicates that different processing conditions and/or the presence of other food components modifies the fat emulsifying properties of SarcoMPC compared to those exhibited by this protein in a simple model system.

5.4.2 Sensory properties

Using the judging criteria developed by the taste panelists in this study to assess sensory properties, the most desirable commercial emulsion-type sausage would be scored as follows: Firmness 5, Cleanness of first bite 0, Rate of breakdown 5, Chewiness 5, Stickiness 0, Juiciness 10, Fattiness 0. Thus by studying these sensory properties of sausages containing different protein additives the overall acceptability of adding MassMPC, MyoMPC and SarcoMPC to

commercial sausages could be evaluated (Figures 4.9 to 4.15).

The addition of SarcoMPC to sausages at the 3% protein replacement level results in a product with sensory properties similar to those found with the addition of GL750 but generally superior to those resulting when caseinates are added. Also the properties of the sausage containing SarcoMPC were generally more desirable than sausages not including any additives.

The addition of MassMPC and MyoMPC at the 3% protein replacement level generally produced sausages with sensory properties inferior to those produced with other additives. As was the case for SarcoMPC, the addition of MassMPC and MyoMPC also produces a sausage that is generally more desirable than sausages not containing additives.

The inferior sensory properties of sausages containing MassMPC and MyoMPC in relation to other additives is consistent with the overall findings of the model system functional property evaluations. It would seem that the superior heat gelation property exhibited by MassMPC and MyoMPC does not give them an overall advantage compared to the other proteins when they are added to emulsion sausages at the 3% protein replacement level. The enhanced properties of sausages containing MassMPC and MyoMPC in contrast to sausages without protein additives, may be attributable to their superior functional properties in comparison with meat protein. Alternatively, a synergistic relationship may exist between these additives and the other ingredients in the sausage which causes them to produce a higher performance measurement than the sum result of their individual capabilities.

The properties exhibited by sausages containing SarcoMPC at the 3% protein replacement level were excellent in relation to other additives and cannot be explained in terms of the functional properties of this material. A synergistic relationship between SarcoMPC and the other sausage ingredients is more likely to account for these results.

The addition of MassMPC and SarcoMPC at the 30% protein replacement level produced sausages which generally exhibited inferior properties compared to soy or caseinate proteins and to sausages containing no protein additives. These observations are probably directly attributable to the poor functional properties exhibited by SarcoMPC and MassMPC in relation to the other proteins. These results also demonstrate that the functional properties of additives become more important as the concentration at which they are included in products increases.

MyoMPC included at the 30% protein replacement level generally produced a product with properties superior to products containing the other meat protein concentrates, and CasN and GL750. The sausage containing MyoMPC at this level had properties similar to sausages containing no protein additives. These superior properties exhibited by MyoMPC in contrast to MassMPC and SarcoMPC may be due to the superior water binding and heat gel properties exhibited by this material. Alternatively they could be attributed to a synergistic relationship between MyoMPC and the other food ingredients.

5.4.3 The correlation between sensory and instrumental textural measurements

The extrusion test, multiple compression test and Warner Bratzler Shear test would be the most useful instrumental measurements for evaluating textural properties considered important in a commercial emulsion-type sausage (Table 4.30). However, the relationship between sensory properties and these instrumental measurements was dependent on the level of addition of protein additives. Thus the relationships identified in this study are of limited use in future studies unless sausages are evaluated which have similar non-additive ingredients and additive incorporation levels. The other drawback to using only instrumental

measurements in future studies, based on the findings of this study, is that not all the properties considered to be important in commercial sausages could be related to instrumental measurements.

In many cases the sensory properties were found to be related to several instrumental measurements which suggests that the sensory properties perceived by the taste panelists are dependent on the interaction between or combination of several different rheological measurements.

Different relationships were found to exist between sensory and instrumental measurements of texture when the level of protein replaced by additives was increased from 3% to 30%.

This suggests that increasing the concentration of additive protein brings about complex changes in many of the rheological parameters which go to make up a given perceived sensory property. When additives are incorporated at low concentrations some of these rheological parameters may change while others remain unaffected.

5.4.5 Relationships identified between functional properties measured using model systems and the properties of an emulsion-type sausage

Successful research into establishing the relationships between empirically determined functional properties of proteins and the properties of meat products incorporating these materials has been limited.

Results from these studies are useful for identifying and estimating the relationships between proteins and food components and thus predicting the performance of proteins in food systems. As pointed out in Section 5.3.7 these results combined with the interrelationships identified between functional properties could lead to a new approach to measuring the functional properties of new protein foods. If significant relationships exist then the numerous model

systems and food system tests currently used to evaluate functional properties could be reduced. Alternatively if no relationships are found then alternative approaches need to be developed.

It was in an effort to expand this field of research that the following investigation was undertaken. Functional properties of seven protein additives were determined using empirical model systems, in the presence and absence of salt, and related to cooking loss, sensory and instrumental textural measurements of sausages containing these additives at both 3% and 30% protein replacement levels (Table 4.32).

A larger number of useful correlations were determined between functional properties and sausage properties when the former was measured in the presence of salt. This is to be expected as the sausages contained salt and salt was demonstrated to affect functional properties in the study made using model systems.

All relationships identified between the functional properties measured in the absence of salt and the properties of sausages containing 3% additive protein were meaningless and/or did not contribute to a better understanding of the relationship between additive proteins and sausage ingredients. When the functional properties measured in the absence of salt were compared with the properties of sausages containing 30% additive protein meaningful relationships between juiciness and solubility, fat loss and solubility and between the extrusion test rupture force, extrusion force and rupture slope and solubility resulted. These also occurred when solubility was measured in the presence of salt (discussed later) which is to be expected because the effect of salt on solubility was limited.

The correlations between the functional properties measured in the presence of salt and the properties of

sausages containing 3% additive were disappointing and do not contribute to the understanding of why the proteins behaved as they did in the presence of the sausage ingredients. The relationship between the Warner Bratzler test deformation and slope values and the emulsion stability moisture loss at 10°C may indicate that moisture lost from the raw sausage batter will directly affect these textural properties irrespective of further moisture loss during cooking. Perhaps the moisture lost from the raw batter modifies the protein network structure.

The lack of relationship between the functional properties of proteins measured in the presence of salt and properties of sausages containing these proteins at the 3% additive protein level may have arisen because:

i) the properties of the sausages were a result of differences between the functional properties of the non-additive ingredients

ii) synergistic and/or antagonistic relationships existed between the protein additives and the food ingredients which were protein type dependent. Synergistic or antagonistic relationships result in performance measurements above and below the sum result of them acting individually. Porteous and Quinn (1979) have reported that these relationships exist between meat homogenate and milk proteins, soy proteins and yeast concentrates and cause deviations from predicted solubility and emulsion capacity results

iii) the relationships between the functional properties and the observed sausage properties are non linear. Optimal sausage properties could be dependent upon critical values for some functional properties above and below which the sausage property is adversely affected. This may be the case for some of the sausage properties evaluated using sensory measurement. For example, cleanness of first bite scores were greatest for the control sausage with

a water binding capacity of 56 and were reduced in sausages with a water binding capacity above and below this value. This effect may however be due to differences in meat ingredient properties.

The most useful correlations resulted when functional properties were measured in the presence of salt using model systems and compared to the properties of sausages containing 30% additive protein. Under these conditions fat loss and moisture loss were both positively correlated to the water binding capacity of the proteins measured at cook temperatures. This indicates that an antagonistic relationship exists between the non-additive ingredients and the protein additives in a sausage which is dependent upon the water binding ability of the protein additive. This relationship is responsible for the observed moisture and fat loss in sausages containing protein additives. The positive correlation between the |WBC predicted - WBC observed| value for the sausages and the water binding capacity of the heat treated proteins supports this hypothesis.

The negative relationship between the solubility of the protein additives and fat loss in sausages is consistent with the theory for fat emulsification in a sausage system (i.e. stabilization of a sausage system is dependent, to some extent, on there being sufficient soluble protein to engage in film formation around the fat droplets). Juiciness of a sausage was found to be dependent on several important functional properties of protein additives incorporated into the product (i.e. heat gel strength, emulsion stability fat and moisture loss, and solubility). These relationships could be useful in future to predict the juiciness of sausages containing a new protein at a replacement level similar to that used in this study. Similarly the negative relationship between water binding capacity and fat loss could be useful in future studies.

Numerous textural properties of the sausages containing 30% additive protein were found to relate to the water binding capacity, heat gel strength, solubility and emulsion stability

fat loss properties of the additive proteins measured in the presence of salt. The relationships between textural properties and moisture loss and gelation properties of proteins supports the findings of Hermansson, (1975). Relationships, such as that found between the compression test slope values and water binding capacity of proteins at 50°C, are meaningless in terms of understanding the interaction of the protein additives with food ingredients and for predicting the effect of adding the proteins to commercial food products. The absence of any relationship between many of the textural properties considered important in commercial sausages and the functional properties of the proteins measured using model systems may be a result of antagonistic or synergistic relationships between the proteins and other food ingredients and/or non-linear relationships between protein functional properties and the sausage properties (as discussed previously).

Thus, some useful relationships were found to exist when the functional properties of proteins measured in the presence of salt were compared to properties of sausages which had 30% of the protein replaced by additive protein. This indicates that satisfactory prediction of some aspects of the performance of a new protein in a food product can be made using model systems alone. In order to do this the physical and chemical conditions in the model systems must approximate those found in the food system.

The fact that not all aspects can be predicted, however, still limits the use of model systems alone for assessing the properties of new protein products. Also these relationships only seem to be significant if the proteins are to be added to food systems in quantities which ensures they play a major role in the structure and stability of this product.

The usefulness of these relationships does not extend to explaining why the important properties of food products are altered by the addition of proteins with different

properties.

Thus if the current approach to evaluating functional properties using model and controlled food systems is continued, no increased understanding of the relationships between proteins and other food components will be gained (particularly when proteins are added to foods in small amounts). If model systems alone are used, misleading results concerning the acceptability of new proteins as food additives may occur. It would seem that a more fundamental approach should be taken to understand these complex relationships so that the possible effects of adding proteins to foods can be assessed. An improved approach would be to assess functional properties of proteins in the presence of the individual food components and combinations of these food components using model systems. In addition to monitoring changes in response values for a given functional property under each of these conditions, the structure of the simple model and multicomponent systems should be studied using electron microscopy techniques. Thus the progressive changes in response values between simple and multicomponent model systems can be measured and explained for a given type of protein.

CHAPTER 6

SUMMARY AND CONCLUSIONS

MassMPC and MyoMPC exhibited similar functional properties which differed from those exhibited by SarcoMPC. When compared to commercial protein products MassMPC and MyoMPC exhibited superior heat gelling properties while SarcoMPC was more soluble and exhibited similar foaming properties to these commercial products.

All three meat proteins could be added to emulsion-type sausages at the 3% protein replacement level without causing detrimental effects to these products. However, the properties of sausages made with MassMPC and MyoMPC were inferior to those of sausages made with soy or caseinate proteins at this inclusion level. Sausages made with SarcoMPC were generally very acceptable in relation to products containing the commercial proteins.

The addition of SarcoMPC and MyoMPC at the 30% replacement level adversely affected sausage properties and produced inferior products compared with those containing soy or caseinate proteins. The performance of MassMPC in sausages at this level was similar to the other protein additives.

From these results it is concluded that in terms of overall functional properties and performance in a food system MassMPC, MyoMPC and SarcoMPC would not be able to compete with protein materials available on the commercial protein market.

The negative relationships determined between solubility and water binding capacity, and between emulsion stability moisture loss and water binding capacity were the most useful relationships established between functional properties measured using model systems. The latter relationship was dependent on the methods used to assess water binding capacity and emulsion stability. The relationships between

functional properties seemed to be protein type dependent and it is concluded that more significant and useful relationships could be identified if only the properties of specific groups of proteins were compared.

An attempt was made to establish which instrumental measurements could be used to assess the commercially important properties of emulsion-type sausages. The extrusion test, multiple compression test and Warner Bratzler Shear test were found to be the best for this purpose. However, relationships between sensory properties and instrumental measurements were dependent on the level of addition of protein additives in the sausages. Therefore the results from this study would have little application in further studies.

Useful relationships between functional properties of proteins measured using model systems in the presence of 2% salt and the properties of a sausage which had 30% of the total protein replaced by additive protein were determined. This indicates that satisfactory prediction of some aspects of the performance of a new protein in a food product can be made using model systems alone. In order to do this the physical and chemical conditions in the model systems must approximate those found in the food system. This is only the case if proteins are to be added to food systems in quantities which ensure they play a major role in the structure and stability of this product.

All properties of a sausage cannot be predicted by only measuring the functional properties of proteins with model systems because; antagonistic and/or synergistic relationships exist between the protein additives and food ingredients; or because the relationships between functional properties of proteins and sausage properties are non-linear.

It is concluded that by using the current approach to measuring functional properties of proteins, using model and controlled food systems, no increased understanding of

the relationships between proteins and other food components will be gained. A more fundamental approach should be taken to understand these complex relationships so that the effects of adding proteins to foods can be assessed. This should involve assessing the functional properties of a protein, in the presence of individual food components and combinations of these food components, using model systems. In addition to measuring response values for a given functional property under each of these conditions, the structure of the simple model and multicomponent systems should be studied using electron microscopy techniques. Thus the progressive changes in response values between simple and multicomponent model systems can be measured and explained for a given type of protein.

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APPENDIX I

Postal Survey Questionnaire

MASSEY UNIVERSITY

A SURVEY ON THE USE OF NON-MEAT PROTEIN ADDITIVES IN MEAT PRODUCTS

Please tick the box showing the correct answer, or write your answer in the space provided.

1. Do you use non-meat protein additives (such as caseinates, skim milk power, etc. (in your meat products?

☐ YES ☐ NO

If no, terminate the questionnaire.

2. Do you use caseinates in your meat products?

☐ YES ☐ NO

If no, go to Question 7.

3. What brand(s) of caseinate do you use?

Please list:

BRAND (e.g. Globus Arlow	CODE NUMBER IPOD 54)

4. Why do you use this particular brand?

_____

_____

5. In what type of products do you use a caseinate? (For example luncheon sausage, fresh sausage etc.)

Please list:

PRODUCT	BRAND USED

(If further room is required please use back of questionnaire).

6. What quantity (percentage by weight) of above caseinate do you add to each of the products?

PRODUCT	% BY WEIGHT ADDED

7. Do you use a soy protein isolate or a soy protein concentrate in your meat products?

Soy Protein Isolate YES ☐ NO ☐

Soy Protein Concentrate YES ☐ NO ☐

If neither product is used, terminate the questionnaire.

8. What brand(s) of Soy Protein Isolate and/or Soy Protein Concentrate do you use? Please list:

SOY PROTEIN ISOLATE

BRAND (e.g. Globus Arlow	CODE NUMBER IPOD 54)
_____	_____
_____	_____

SOY PROTEIN CONCENTRATE

BRAND	CODE NUMBER
_____	_____
_____	_____

9. Why do you use these particular brands? _____

_____

_____

_____

10. In what type of product(s) do you use a Soy Protein Isolate and/or a Soy Protein Concentrate? Please list:

(e.g. luncheon sausage, fresh sausage etc.)

10. (ctd)

SOY PROTEIN ISOLATE

PRODUCT	BRAND USED

SOY PROTEIN CONCENTRATE

PRODUCT	BRAND USED

11. What quantity (percentage by weight) of Soy Protein Isolate and/or Soy Protein Concentrate do you add to each of the above products.

SOY PROTEIN ISOLATE

PRODUCT (e.g. Luncheon Sausage	% BY WEIGHT ADDED 3%)

SOY PROTEIN CONCENTRATE

PRODUCT	% BY WEIGHT ADDED

Thank you for your co-operation.

S.H. NICKLIN

APPENDIX II

Calculations of the Relative Standard Deviation for Test Methods

The relative standard deviation (R.S.D.) is used as a measure of data precision (McFarren *et al.* (1970) and is calculated using Equation II.1

$$R.S.D. = \frac{\sigma}{\bar{x}} \times \frac{100}{1} \qquad \text{II.1}$$

where σ = standard deviation of sample

$\bar{x}$ = mean of sample

Calculations of the R.S.D. from data obtained from each test method using one of the specified test conditions were made.

II.1 Percent soluble protein

Five percent soluble protein data were obtained using the method described in Chapter 3.5 for a dispersion of SV02 (1% protein (m/m) heated to 70°C)(Table II.1). The R.S.D. for this data was 2.87%($\frac{2.08}{72.48} \times \frac{100}{1}$)

TABLE II.1

Percent soluble protein data for R.S.D. calculation

Percent Soluble Protein	
	75.21
	71.94
	70.43
	74.05
	70.77
$\bar{x}$	72.48
σ	2.08

II.2 Heat gel strength

Ten heat gel strength data were obtained using the method described in Chapter 3.6 for a dispersion of SV02 (protein to water ratio 1:5 m/m) (Table II.2). The R.S.D. was calculated from this data and found to equal

$$5.00\% \quad \left(\frac{2.94}{58.84} \times \frac{100}{1} \right).$$

TABLE II.2

Heat gel strength data for R.S.D. calculation

Heat Gel Strength (g)
59.4
56.0
56.7
63.9
60.0
58.4
60.1
54.8
62.6
56.5
$\bar{x}$ 58.84
σ 2.94

II.3 Water binding capacity

Ten water binding capacity data were obtained using the method described in Chapter 3.7 for a dispersion of SV02 (protein to water ratio (1:5 (m/m) heated to 70°C) (Table II.3). The R.S.D. was calculated from this data and found to equal 3.07% ($\frac{0.05}{1.63} \times \frac{100}{1}$).

TABLE II.3

Water binding capacity data for R.S.D. calculation

Water binding capacity (g water bound/g protein)	
	1.65
	1.65
	1.73
	1.57
	1.65
	1.57
	1.65
	1.57
	1.65
	1.65
$\bar{x}$	1.63
σ	0.05

II.4 Emulsion capacity

Ten emulsion capacity data were obtained using the method described in Chapter 3.8 for a dispersion of SV02 with a protein to water ratio of 1:5 (m/m), in the presence of 2% (m/m) salt (Table II.4). The R.S.D. for this data was 3.46% ($\frac{0.60}{17.32} \times \frac{100}{1}$).

TABLE II.4

Emulsion capacity data for R.S.D. calculation

Emulsion Capacity (ml oil/g protein)
17.87
18.01
16.67
16.95
16.46
17.40
18.23
17.54
17.32
16.79
$\bar{x}$ 17.32
σ 0.60

II.5 Emulsion stability (moisture loss)

Ten emulsion stability (moisture loss) data were obtained using the method described in Chapter 3.9 for a dispersion of SV02 with a protein to water ratio of 1:5 (m/m), heated to an internal temperature of 70°C (Table II.5). The R.S.D. for this data was 13.72% ($\frac{0.76}{5.54} \times \frac{100}{1}$).

TABLE II.5

Emulsion stability data for R.S.D. calculation

	Moisture loss (%)
	5.90
	6.96
	5.71
	4.60
	6.42
	5.20
	5.85
	4.90
	4.84
	5.03
$\bar{x}$	5.54
σ	0.76

II.6 Foaming capacity and foaming stability

Ten foaming capacity and foaming stability data were recorded, 30 seconds and 90 minutes post shaking respectively, for a 2% (m/m) protein dispersion of CasN using a shaking time of 2 minutes (Table II.6). The R.S.D. for the foaming capacity data was 4.39% ($\frac{3.14}{71.50} \times \frac{100}{1}$) and 78.8% ($\frac{1.26}{1.60} \times \frac{100}{1}$) for the foaming stability data.

TABLE II.6

Foaming capacity and foaming stability data used for R.S.D. calculations

Foam capacity (ml) (30 seconds post shaking)	Foam stability (ml) (90 minutes post shaking)
69	2
74	3
77	1
70	0
70	1
68	4
72	2
76	1
70	2
69	0
$\bar{x}$ 71.5	$\bar{x}$ 1.60
σ 3.14	σ 1.26

APPENDIX III

Equal Interval Taste Panel Evaluation Sheet

TEXTURAL EVALUATION OF SAUSAGES

BIOTECHNOLOGY DEPARTMENT

Name .....

Date .....

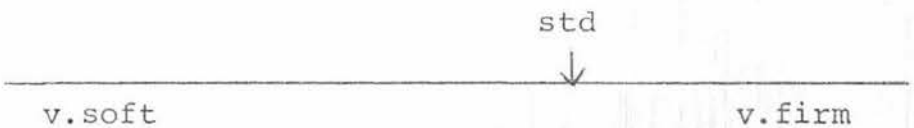
You have been presented with ..... samples of sausage coded ..... I would like you to score each property using the line scale:

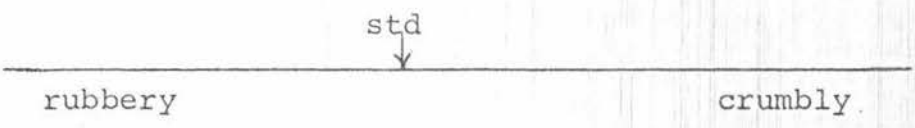
v.soft — v.firm

e.g. If sample 36 is v.firm, 12 is v.soft, and the firmness of sample 68 is midway between them, your judgement, might be as follows:

A. Primary Textural Properties

1. Firmness

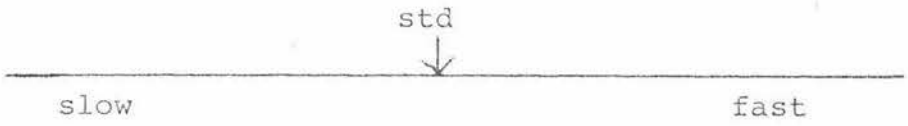




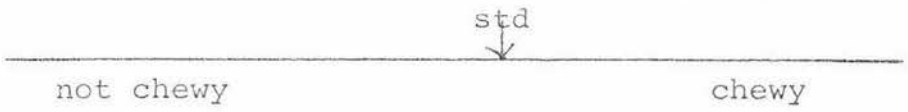
2. Cleanness of first bite

B. Mouthfeel Properties

1. Rate of breakdown in the mouth

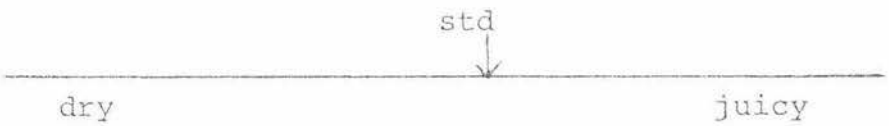


2. Chewiness



3. Stickiness

4. Juiciness



5. Fattiness

APPENDIX IV

Percent soluble protein data

TABLE IV.1 Percent soluble protein data

Temperature (°C)	Salt (present or absent)	% Soluble protein						
		SV07	SV02	CasN	GL750	MassMPC	MyoMPC	SarcoMPC
10	absent	74.8	71.9	73.5	12.0	1.1	0	99.7
20	absent	76.0	72.8	74.4	12.1	1.1	0	97.2
30	absent	75.9	72.3	73.7	12.7	1.1	0	92.5
40	absent	75.5	72.9	73.5	13.2	1.0	0	89.0
50	absent	76.1	72.4	73.2	14.3	0.9	0	51.3
60	absent	75.9	72.5	74.1	14.5	0.7	0	43.7
70	absent	75.5	73.0	73.9	15.3	0.5	0	30.5
10	present	66.1	78.0	83.2	10.3	1.0	0	*
20	present	66.9	79.8	81.6	10.3	0.9	0	
30	present	66.8	79.0	79.5	10.8	0.9	0	
40	present	65.5	79.9	78.7	11.1	0.9	0	
50	present	66.2	79.6	77.8	12.1	0.9	0	
60	present	67.3	78.9	75.8	14.3	0.5	0	
70	present	66.0	79.1	74.0	14.7	0.3	0	

* SarcoMPC was not studied in the presence of salt

APPENDIX V

Water binding capacity data

TABLE V.1 Water binding capacity data

Temperature (°C)	Protein to water ratio (m/m)	Presence or absence of salt	Water bound (g)/gram protein						
			SV07	SV02	CasN	GL750	MassMPC	MyoMPC	SarcoMPC*
10	1:5	absent	5.00	5.00	5.00	4.96	5.00	5.00	0
20	1:5	absent	5.00	5.00	5.00	4.96	5.00	5.00	0
30	1:5	absent	5.00	5.00	5.00	4.96	4.68	5.00	0
40	1:5	absent	5.00	5.00	5.00	4.96	4.19	5.00	0
50	1:5	absent	5.00	3.20	4.04	4.96	3.46	4.96	0
60	1:5	absent	4.94	2.34	2.85	4.96	3.06	3.81	0
70	1:5	absent	3.14	1.65	1.65	4.39	1.14	1.84	0
10	1:10	absent	10.00	2.31	8.25	7.88	7.59	7.61	0
20	1:10	absent	4.34	1.71	8.02	7.88	7.29	7.61	0
30	1:10	absent	4.04	1.63	7.93	7.46	6.69	7.04	0
40	1:10	absent	3.74	1.45	7.04	7.46	6.39	6.98	0
50	1:10	absent	3.14	1.41	1.85	7.32	4.67	5.07	0
60	1:10	absent	1.65	1.20	1.35	7.03	4.40	4.96	0
70	1:10	absent	1.41	1.20	0.81	5.34	3.69	4.41	0
10	1:5	present	5.00	5.00	5.00	4.96	5.00	5.00	
20	1:5	present	5.00	5.00	5.00	4.96	5.00	5.00	
30	1:5	present	5.00	5.00	5.00	4.96	5.00	5.00	
40	1:5	present	5.00	5.00	5.00	4.96	5.00	5.00	
50	1:5	present	5.00	4.64	4.34	4.96	4.07	4.17	
60	1:5	present	5.00	4.34	4.04	4.96	2.96	3.66	
70	1:5	present	5.00	3.20	3.75	4.39	2.26	2.66	

TABLE V.I ctd.

Temperature (°C)	Protein to water ratio (m/m)	Presence or absence of salt	Water bound (g)/gram protein						
			SV07	SV02	CasN	GL750	MassMPC	MyoMPC	SarcoMPC*
10	1:10	present	10.00	8.59	9.73	7.42	8.00	7.96	
20	1:10	present	9.79	6.14	9.61	7.36	7.67	7.71	
30	1:10	present	9.73	5.60	8.76	7.22	7.00	7.00	
40	1:10	present	9.73	5.94	7.95	7.22	7.00	7.00	
50	1:10	present	9.73	4.34	2.85	7.17	5.23	5.90	
60	1:10	present	9.13	4.04	2.11	7.10	4.08	4.82	
70	1:10	present	8.89	3.14	1.95	5.34	3.32	3.55	

* The water binding capacity of SarcoMPC was not determined in the presence of salt

APPENDIX VI

Emulsion stability data

TABLE VI.1 Mean (n = 3) emulsion stability moisture loss data

Temperature* (°C)	Salt* (present or absent)	Moisture loss % SV07	SV02	CasN	GL750	MassMPC	MyoMPC	SarcoMPC*
overnight	absent	1.32	1.50	3.90	4.60	4.02	1.77	23.51
20	absent	2.00	2.13	1.69	2.42	2.00	3.23	4.40
30	absent	2.34	1.94	3.17	3.95	3.74	4.41	8.68
40	absent	2.81	2.49	5.87	5.60	6.23	6.32	25.68
50	absent	3.00	3.78	6.83	6.23	8.74	10.18	46.55
60	absent	3.20	4.22	7.93	8.79	9.44	13.76	47.74
70	absent	3.11	5.63	11.62	12.52	18.98	26.60	56.99
Total 70***		4.43	7.13	15.52	17.12	23.00	28.37	90.50
overnight	present	1.45	1.66	1.10	1.10	1.57	0.84	**
20	present	1.92	2.05	2.34	4.72	3.30	2.30	
30	present	2.40	2.00	2.31	5.60	3.30	3.05	
40	present	2.73	2.61	5.90	9.01	4.74	5.88	
50	present	3.11	3.73	8.26	10.91	6.31	6.95	
60	present	3.42	4.60	8.15	11.70	7.10	8.17	
70	present	3.18	5.20	8.13	17.18	16.98	19.57	
Total 70***		4.63	6.86	9.23	18.28	18.55	20.41	

Pooled $S\bar{x}$ = 0.31

* Significant main effect differences ($P < 0.001$). Interactions (protein x salt, protein x temperature, salt x temperature, and protein x salt x temperature) significant ($P < 0.001$).

** SarcoMPC was not examined in the presence of salt

*** This includes overnight moisture loss

APPENDIX VII

Group discussion taste panel scores

TABLE VII.1 Group discussion taste panel scores

Sausage	Sensory property						
	Firmness	Cleanness of first bite	Rate of breakdown	Chewiness	Stickiness	Juiciness	Fattiness
WBC60	4.13	5.25	6.50	2.79	4.17	3.75	4.75
WBC56	3.08	7.17	7.37	2.84	5.50	5.50	5.33
WBC40	2.08	6.58	7.20	2.50	5.33	5.50	4.50
SV07(30)	4.67	4.92	7.92	3.92	3.63	6.13	3.33
SV02(30)	2.00	5.88	7.83	1.88	4.83	5.92	4.67
CasN(30)	2.00	5.88	8.42	1.58	6.17	6.17	5.04
GL750(30)	2.08	6.58	8.75	2.54	6.13	5.50	4.50
Mass(30)	2.33	7.13	8.33	1.92	6.83	4.75	5.25
Myo(30)	3.33	5.67	6.92	3.54	4.17	4.25	5.17
Sarco(30)	2.25	4.92	8.08	2.00	5.58	6.42	5.61
SV07(3)	3.42	5.83	6.70	4.46	2.92	4.04	2.83
SV02(3)	4.58	6.50	6.58	3.29	5.58	3.75	5.63
CasN(3)	3.42	5.79	7.71	3.92	4.38	5.42	3.33
GL750(3)	4.00	4.92	6.50	2.96	4.75	5.00	2.50
Mass(3)	3.83	6.50	6.25	3.29	3.92	6.17	5.63
Myo(3)	2.92	6.67	7.25	2.42	5.54	5.83	4.00
Sarco(3)	4.50	5.50	5.71	2.58	3.54	5.50	4.33