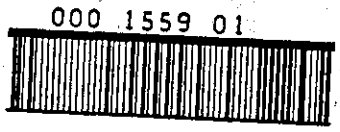

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PROCESSING AND FUNCTIONAL PROPERTIES OF BOVINE PLASMA

BY

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A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy in the Department of Chemical Engineering of Loughborough University of Technology.

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PROCESSING AND FUNCTIONAL PROPERTIES OF BOVINE PLASMA

S. E. Hill

A powdered product that was light in colour and had no off flavour or odour was made by the separation, ultrafiltration and spray drying of bovine blood. To establish the variability of the production methods samples were taken at stages during processing. A one year stability trial on one batch of powder was also done. Samples were analysed to indicate their physical characteristics, approximate composition and microbiological content. Model systems were established and used to assess the functional properties of the powder. Ten bovine plasma powders were produced with little indication of variation between batches. The powder was stable.

Other protein products, to act as comparisons for the bovine plasma powders, were analysed by the same model systems. The protein products used were other blood products, egg proteins, vegetable proteins and a fish protein. The bovine plasma sample has gelling, emulsification and fat absorbance properties equal to, or better than, egg white. The bovine powder would foam and was highly soluble in water at all pH values.

Variations in the methodology used to establish values for emulsification, foaming, gelation and fat absorbance were studied. The effect of the salt and sugar concentration, the protein concentration, temperature and pH were also examined. The results showed that different methods would give diverse numerical results. Even slight differences, either physical or chemical, could radically alter the results obtained for a functional property.

Keywords

Functionality, Processing, Bovine plasma, Emulsion, Foam, Gel, Fat Absorbance

DEDICATION

To two special men,

RMC and RJH,

with my love and thank you.

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PROCESSING AND FUNCTIONAL PROPERTIES OF BOVINE PLASMA

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CHAPTER 1

CHAPTER 1

PROTEIN IN FOODS

1.1 Functionality

Foods are consumed to satiate hunger and to obtain essential nutrients. However, as Young (1949) pointed out "although food selections often are in accord with nutritional needs, the correlation between need and acceptance is far from perfect. Food acceptance is regulated by the characteristics of food object (palatability)". It is the functional properties of the protein that provide the aesthetic appeal of many of the food systems. The term functionality was defined by Pour-El in 1979 as: "any property of a food or food ingredient except its nutritional ones that affects its utilisation". This covers a wide range of properties and some of the categories are listed in Figure 1.

Epstein (1967) went as far as to say that "Where food is abundant and choice wide, man eats first for palatability and only secondarily for nutritional benefit". It is therefore, not surprising that the food industry is always seeking less expensive proteins that possess a broad range of functional characteristics.

Proteins as dry powders have very little appeal to potential users or consumers. It is necessary for the product to impart desirable characteristics to the food system for its maximum utilisation. The amount of protein used for its functional rather than its nutritional value is thought to be 20% of the total food protein. The type of functional property required in a protein or a protein mix will vary with the food system, each food system requiring different characteristics. Figure 2 gives examples of the different types of properties that are required.

The factors that affect functionality are the factors that affect the physico-chemical characteristics of the protein. Any changes in the amino acid structure or the forces and bonds that form the structure of the protein will change the functionality of the protein. This also means that many of the processing methods will influence the functional properties.

FIGURE 1

General Classes of Functional Properties of Proteins Important in Food Applications*

General Property	Specific Functional Term
Organoleptic Kinesthetic	Colour, flavour, odour, texture, mouth-feel, smoothness, grittiness, turbidity, etc.
Hydration	Solubility, dispersibility, wettability, water absorbance, swelling, thickening, gelling, rheological, water holding capacity, syneresis, viscosity, dough formation etc.
Surface	Emulsification, foaming, aeration whipping, protein/lipid film formation, lipid binding, flavour binding, stabilisation etc.
Structural Textural Rheological	Elasticity, grittiness, cohesion chewiness, viscosity, adhesion, network cross-binding, aggregation stickiness, gelation, dough formation, texturizability, fibre formation, extrudability, elasticity
Other	Compatibility with additives, enzymatic, inertness, modification properties.

* from Kinsella (1976)

1.2 Measurement of Functionality

As functionality is such an important feature of the behaviour of a protein it needs to be assessed and routinely monitored. However, this is not a simple matter as pH, concentration, ionic medium, temperature, all affect the nature of the protein. Kinsella (1976) stated that some systematic sequence of uniform testing to assess the influence of the variable factors (preparation, processing environment, concentration, additives, etc.) on protein functionality should be established.

Functionality can be assessed by using Utility systems or food tests. These evaluate the functional property in a system that mimics a food preparation in all its particulars. Such systems are time consuming, often use large quantities of material, are difficult to quantify and only allow the assessment of gross changes, making differentiation between similar products impossible. In a Model system or Model test an evaluation of one or more functional properties is assessed, it does not mimic completely the steps and ingredients of an actual food preparation, (Pour-El, 1981). Hermansson(1975) concluded that model systems, when properly used, were important for the identification and estimation of relationships and were useful as predictors of possible effects of added proteins in processing. Yatsumatu (1972) also concluded that data from model systems correlated well with the results obtained in utility tests. However, Harper (1978) found disparity between the model test results and the actual functionality of the protein in utility systems. As model tests are carried out under different conditions it is not possible to correlate results from different studies.

The development of standard tests seems well overdue, but it is not easy to define a methodology for a model system that is suitable for the wide range of protein products and can be reproducibly used by all operators. Until standardized methods are adopted,

it would seem advisable to use a well characterized protein for the comparative evaluation of functionality in novel proteins (Wang, 1976). As the estimations can vary with a number of parameters the use of more than one control product would seem beneficial. The estimation in a model test should correlate well with the food system, but for final assessment of a functional property the protein must be investigated in the processing procedure that is to be used.

The use of model systems can be used not only for the assessment of a protein's likely behaviour in a complex food system, but also for its routine monitoring. If a protein is to be of use to the food industry the storage stability and the reproducibility of the product must be known (Kinsella, 1976). As its most important role in the system is its functionality this has to be routinely monitored with quick, reliable and indicative methods. Much work on the use of novel proteins has used material from different sources with no knowledge of the performance of the individual batches.

Some of the analysis that is required for the assessment of a model system does not involve the measurement of functionality. Chemical analysis for general composition, especially for protein and moisture, are essential for the comprehension of the results of the model tests. Physical analysis for size of particle, appearance, and density will also aid in the interpretation of the results for functionality.

FIGURE 2

Types of Food and Their Related Functionalities *

Type	Functionality
Beverages	Solubility, grittiness, colour
Baked goods	Emulsification, complex formulation, foaming, viscoelastic properties, matrix and film formation, gelation, hardness, absorption.
Dairy substitutes	Gelation, coagulation, foaming, fat holding capacity
Egg substances	Foaming, gelation
Meat emulsion products	Emulsification, gelation, liquid holding capacity, adhesion, cohesion, absorption
Meat extenders	Liquid holding capacity, hardness, chewiness, cohesion, adhesion
Soups and gravies	Viscosity, emulsification, water absorption
Topping	Foaming, emulsification
Whipped desserts	Foaming, gelation, emulsification

* taken from Pour-El (1981)

1.3 Protein

In a food product it is the protein that contributes the functional properties. Any study on functionality is an investigation of the action and reaction of the proteins in different environments.

1.3.1 Protein Composition and Structure

All living things contain protein which is formed from approximately 20 amino acids. The amino acids contain two reactive groups, the α-carboxyl and α-amino and may have a third group or side chain. Peptide linkages, between the α-carboxyl and α-amino functions of adjacent amino acids, join the amino acids in a legion number of permutations and combinations to form unbranched long chains. There is only one other type of covalent linkage between amino acids and that is the disulphide bond (S-S) cystine. It is the number, nature and sequence of the amino acids in the peptide chain that is referred to as the primary structure.

The secondary structure of a protein is determined by the hydrogen bonding between the components of the primary chain. These bonds can occur either between different peptide chains or within one polypeptide chain. One of the simplest structures is the helix.

The tertiary structure of a protein is defined as its total three-dimensional structure. This is the coiling of long structures to give a compact form. The formation of these tertiary structures is believed to be very dependent on hydrogen bonds, but ionic bonding and hydrophobic association may also play a role. The formation of disulphide bonds between two cysteine residues, although forming part of the primary structure, may be thought of as

maintaining the tertiary structure of the protein. Bovine serum albumin (66 K daltons) has 17 S-S bonds (Kinsella, 1982). In some proteins the disulphide bond cross-links between two separate polypeptide chains (interchain) or between loops of a single chain (intrachain). The tertiary structure is probably the most thermodynamically stable shape.

Quaternary structure refers to how polypeptide chains of a protein, having two or more chains, have them arranged in relation to each other. The forces maintaining the quaternary structure are similar to those involved in the tertiary structure but the association of the subunits is in general more flexible.

The term conformation is used to refer to the combined secondary, tertiary and quaternary structure of protein. In solution a globular protein probably has most of its non-polar (hydrophobic) residues inside the molecules (to avoid contact with the surrounding water) and most of its charged (hydrophilic) groups outside, in contact with the water. Polar, but uncharged groups could be both inside and out, but probably hydrogen bonded in either place to other parts of the protein or to water molecules (Bigelow, 1967). In most native proteins, a few hydrophobic groups remain at the molecular surface or in crevices, also hydrophobic side chains can occur at the surface.

1.3.2 Denaturation

Treatment of protein with acids, bases, high concentrations of salt or organic solvents, heat, radiation. etc., cause the high-order protein structures to be lost to a variable degree. This process is termed denaturation, the secondary and tertiary structure can be completely lost without breaking the primary structure.

One of the most common methods for denaturing a protein is by the application of heat, especially in the presence of moisture. Bovine plasma is thought to start denaturing at about 55^{o}C (Hermansson, 1983).

1.3.3 Salt Effects

Neutral salts are known to exert striking effects on the stability of native globular and fibrillar structures.. In low concentration salts increase solubility of many proteins, a phenomenon called salting in. If the ionic strength is increased further the solubility decreases, salting out. It is possible to arrange ions according to their effectiveness in salting out proteins, this is called the Hofmeister or lyotrophic series. The reaction to salt and concentration does vary with the type of protein being investigated. Three classes of protein were defined by Shen (1981) depending on their behaviour to increasing salt concentration. Shen (1981) also describes the Melander and Horvath theory that accounts for the effects of neutral salts on the electrostatic and hydrophobic interactions in the salting out of proteins.

1.3.4 pH Effect

The titration of a single amino acid reveals two pK ($-\log_{10}$ (acidity constant)) values. For proteins the titration curves are complex as there are large number of titratable groups present in each molecule. The value of pK for each of the titratable groups of protein may vary by one or more pH unit from that for the simple amino acid (Mahler and Cordes, 1966).

The isoelectric point (pI) is the pH at which the mean charge on the protein is zero and the protein fails to move in an electric field. However, due to the ability of proteins to bind ions and the alteration in pK due to ionic strength effects, the isoelectric point is a function of the nature and concentration of the solutes present. The solubility of most globular proteins is profoundly altered by the pH of the system and the protein is least soluble at its isoelectric point. At the pI there is no electrostatic repulsion between neighbouring protein molecules and they tend to

coalesce and precipitate. Since different proteins have different pI values they will have different solubilities over a range of pH values.

1.3.5 Functional Properties and Protein Groups

Protein has been reported to be primary responsible for the water absorption in protein rich powders, although other constituents may have an effect. The water retention is related to the polar groups, i.e. carbonyl, hydroxyl, amino, carboxyl, and sulphydryl groups. Conformational changes in the protein structure can affect the nature and availability of the hydration sites.

Correlations exist between the hydrophobic nature of a protein and its ability to perform as a functional protein. Calculations for average hydrophobicity of proteins, based on the polarity of the constituent amino acids, have been proposed (reviewed by Bigelow, 1967). However, the correlation between the theoretical hydrophobicity and the functional measurements were not good. Keshaverz and Nakai (1979) proposed that "effective hydrophobicity" should be measured as this would take into consideration the flexibility and conformation of the protein molecule. They proposed methods of hydrophobic partitioning and hydrophobic affinity chromatography. Other workers (Sklar, Hudson and Simoni, 1977; Nakai et al. 1980b) employed fluorescent probes to study the relationship between hydrophobicity and functional properties. The ability to disperse and to act as emulsifiers were related to the effective hydrophobicity.

1.3.6 Proteins at Interfaces

A mixture of polar and nonpolar side chains causes proteins to be concentrated at interfaces. The action of proteins at interfaces has been reviewed by MacRitchie (1978); Graham and Phillips (1979); and Halling (1981). Properties of disperse systems

e.g. foaming and emulsion formation, are dependent on the interface and any action that affects the protein could change that interface and therefore the functional property.

1.4 Dietary Requirement for Protein

As well as being the main substance involved in functionality, protein is also eaten to obtain essential nutrients. There is no dietary requirement for protein per se, the requirement is for the building materials to make protein. Proteins formed by the body perform a number of roles including, structural components, catalysts of chemical reactions, transport agents, hormonal messengers, osmotic regulators, buffers, etc.

Dietary proteins, because of their large molecule size, do not cross the intestinal wall to any significant amount in the adult. Denaturation of the protein molecule and some digestion by the enzyme pepsin occurs in the stomach. The major site of enzyme breakdown of the protein and protein digestion is in the small intestine.

For protein synthesis to take place all the necessary elements must be present. L-lysine, L-threonine, leucine, isoleucine, valine, tryptophan, phenylanine and methionine are the essential amino acids. The skeleton of an essential amino acid cannot be synthesized by the body and must therefore be provided by the diet (Freedland and Briggs, 1977). In the early stages of development, higher animals may also require arginine and histidine. The quality of a dietary protein can be estimated by how nearly its amino acid profile matches the amino acid requirements. Martinez (1979) suggested that nutritive value of a product should be included in its functionality. During food preparation certain amino acids may be destroyed or rendered unavailable. Lysine is particularly subject to alteration during heating.

CHAPTER 2

CHAPTER 2

FUNCTIONAL PROPERTIES

2.1 Introduction

> "No subject in the general area of food chemistry and technology has suffered more from inconsistency, confusion, and ambiguity than the field of functionality. Even the term functionality often is applied indefinitely to some related subjects. Until now, no rigorous treatment of its coverage has emerged. Each researcher and/or author has attempted to fit the name to his/her current interests of work. The methodology has not been standardized; properties often are confused with each other and very frequently wide conclusions are drawn from data obtained by ad hoc assays of uncertain validity".

The above statement is the opening paragraph to the paper of Pour-El at the 179th Meeting of the American Chemical Society, Houston, Texas in 1980. It indicates the lack of standard methods, the problems in defining the term functional property and the lack of knowledge relating functional properties to structure and the action of proteins in food systems.

Functional properties refer to the overall physical behaviour or performance of proteins in foods and reflect interactions that are influenced by protein composition, its structures, intermolecular association(s) of protein(s) and other food ingredients and by the nature of the environment in which these are measured (Kinsella, 1977). The types of functional properties and the foods in which they can play an important role are given in Figure 2. They can be grouped into broad categories given in Figure 1.

The functional properties most assessed for a protein are; organoleptic quality, solubility, dispersibility, water absorbance, viscosity, fat absorption, emulsification, ability to form foams and the gelation characteristics.

Circle (1950) first recognized the significance of functional properties in novel proteins. Since then many workers (Briskey, 1970; Mattil, 1971; Hermansson, 1973; Kinsella, 1976; Betschart, Fong and Hanamoto, 1979; Manak, Lawhorn and Lusus, 1980; Pour-El, 1981) have stated the need for standard tests for functional measurement, however these standard methods are not yet available. The complexity, variability and range of food systems, the dissimilarity of the proteins and their variable history makes the development of standard tests for measuring specific functional properties difficult.

Food systems are usually composed of several different proteins, the solubility, isoelectric point, susceptibility to denaturation of each protein being different. The functional properties associated with certain preparations may not reflect the properties of the heterogeneous mixture of proteins, but of one of the components. The literature is replete with papers on the functionality of a wide range of proteins from various sources, the review by Kinsella (1976) summarises much of the work. The different systems used to assess the proteins make it difficult to compare results obtained by different workers.

2.2 Organoleptic Properties

Colour, texture, flavour and odour are the key attributes of a new protein that determine whether it can be used in a range of products.

Colour, and other aspects of appearance influence food appreciation and quality, especially by the consumer (Amerine, Pangborn and Roessler, 1965). Colour is not just a physical property of the object, it is a sensory property dependent on the physical and psychological factors relating to that object. To define colour three fundamental quantities: hue, purity and luminance, must be specified (Blouin, Zarins and Cherry, 1981).

Texture is often one of the most important aspects of food quality. The characteristic sense of feel of food products is a composite property, related to the viscosity, elasticity and other physical properties of food, but the relationship is complex. Szczesniak (1963) classified texture into primary and secondary characteristics. The description of the textural characteristics perceived in a food product, the intensity of each and the order in which they are perceived is known as the Texture Profile (Larmond, 1976a). Larmond (1976b) said that texture was perceived in four stages: initial perception; initial perception on the palate; during mastication and a residual masticatory impression.

The texture of a food product is closely related to its other functional properties in a food system. Food which is processed, so that it has lost the native structure, is often of a colloidal nature in the forms of gels, emulsions and foams (Krog, 1976). Flavour is often considered the most important property in the determining of a foods acceptability (Kinsella, 1976; Amerine et al, 1965). As Brozek (1957) noted "flavour is a complex sensation, with taste, aroma and feeling as the three categories of the components". The flavour aspects of protein, whether of desirable or undesirable nature, are influenced by factors which include protein source, presence of contaminants of the protein itself, processing and storage methods, and the presence of other food ingredients such as lipids. These factors are normally studied in terms of off flavouring. Deterioration of flavour caused by an oxidation of lipids is a general problem with protein concentrates (Ory and Angelo, 1975). In addition to having flavour, proteins will absorb and retain flavour components.

Odours can attract or repel consumers and are therefore important for the acceptance of a product. The sense of smell is extraordinarily sensitive in differentiating odourous material. Odour appears to have three elements the intensity, type and variety.

The response to smell is so subjective that communication about its quality is poor, even using forms of classification.

Measurement

Numerous visual, instrumental methods and colour scales have been devised for the measurement and expression of the three colour quantities necessary for colour determination. The classification of texture characteristics was intended for both instrumental and sensory measurement. Texture profile panels can assess food products from the initial impression through complete mastication and swallowing. Civille and Szczesniak (1973) described the sensory techniques for the evaluation of different textural characteristics.

The instrumental texture profile attempts to quantify as many textural paramenters as possible. The Instron Universal Testing machine will produce a force-distance curve and will give values for hardness, cohesiveness, springiness; fracturability and gumminess can be calculated. An example of the texture profile obtained using an Instron machine is given in Figure 6. P35. Individual methods are available for the measurement of factors that make up the texture profile.

Objective and subjective tests are applied to indicate the flavour of new products. Caul (1957) comprehensively reviewed the methods for descriptive sensory analysis of flavours. Odours cannot be measured quantitatively by the nose and their classification is difficult. Some classifications for odours have been suggested, but they are normally described in relation to one another. (see Amerine, Pangborn and Roessler, 1965). Techniques for the sensory evaluation of odours in food materials is reviewed by Stone, Pangborn and Ough,(1965) The relation of odour quality to molecular weight, volatility and chemical structure is an intimate one, but no precise rules have been formulated (Amerine, Pangborn and Roessler, 1965).

Organoleptic qualities of products are often assessed in Laboratory studies. With trained panels, well organised procedures and correct statistical analysis, reproducable results can be obtained. Consumer studies involving many people will indicate acceptance and preference for food products. Clydesdale (1985) pointed out that instrumental measurements, although an invaluable aid, must correlate with what the sensory organ can perceive.

2.3 Solubility

Many of the important functional properties of food proteins relate to protein-water interactions, i.e., solubility, viscosity, gelation, foaming and emulsification. For this project solubility is being defined as the amount of product that can be converted into a liquid state. Many workers have only investigated the protein in products for their solubility. The solubility is very dependent on the prior treatment of the protein (Lawhon and Cater, 1971; Betschart, 1974; Wu and Inglett, 1974; Tybor, Dill and Landmann, 1975; Nakai et al, 1980a). The extent of denaturation should be studied at more than one pH value (Lawhon and Cater, 1971).

Studies of pH and solubility have been carried out by Morr, Swenson and Richter, 1973; Tybor, Dill and Landmann, 1973; Delaney, Donnelly and Bender, 1975; De Vuono et al, 1979; Wang and Kinsella, 1976; Hermansson and Tornberg, 1978; Betschart, Fong and Hanamoto, 1979; Burgess and Kelly, 1979; McWatters and Holmes, 1979a,b,c ; Schachtel, 1981. The pH values used in production are also important for the final solubility of the protein (Lawhon and Cater, 1971; Betschart, Fong and Hanamoto, 1979). The pH solubility profile is often the first functional property measured, and it should be made at each stage of preparation and processing (Kinsella, 1976).

Other parameters are also important for the solubility of the protein products. The ionic concentration was found to be relevant in the studies by Hang, Steinkraus and Hackler, 1970;

Mattil, 1971; Betschart, 1974; Delaney, Donnelly and Bender, 1975; Wang and Kinsella, 1976; McWatters and Holmes, 1979a,b. The type of ion in solution and the temperature of the solvent will effect the solubility of a protein (Shen, 1981). Nakai et al (1980a) demonstrated that anionic surfactants were effective in making rape seed protein soluble. Tybor, Dill and Landmann (1975) investigated the solubility of proteins when carbohydrates were present. The protein concentration is another critical factor for solubility (Mattil, 1971; Betschart, 1974; Wang and Kinsella, 1976).

Several terms are used to discuss solubility: WSP = water-soluble protein, WDP = water-dispersible protein, PDI = protein dispersibility index, NSI = nitrogen solubility index. The latter two parameters are commonly employed as the American Oil Chemists Society (AOCS, 1980) official methods.

Much work has been done to improve the solubility of some protein products. Partial hydrolysis, either chemically or enzymatically of denatured protein aids in its solubility (Kinsella, 1976). Enzymatic treatment using protease, trypsin and pepsin was found to be effective in making rape seed protein soluble, while papain, bromelain and α-chymotrypsin were ineffective (Nakai et al, 1980a).

NSI and PDI are often used as quality control tests in preparing products such as soy flours as there is some correlation between solubility and functional properties. However, Wolfe and Cowan (1971) pointed out that even if the solubility index figures were the same, but the powders had been produced under different conditions, then their functionality would probably not be equal.

Measurement

The official methods for the estimation of solubility, the PDI and NSI, have been criticised because they are subject to

several errors arising from the pH of the solvent, extraction methods and prior treatment of the protein, etc. Descriptions of suitable methods to establish solubility are given by Regenstein and Regenstein (1984).

In determining the solubility of plasma powder the amount of protein varies, values of 0.2 to 2% have been used. There have been differences in the medium of suspension and the time, temperature and method of mixing. The manner in which the pH is altered varies, some workers using dilute acidic and basic solutions, others more concentrated. Buffers have also been used to establish the pH value. Methods of centrifuging, the duration and speed add other variables. The nitrogen estimation has been made on the pellet and the supernatant using micro-Kjeldahl. The supernatant has also been assessed using the Biuret colour estimation. Estimations on the total nitrogen and the protein nitrogen have been differentiated in some cases.

The most quoted method for the estimation of solubility was that of Lawhon and Cater (1971), which they had based on the method on that of Lyman, Chang and Couch, (1953).

2.4 Ability to Disperse

The rate and ease of a powder going into solution can be an important feature . Different mixing methods and times being required for powders to disperse in the aqueous medium and then to dissolve. The ability to disperse is an investigation of the dried product and its ability to absorb water to such a degree that hydration can occur. Characteristics of the powder such as formation of agglomerates, will be of consequence.

Measurements

Some of the methods normally described for solubility and water absorbance tests could be applied for an estimation of the ability to disperse.

2.5 Water Absorbance

Most functional products are neither completely soluble nor completely insoluble, and the food systems into which they are incorporated are water swollen systems. The amino acid composition and the conformation of the protein will effect the water interaction.

Various terms are used to discuss the uptake of water. Often the term used depends on the method by which it was estimated. Terms used include: water binding; water absorption; water hydration capacity; water holding and swelling. Some of the theory of water sorption is given by Watt (1983) and the practical applications for the food industry were reviewed by Gal (1983).

Measurements

Various methods have been described in the literature (see Hutton and Campbell, 1981). Four general methods are used to establish water absorption: relative humidity; swelling; excess water and water saturation methods.

2.6 Viscosity

The purpose of many food processes is to create structures which give food its characteristic rheological properties. Many proteins absorb water, swell and cause an increase in viscosity. The thickening power of a product can be very important in such items as soups, beverages and batters.

Viscosity is the term used for liquids to define their resistance to flow, and reflects the relationship between the shearing stress and the rate of shear. For further details of the principles behind viscosity estimations see De Mann et al, (1976). Flow properties are governed by the molecular size, shape, charge, solubility and swelling capacity of the protein molecules which are effected by the environmental conditions which can include the temperature, pH, concentration, ionicity, and the previous processing history of the protein.

Viscosity can be a useful index of structural changes in proteins, and subsequently, of the hydrodynamic/rheological properties of modified food proteins (Kinsella, 1976). The denaturation of a protein has been followed by assessing the viscosity (Hermansson, 1983). Flow properties and viscosity of protein dispersions can be of importance during processing. Viscosity of plasma during ultrafiltration was studied by Dobromirov (1979) and Hurst (1980).

Measurement

Viscosities of protein solutions are usually studied using standard capillary viscometers, such as the Ostwald, Cannon-Fenske or Ubbelohde viscometers. Dispersions, slurries and pastes can be assessed for viscosity by using rotational viscometers, the Brookfield viscometer with the Helipath stand was used successfully by Hermansson (1975)in the study of the flow properties.

2.7 Fat Absorption

Of all the functional properties fat absorption has been studied least, yet, it has a role in a wide variety of food systems including meats, sausages and doughnuts. Bound fat is said to improve mouthfeel, enhance flavour retention and also to improve

the flavour carry over in simulated foods. The ability of a protein to bind fat is particularly important for such applications as meat replacers and extenders. A little understood phenomenon of decreased fat absorption has lead to soy flour being added to pancakes to prevent excessive fat absorption during cooking (Wolfe and Cowan, 1971).

Fat absorption has been mainly attributed to the physical entrapment of oil. It is known that the protein amount and source effects the assimilation of fat. The primary protein structure and that of the subunits will effect the binding of the lipid. The forces involved in the fat absorption are the non-covalent bonds, such as hydrophobic, electrostatic and hydrogen (Cornwell, and Horrocks, 1964).

The amount of fat absorption is normally attributed to the protein (Hutton, 1977) while Nath (1981) found that carbohydrate inhibited fat absorption. Several authors have investigated the relationship between the amount of soluble protein, as compared with the total protein, and fat absorption, but no correlation can be found (Lin, 1974; Schachtel, 1981). As fat absorption also relies on the physical entrapment of oil the characteristics of the particles of powder should be relevant. Good correlations between bulk density and fat absorbance were established by Wang and Kinsella (1976) and Schachtel (1981). The greater the bulk density the less oil was absorbed. Jasmin (1983) showed that fat absorption was also dependent on particle size. Hutton (1977) also found that small soy particles absorbed more oil than the large particles.

Measurements

The methods for the measurement of fat absorption have varied, but most methods are based on that of Lin, Humbert and Sosulski (1974). That method described the use of 0.5 g of sample mixed with 3 ml of oil in a conical centrifuge tube. Sosulski et al (1976) used increased volumes. The mixture was stirred for 1 minute with a brass wire and held for 30 minutes before centrifuging

at 1610g for 25 minutes. The volume of free oil was then read.

2.8 Emulsification

One of the primary functional requirements in several food systems is the ability to form emulsions. An emulsion is defined as a dispersion or suspension of two immiscible liquids. An emulsion has three parts. One of which forms the continuous or dispersion phase, one the discontinuous, dispersed or globular phase and the third part is an adsorbed surfactant. The surfactant or emulsifier stabilizes the dispersion of the two immiscible liquids which would otherwise rapidly separate into two distinct phases upon standing. On shearing the liquids the emulsifier lowers the surface tension of one liquid which becomes the continuous phase. The other substance will form globules which would readily coalesce except for the presence of the emulsifier. The surfactant must contain hydrophilic and lipophilic portions which can orientate and be selectively adsorbed at the globule/continuous phase interface, thus preventing the globules coming into contact with each other. Proteins form an important group of emulsifiers because they are able to form mechanically strong monolayer films at interfaces. The action of the protein in emulsions was reported by MacRitchie (1978) and Graham and Phillips (1979).

In time an emulsion will undergo processes which will result in the separation of oil and water phases. These processes include flocculation, coalescence, creaming and oiling-off and may occur singly or in combination. Flocculation has been said to have occurred when the emulsified droplets become associated in flocs without destruction of the individual droplets. When the walls of the droplets are destroyed and larger drops are thus formed the process is known as coalescence. When the dispersed phase is at a lower density than the continuous phase creaming takes place, but this is not flocculation.

The theory of stability as a balance between forces of attraction and repulsion was developed by Deragin and Landau, Verwey and Overbeck and is commonly known as the DLVO theory. The theory ascribes emulsion stability to a balance of attractive Van der Waals forces and electrostatic repulsive forces. With the emulsion being stable as long as the repulsive forces are greater than the attractive. Electric charges on droplets in emulsions can arise by ionization, absorption or frictional contact. In the absence of emulsifiers only frictional contact appears capable of charging droplets. The electrical charge surrounding the globules provides a degree of stability by the repulsive interaction of droplets possessing like charges.

There are a number of factors which influence the stability of emulsions:-

1. Interfacial tension between the two phases.
2. Characteristics of the adsorbed film in the interface
3. Magnitude of the electrical charge on the globules
4. Size and surface/volume ratio of the globules
5. Weight/volume ratio of dispersed and dispersion phases
6. Viscosity of the dispersion phase

Measurements

There are a variety of tests for indicating the value of a protein in an emulsion.

2.8.1 Emulsion Capacity (EC)

Emulsion capacity is the parameter most commonly estimated in the various studies on oil in water emulsions. When an aqueous solution containing a protein is vigorously stirred while oil is added an emulsion is formed. After a certain volume of oil has been added, the emulsion undergoes a sudden change referred to as inversion or breaking. The EC gives the maximum amount of oil that is emulsified before inversion, under specific conditions, by a standard amount of protein and is normally expressed as the amount of oil emulsified

by unit weight of protein. The system introduced by Swift, Lockett and Fryer (1961), mixing oil with a protein until the emulsion formed breaks, is the method most commonly adapted to estimate EC.

There are three indicators of the inversion of the emulsion, the change in visual appearance, the sudden drop in viscosity and a sudden increase in electrical resistance. The sudden drop in viscosity which occurs as the emulsion collapses has been used as the end point by many workers. However, the method requires an experienced operator and is not applicable to emulsions of low viscosity (Swift, 1961). To aid in the visual detection of breaking Marshall, Dutson and Carpenter (1975) added oil-red-O to the oil before mixing.

Webb et al (1970), Satterlee, Zachariah and Levin (1973) and Kato et al (1985) investigated the sudden increase in electrical resistance as the oil phase became continuous for the objective determination of end-points in emulsification. The perception of the drop in amperage required to drive the mixing apparatus which occurs at the breaking point has also been investigated (Crenwege et al, 1974).

2.8.2 Oil Phase Volume (OPV)

When the maximum amount of oil is incorporated into an oil in water emulsion, theoretically the addition of more oil would form a water in oil system. According to Ostwald's geometric phase theory the critical value for inversion, if the droplets are spherical, is 0.74 (dispersed phase/total volume) (Halling, 1981). Different techniques and stabilizers for emulsifying can induce deformation of the spheres producing polyhedra droplets and/or heterogenous sizes for the droplets. Emulsions have been prepared containing up to 99% internal phase (Becher, 1966).

Acton and Saffle (1972) reported a narrow range of oil phase volume values when the protein concentrations widely differed and suggested that emulsifying capacity expressed in "oil phase volume" would minimize the variations caused by protein concentration and allow results to be compared.

2.8.3 Emulsion Stability (ES)

Different procedures have been used to measure emulsion stability, but it is commonly measured in terms of the oil and/or cream separating from an emulsion during a certain period of time at a stated temperature and gravitational field.

Some methods(Inklaar and Fortuin, 1969; Yatsumatsu et al, 1972; Hutton and Campbell, 1977; Betschart, Fong and Hanamoto, 1979; Schachtel, 1981) are based on taking an aliquot of emulsion before it breaks and heating it (up to 90^{o}C) and centrifuging the sample. Tornberg and Hermansson (1977) realizing that to be able to measure oil release from an emulsion only weak or centrifuged emulsions could be studied, developed a creaming stability test which was based on the percentage change of fat in the lower portion of an emulsion after mild centrifugation. Only low viscosity emulsions were used. Other workers have used this method (Hayes, Stanaghan and Dunkerley, 1979; Yamauchi et al, 1980).Kato et al, (1985)maintained that a value for emulsion stability could be extrapolated from the reciprocal of the initial slope of the electrical conductivity curve for an emulsion after it is mixed. Pearce and Kinsella (1978) proposed a turbimetric method for the determination of emulsion stability.

2.8.4 Emulsifying Activity (EA)

Emulsifying activity is said to reflect the ability of the protein to aid formation and stabilization of newly created emulsions (Ivey, Webb and Jones, 1970). EA is measured by determining the particle size distribution of the dispersed phase.

This can be done by microscopy, Coulter Counting or by spectroturbidity estimations (Walstra, Outwijn and de Graaf, 1969). This work was taken further by Pearce and Kinsella (1978) where they defined an emulsifying activity index (EAI).

Emulsifying activity has also been used in other contexts. Kato et al (1985) defined emulsion activity of proteins as the difference between the conductivity of the protein solution and the minimum conductivity of the emulsion during homogenization for 1 minute. The idea of EA as an indication of emulsion capacity of protein was developed by Yatsumatsu et al (1972), but the method is the same as for emulsion stability except no heat is involved.

2.8.5 Measurement of Droplet Size

A direct approach in the evaluation of the emulsifying properties of a system is by measuring the individual droplets. Microscopy can be used to follow the variation in droplet size with time due to coalescence in a protein stabilized emulsion.

The normal method for protein size estimation by microscopy is to dilute the emulsion with a solution that will gel. A thin film is formed on a slide and covered, a haemocytometer cell can be used (Walstra, 1968; Howell, 1981). The size of the globules can then be estimated. Walstra (1969) concluded that microscopic counting and measurement was difficult (mainly due to the poor detection of small globules), tedious and time consuming and the results showed poor reproducibility. Fluorescence microscopy was considered slightly preferable and a Coulter Counter, used for a limited size range, could determine globule size and size distributions with good replication and little bias.

The method most favoured by workers to estimate globule size distribution of oil in water emulsions is by spectro-turbidity. The emulsion can be regarded, in its light scattering behaviour, as a collection of spheres that fulfills the conditions of the Mie theory (Walstra, 1968). In the method of Walstra (1968) the optical density of the emulsions is measured at several wavelengths and using data on concentration and reflective indices a specific turbidity spectrum is calculated and plotted. This is compared with theoretical spectra that are computed for assumed globule size distributions.

Pearce and Kinsella (1978) defined an EAI which had units of area of interface stabilized per unit weight of protein and was estimated from the turbidity of the emulsion at a wavelength of 500 nm. Ivey, Webb and Jones (1970) used the term IFT, which is an approximation for the hydrated Interfacial Film Thickness and is dependent on the diameter of the droplets.They found that the stability of emulsions was related to the IFT.

2.8.6 Other Methods

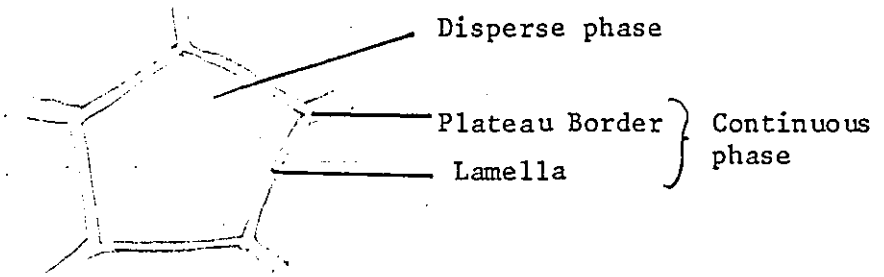
Other techniques to study the role of proteins as emulsifiers have been described by Cante, Franzen and Saleeb (1979). These include microtechniques, aimed at single interfaces (film balance) and double interfaces (tensiolaminometry), to macrotechniques aimed at gross emulsions (microcalorimetry, electrophoresis, pulsed NMR and microwave irradiation).

2.9 Foam Formation

Angel cakes, sponges, soufflés, whipped toppings, meringues, fudges all incorporate a gas into their matrix to produce the light texture required. The ability of a protein to stabilize a foam,

which is a mixture of gas bubbles in a liquid, is an important functional property. Like emulsions a foam is a disperse system with the liquid as the continuous phase and the gas bubbles as the disperse phase. Proteins tend to accumulate at the air/water interface. At low phase volumes the bubbles of air are spherical, but at high phase volumes the bubbles are distorted into polyhedra. The continuous phase liquid separating the faces of the two adjacent polyhedra are known as 'lamella' while the thicker channels where three lamellae meet are known as plateau borders. (see Figure 3)

Figure 3 Structure of High Phase Volume Foam



Cumper in 1954 described adsorption of protein at air/water interfaces occurring in three main stages.

1. Diffusion of the native globular molecules to the interface and the adsorption.
2. Uncoiling of the polypeptide chains at the interface (surface denaturation).
3. Aggregation of the surface denatured protein into a coagulum largely devoid of surface activity.

Figure 4 gives the sequence of events occurring during foam formation and coalescence. The stability and elasticity of the protein film around the air bubble depend on the degree of denaturation of the protein. Too much denaturation gives a fragile film structure and the foam will collapse.

A foam is a dynamic multiphase system stabilized by a protein which lowers the surface tension of the disperse phase and forms the cohesion and elasticity of a film at the interface. The action

Figure 4.

Sequence of Events occurring during Foam Formation and Coalescence*

1. Denaturation: uncoiling of protein polypeptides.

2. Adsorption: formation of a monolayer or film of denatured protein at the surface of the colloidal solution.

3. Entrapment: surrounding of gas at the interface by the film and formation of bubbles.

4. Repair: continued adsorption or formation of a second monolayer around the bubbles to replace coagulated regions of the film.

5. Contact: protein films of adjacent bubbles come in contact and prevent flow of the liquid.

6. Coagulation: interacting forces between polypeptides increase causing protein aggregation and weakening of the surface film followed by bursting of the bubble; weakening of the film also occurs when the Repair step ceases because of a deficiency of denatured protein.

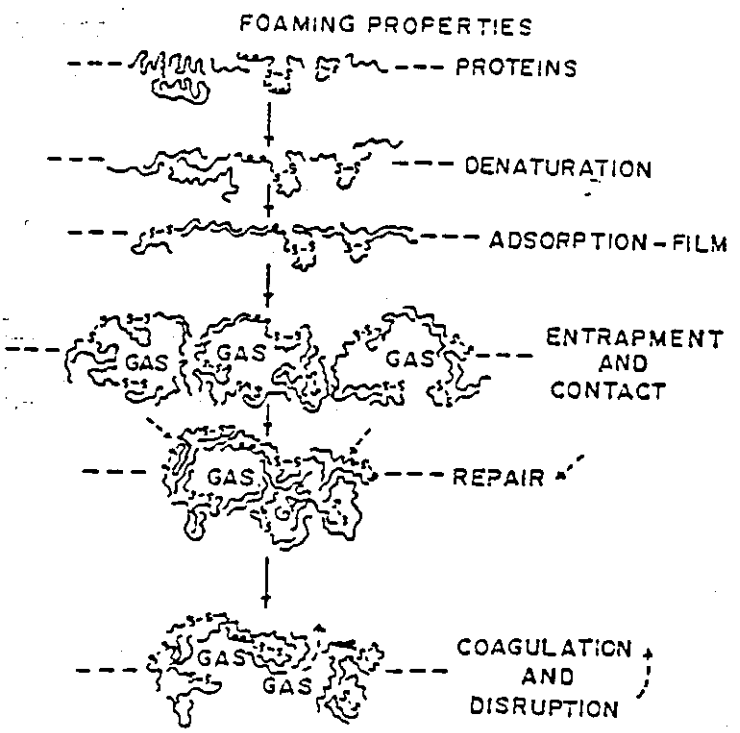


Diagram of foam properties

* Cherry and McWatters (1981)

of the protein at the interface is reported by Halling (1981).

Measurements

Like the other functional properties there is no standard method for the estimation of the foaming ability of a protein. Workers measure whippability or foamability using a variety of methods each measuring different parameters under various conditions.

2.9.1 Foam Production

There have been three main methods for the creation of a foam:- bubbling/sparging, whipping/beating and shaking. Sparging produces foams of uniform small bubbles from little protein. Foam formation with shaking tends to be slow and depends on the frequency, amplitude, volume and shape of the container and the properties of the liquid. Whipping is the method most commonly used and can be carried out with a variety of devices that vigorously agitates a liquid and its interface with the bulk phase. Each bubble as it is formed remains subject to severe mechanical stress throughout the residual whipping time, and could be broken up to form bubbles of smaller sizes. In foam formation an excess of the dispersed phase is normally available and measurements are not normally made until the foam has separated as a layer. This layer will have a high disperse phase volume and the bubbles will therefore be distorted from the spherical form.

Halling (1981) reports that there have been no systematic studies of foams produced from the same protein solutions by different methods. However, the widely differing amounts of protein required for the bubbling and whipping methods and the varying stability data would indicate that foam is markedly affected by the method of formation.

2.9.2 Parameters

Volume

Estimations are based on a variety of ways to measure volume.

Section 5.5 gives the measurements and definitions used in this study, the same terms have been used for different values by some workers. Foam volume is often called foam expansion. Bubbling methods have been used to measure "dynamic foam lifetime" and for studies of the lifetime of individual bubbles (Bickerman et al, 1953). The term "Foaming Power" was used by Kitabatake and Doi (1982) to express the ratio of gas volume to liquid volume in the foam. Photographic methods have also been employed to investigate bubble size.

Stability

The most fundamental method for the measurement of foam stability is that which follows the change in surface area of foam with time. However, the two most common measurements of foam stability are the assessment of leakage or syneresis i.e. measurement of liquid drained from foam, and the decrease in volume of the foam column.

Clarke and Blackman (1948) investigated the loss of light on transmission through a layer of foam and found that a method could be developed to indicate rate of change of bubble size. Also, continuous measurement of electrical conductivity to estimate the foam stability has been investigated(Clarke and Blackman, 1948).

Other estimations

Many workers have investigated foam strength or stiffness. The estimations have been made using: Brookfield viscometers, rate of fall of a perforated weight, penetration by a cone or the ability to support a series of specific weights.

Surface tension has been monitored as foaming is dependent on the surfactant properties of a protein to lower the interfacial tension. Kitabatake and Doi (1982) found that foaming power correlated

well with the rate constant of surface tension decay of protein solutions. The viscosity of the protein solution also plays a role in the stability of a foam and therefore workers have measured viscosity of the protein solution before whipping and of the drained solution. The density of foam has also been estimated in some studies. The amount of time required to produce a foam has also been used for the assessment of the foaming properties of a protein.

2.9.3 Angel Cake Preparations

Some studies (MacDonnell et al,1955; De Vilbiss, 1974) have used an Angel cake preparation, a Utility test, for the estimation of a protein's functional properties. Although the model systems indicate foam volume and foam stability the preparation of a true food product does show other requirements. The foam must be strong enough to support the weight of the flour, bubbles in the foam must be elastic and the foam must set on heating (West and Weir, 1979).

MacDonnell et al (1955) reported that damaged egg whites would produce cakes of optimum volume at different stages of foam stiffness and therefore specific gravitities of meringues could not be substituted for cake volume in assessing the protein.

2.10 Gelation

The structure, mechanical properties and sensory quality of foods are closely related. The ability of a protein to form a gel is an important functional property, as it adds bite and texture in such products as baked goods, processed meats, sausages, some confectionery products and some oriental textured foods e.g. tofu.

Gelation is said to have occurred when a small amount of solid is dispersed in a relatively large amount of solvent, usually water, by the property of mechanical rigidity. The gel is characterized by relative high viscosity, plasticity, elasticity and yield values (Paul and Palmer, 1972). The theoretical definition of the protein gel was given by Schmidt (1981) and states that gelation may be defined as a protein aggregation phenomenon in which attractive and repulsive forces are so balanced that a well ordered tertiary network or matrix is formed. If the forces are not quite balanced a protein coagulum may occur.

Stanley and Tung (1976) investigated various gels using an electron microscope technique and found milk gels, egg white gels and tofu were characterized by distinct particles joined through some cementing or connective material. Gelatin gels were formed by thin compact sheets with warped surfaces. Macroscopically it is hard to distinguish between a true gel and a coagulum (cogel). Gel formation was said to have occurred in this study if solid material, either a true gel or cogel, was observed after heating a protein solution.

2.10.1 Theory of Gelation

Coagulation and gelling can be brought about by heat, high pressure, salts, acids, alkalies, alcohols or denaturing agents such as urea.

Ferry (1948) studied gelation and came to the conclusion that the three dimensional network of fibrous proteins forming a gel occurred by a two step procedure. Initially there must be a denaturation of the protein causing it to unfold. This is followed by an aggregation and association step which results in gel formation, provided that the attractive forces and thermodynamic conditions are suitable. The second step may be reversible and then the gel is said to be thermoset or reversible.

For the formation of a highly ordered matrix, it is essential that the first step proceeds at a faster rate than the aggregation (Hermansson, 1978). In complex protein systems the aggregation may occur more randomly and simultaneously with the unfolding step. In these cases a cogel can be formed and this is made of strands where spherical aggregated particles predominate as described by the "string of beads" model by Tombs (1970). This type of gel, the cogel, is characterised by higher opacity, lower elasticity and greater syneresis compared with more highly ordered gels.

2.10.2 Crosslinking

The characteristics of strength, elasticity and flow behaviour of protein gels are affected by the fluidity of immobilized solvent and by the intra and inter-strand crosslinking. The crosslinking is due to non-covalent and covalent bonding.

Investigations of covalent bonding in heat induced gels have primarily focused on the disulphide bridges. Heat treatment can result in cleavage of existing disulphide bonds or by participation of sulphydryl groups after unfolding of the protein. Utsumi and Kinsella (1985) reported that hydrogen bonding and disulphide bonds were important in maintaining soy protein isolate gels. Howell and Lawrie (1985) investigated plasma and egg albumen gels after chemical modification. They demonstrated that gelation was predominantly caused by disulphide bonds. Hydrophobic and hydrogen bonds were also of importance in the mechanism for plasma gelation, but their action in the egg gels was different.

2.10.3 Factors Affecting Gels

Catsimpoolas (1971) studied the effect of the dielectric constant of the medium in which the protein was dissolved. Some of the changes in gelation were pronounced, however the ability

of alcohols and glycols to unfold the protein may play an important role in some gelation systems. The effects of electrostatic charge, protein concentration, formation of disulphide bonds and composition of the protein in gel formation was reviewed by Gossett, Rizvi and Baker (1984).

2.10.4 Description of a Gel

Many different parameters are required to characterize a protein gel and some of the terminology in Figure 5 is used. Some of the terms can be interpreted from a force/distance curve from the compression measurements on a protein gel using the Instron Universal Testing Machine, see Figure 6.

Hermansson (1982,a) studied the relationship between gel hardness and water binding capacity of the gel and found that the gel structure may affect texture and water binding quite differently.

2.10.5 Measurements

A gel can be characterised in a variety of ways: the ability to hold water, gel strength, breaking strength, viscosity, the melting point of reversible gels., resistance to penetration, gravimetric analysis, study of structural changes, the appearance and disappearance of different bands during electrophoresis. Some of these methods are reviewed by Gossett, Rizvi and Baker (1984) and the paper includes a description of their nondestructive technique involving the use of an electrobalance.

2.10.5.1 The Penetration Test

Bourne (1966) defined the term "Penetration Test" as a measure of the depth of penetration of a punch into a food under a constant force in a given time. The test was used initially as an empirical indicator, but much work has been done to define the compression coefficient and shear coefficient in a penetration test.

Figure 5

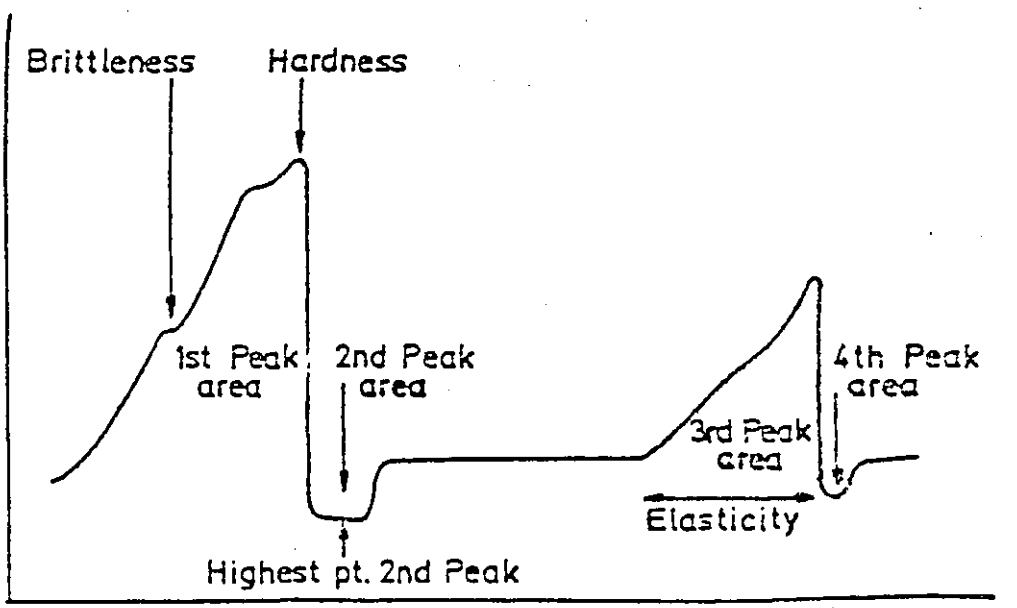
Relations between Textural Parameters and Popular Nomenclature*

Primary parameters	Secondary parameters	Popular terms
Mechanical characteristics		
Hardness		Soft→firm→hard
Cohesiveness	Brittleness	Crumbly→Crunchy→brittle
	Chewiness	Tender→chewy→tough
	Gumminess	Short→mealy→pasty→gummy
Viscosity		Thin→viscous
Elasticity		Plastic→elastic
Adhesiveness		Sticky→tacky→gooey
Geometrical characteristics		
Particle size and shape		Gritty, grainy, coarse etc
Particle shape and orientation		Fibrous, cellular, crystalline, etc.
Other characteristics		
Moisture content		Dry→moist→wet→watery
Fat content	Oiliness	Oily
	Greasiness	Greasy

* Szczesniak (1963)

Figure 6

Texture Profile using the Instron



Cohesiveness = Peak 3 / Peak 1

Tanaka (1971) used a cone-shaped probe to measure food texture and equated the force on the cone, to the sum of the plastic and viscous sample reactions. De Man (1976) said that in strongly bound networks the main factors in the penetration were shear and flow. In the studies by Kalab (1971a) a variation of 3.3% for the penetration readings occurred.

CHAPTER 3

CHAPTER 3

PROCESSING BLOOD

3.1 Blood, a Source of Protein

There are 29 million cattle, sheep and pigs slaughtered each year in the UK. This results in the production of 100000 tonnes of blood, equivalent to 7000 tonnes of high grade protein. Only 900 tonnes of blood is used for human consumption, another 25000 tonnes is used in pet food manufacture. Some 4000 tonnes is dried and sold as animal feeds while 19000 tonnes goes as fertilizers. The largest quantity of blood, 49000 tonnes is discarded into the sewage system, causing serious pollution problems. As Bates (1974) points out the recent emphasis upon pollution abatement has dictated that formerly accepted (or tolerated) food industry waste disposal practices be drastically curtailed. The use of the blood for human food consumption would therefore not only be utilizing a protein rich source, but lessening the disposal problems of a polluting waste product. This study deals with the processing of blood to produce a plasma powder and the functional properties of that powder.

The report written by Bright (1977) gives a survey on the collection, processing and utilization of blood. The main points of interest for this project are:

a) Most blood is treated as a waste product
b) Blood could be collected under conditions so that it is suitable for human consumption
c) Labelling laws allow blood/plasma to be used in meat products
d) Blood has been utilized for many different processes, but requires careful processing if it is to fulfill its potential as a food product.

3.2 Blood

Blood is a highly specialised tissue containing several types of cell suspended in a fluid medium called plasma. The blood forms 8% of the total weight of the carcass. The red blood cell fraction of bovine blood is approximately 28-38% by volume, the total number of erythrocytes being 5.0-9.0 * $10^6/\mu l$ The total white blood cell count for healthy cattle is 4.0 - 10.0 * $10^3/\mu l$. Plasma is mostly water with a variety of substances dissolved in it and plasma forms the medium of transportation from one part of the body to another.

Plasma contains about 9% solids out of which 6-8% in cattle and between 5 and 8% in pigs are protein (BCL, 1983). Approximately 0.9% of the solids are inorganic salts, mostly sodium chloride, but the cations potassium, calcium and magnesium are also present. The electrolytic balance is maintained by bicarbonate, with phosphate and sulphate ions also participating. Other constituents in the plasma include cholesterol, glucose, urea and vitamins.

The plasma proteins can be divided into three main types; fibrinogen, albumin and globulin. Albumin and globulin consist of a group of proteins with similar electrical properties. Fibrinogen, of molecular weight 330000 is the precursor of the fibrin of the blood clot. If a blood sample is allowed to coagulate, the fibrinogen is used in the clot formation, the remaining fluid is serum. The albumins, of molecular weight 68000 are either equal to or lower than the globulin concentrations in porcine and cattle samples. The globulins are designated $\alpha 1$, $\alpha 2$, β and γ-globulins and cover a wide range of molecular weights from 90000 to 1300000 or more. The proteins forming the groups within the globulins can be further subdivided into a range of proteins.

3.3 Examples of Processed Blood

Plasma can be successfully separated from whole blood and the potential for this material was considered extensive by the meat product manufacturers for their open pack and canned products. The use of plasma as a substitute for egg albumen in the baking industry was shown to be feasible in Germany during World War II (Akers 1973). Halliday (1973) reports that since the second World War sufficient interest was shown by the continental slaughter houses to establish a process system for the collection and separation of whole blood. As the volume of plasma being utilised increased the process was extended to concentrate the separated fractions, either by a form of spray drying or by roller drying. In some parts of Europe 90% of the blood from cattle was being processed. Two plants were in operation in Ireland for the production of blood albumin, but no operational plants were established in the UK.

In 1959, Brooks and Ratcliff investigated the storage of spray dried bovine plasma and found that it acquired a fishy flavour. The frozen plasma did not become fishy. The trials incorporating some plasma instead of egg white into cakes and nougat produced a satisfactory product. However, it was concluded that concentrated plasma might not be acceptable as an extender for dried or fresh egg white because of its pronounced red colour.

Whole blood was used by Vickery(1968) to produce a product that was bland in taste and odour and contained 90% protein. An isolate that was light tan in colour and contained 90% protein was made by Pals (1970) from bovine plasma by spray drying. In Texas whole bovine blood was centrifuged and the resulting plasma spray dried by Tybor, Dill and Landmann (1975). They found that the concentrate contained 71% protein but, as sodium citrate had been added as the anti coagulant, the salt content was high. The solubility of the dried plasma was reduced but the incorporation of lactose was said to improve this feature (Tybor et al, 1973). This product was also used to make cakes and bread and low levels of its substitution for egg white was found to be acceptable (Khan, Rooney and Dill, 1979).

Porcine plasma was concentrated by ultrafiltration and spray dried in the study of Delaney, Donnelly and Bender (1975). The solubility of the powder they obtained was compared with a non-specified commercial product. The experimental product was more soluble than the commercial. This was thought to be due to the differences in the drying conditions.

In Brazil a study on the functional properties of bovine blood was undertaken by De Vuono et al,(1979). The product used was separated by decantation followed by centrifugation, then freeze dried. In Chile an investigation of animal blood as a source of proteins was studied by Borghesi and del Aguirre (1980). The acceptability of dried plasma in processed meat products produced by Nakamura et al (1983) in Japan was dependent on the type of dried plasma used. Other recent reports of workers producing a product from animal blood suitable for human consumption include papers from Italy (Langhoff, 1980) Yugoslavia (Sabljak-Uglesic and Prilika, 1979), France (Bailly, 1982) and Hungary (Kormendy, 1984). The problems of blood collection from abattoirs has been discussed in papers by Bourgeois (1975), Poma (1981), Dehaumont (1982) and Fal and Illes (1984).

3.4 Powder Production

The processing of animal blood to form a stable dried product can be divided into the following stages: blood collection, separation of the plasma, concentration of the plasma and drying of the plasma.

3.4.1 Microbiology

It is necessary to maintain a high level of hygiene throughout the processing of the blood. Blood in its natural state is usually sterile. The incidence of aerobic bacteria contaminating the plasma is due to contamination during collection, processing and post processing procedures (Tybor, 1975). The standard for the final

product should be a total count of $<10^4$/g, a coliform count of <10/g, a count for yeasts and moulds of <10/g and no indication of salmonella (Davis and MacDonald, 1953).

The conditions of processing and storage of blood products from the microbiology of the product has been studied by Uchman, Challaz and Pezacki (1980); Walkowiak (1980) and Suzuki and Shimizu (1982).

3.4.2 Blood Collection

The plasma must be collected under sanitary conditions if the product is to be used as a food. The majority of animals are slaughtered by the use of the captive bolt. The blood is then drained from the neck. This is best done using a hollow knife system as described by Akers (1973). In some systems the anti-coagulant can be incorporated into the sticking knife. An anti-coagulant must be used if the fibrinogen and thrombin are not to start forming clots. Many materials are available and include EDTA, heparin, citrate and oxalates. The most common agent used is sodium citrate. Other methods to stop clot interference are defibrination, heat treatment, bubbling with ozone, treatment with an electrical current and resolubilising the clot (Bright, 1977).

Blood is normally stored in small tanks containing blood from a few animals, often five cattle or twenty pigs (Akers, 1973). If any of the carcases are rejected as unfit for human consumption then the blood recovered from the group of animals containing the rejected one must be discarded. Plaschke and Biewald (1969) found that cooling was necessary if the blood was not to be used soon after collection. Some work on the stabilisation of whole blood has been done, although this is normally only carried out when the product is to go for animal feed.

3.4.3 Separation of the Plasma

To obtain plasma the cellular constituents of the blood have to be removed. Care must be taken not to lyse the red blood cells during this process as the haemoglobin will form a continuous phase with the plasma and cannot be removed. The specific gravity of cells is 1.08-1.09 and for plasma 1.027-1.034 (Johnson, 1974) it is therefore not difficult to separate these two fractions by centrifugal force. Centrifugation can be carried out with plate type separators or with the newer form of continuous cylinder machines. The newer equipment can cater for 1,000-2,000 litres per hour and can be operated for a longer duration than the plate type separators before being opened up for cleaning (Halliday, 1973).

3.4.4 Concentration

The liquid plasma is too dilute for some purposes at a protein concentration of 8-9% solids. Brooks and Ratcliff (1959) noted that even egg white at 12% solids was not so useful for the sugar confectioner as dried egg.

A process used to concentrate the plasma is ultrafiltration (UF). In UF a high enough pressure is applied against the osmotic pressure, occurring across a membrane, so that molecules small enough to pass through the membrane go from the concentrated material to the dilute, thus causing further concentration. Ultrafiltration separates relatively high molecular weight solutes (molecular weights of 300 to 0.5 μm in diameter) from solvents (Michaels, 1968). The high molecular weight products do not exert high osmotic pressure and therefore low operating pressures can be used. Wong, Jelen and Chong (1984) state that UF is an attractive process for the preparation of blood protein concentrates because it is non-thermal and non-chemical. Also the concentration, fractionation and purification are carried out at the same time and the operational costs are relatively low.

The structural and mechanical properties of blood and plasma were investigated by Dobromirov (1979). The influence of these properties on the ultrafiltration of bovine plasma was studied by Eriksson and von Bockelmann (1975); Hurst (1980); Quaglia and Tasselli (1980) and Wong, Jelen and Chong (1984).

3.4.5 Drying the Plasma

To maximise the storage and stability of plasma it must be in a solid form. Processes have been developed to dry blood products without too much denaturing of the protein and include: drying the protein in a hot air stream (Maeda, Nishijima and Nishijima, 1975); drying between two heated surfaces (Craven, 1967); whole blood has been dried using air ring driers (Perry, 1967); a method to dry plasma was established by Regal Foods, it involved drying the plasma on a bed of spheres at temperatures not exceeding 50°C (Anon, 1974); fluidized bed drying for whole blood has been used (Haughey, 1971). Spray driers which have been widely used to dry whole blood, plasma and plasma fractions, do not extensively denature the protein.

Spray drying is a process where a pumpable fluid is fed into a flow of hot air. The resulting rapid evaporation maintains the low temperature of the spray droplets so that heat sensitive products can be dried without heat degradation (Masters and Vastergaard, 1978). There are four process stages: atomization, spray/air contact, evaporation and separation of dry product from air. Each stage is carried out according to dryer design and operation, and together with the physical and chemical properties of the feed determines the characteristics of the dried powder.

Plasma samples have also been obtained by freeze drying. Solidification of plasma has also been achieved by various freezing techniques. One method of processing is by freezing on a refrigerated rotating drum, resulting in flakes about 3 mm thick which can be packed into polythene bags and stored at -30°C (Halliday, 1973; Akers, 1973).

3.4.6 Further Processing

Further process developments for animal blood for human consumption have also been investigated. Young and Lawrie (1974) found that plasma could be spun to form a textured product. This product may have been acceptable as a meat analogue.

3.5 Uses of Plasma as a Food Ingredient

Papers on the uses of animal blood in meat products have been discussed by numerous workers (Inklaar and Fortuin, 1969; Frentz and Perron, 1971a, 1971b; Suter et al, 1976; Ranken, 1977; Hermannson, 1978; Terrell et al, 1979; Wismer-Pederson, 1979; Pinel and Dorval, 1980; Zharinov et al, 1981).

There have also been papers on the incorporation of blood products in bakery goods (Bates, Wu and Murphy, 1974; Khan, Rooney and Dill, 1979; Johnson et al, 1979). Earlier descriptions on the utilisation of animal blood are given in the report by Bright (1977) and the thesis of Howell (1981).

3.6 Economics of Blood Processing

At the moment the blood from slaughtered animals mostly goes to waste and the disposal is expensive. In many countries throughout the world the production of dried products from blood has proved environmentally satisfactory, a source of protein and a profitable exercise. If processing is to be profitable in the UK then the price the dried product could command is of prime importance. The ability to market the product as a functional material rather than for its nutritional qualities is essential.

Labelling legislation may also play an important role in the marketability and therefore the price a dried blood product can command. At the moment plasma can be counted as "permitted offal", (Food Standards Committee report on Offals in Meat Products, 1972). This will aid its incorporation into meat products. However, if

the product has to be declared on the labelling of non meat products there could be some consumer resistance to its use.

Processing of blood would be a better proposition if the whole of the blood could be utilised. It is the red cell fraction that causes the greatest of pollution. Halliday, (1973) reported that continental manufacturers would only sell dried plasma if the less demanded red cell fraction was also bought. The erythrocyte fraction may have to be decoloured before it is of much use to the food manufacturers.

The capital investment to set up and run a plant for the production of dried plasma could be repaid within three years according to the costings of Sakellariou and Norwood (1982). Other recent reports of processing plants for the production of dried plasma seem to be show economic viability (Petrovics, 1984).

CHAPTER 4

CHAPTER 4

OBJECTIVES OF RESEARCH

A valuable source of protein, blood, is being expensively disposed of via the sewage system. Products containing plasma are widely excepted in continental markets. However, for plasma to command a high value it must be produced to a known quality, must be easy to store, and exhibit properties that enhance food systems. The plasma therefore, has to be dried and still retain its functional characteristics.

Much work has been done drying plasma, but the studies have concentrated on the mechanisms and economics of the processing. The literature abounds in ways to define functionality in many protein products, however, there are no definitive tests and results can not be compared directly and often do not agree even in the broadest terms.

The object of this work was to study systematically the processing of plasma to establish if it can be made in a powder form, in reproducible quality for its use in food manufacture. The functional characteristics that the dried plasma could impart to food systems were also to be ascertained.

Blood from the abattoir, plasma after separation, concentrated plasma and the final powder need assessing to ensure their quality. The powder during production and storage should be evaluated in case of variation. Enough samples should be analysed to establish the deviation in product quality.

There are no standard tests for functional assessment. A closer investigation of some of the proposed functional assays would

be necessary to establish their suitability and reproducibility in assessing plasma powders. The functional properties to be studied in detail are foaming, emulsion formation, fat binding and gelling.

As functionality is difficult to define other protein powders need to be assessed under the same conditions so that direct comparisons can be made. The action of the assay procedure needs to be understood so that reasonable interpretation of the results is possible.

CHAPTER 5

CHAPTER 5

METHODS

5.1 Preparation of Bovine Plasma Powders (BPP)

The processing of fresh blood to a dried powder was normally carried out within one working day. This was to ensure that the BPP was as free as possible from contamination and products of degradation.

Blood Collection

Bovine blood was collected from the slaughter house of W. & J. Parker Limited, Leicester. Churns, 50 litre capacity, containing 3.75 Kg of sodium citrate dissolved in 500 ml distilled water were prepared for blood collection. As the cattle were hung and bled, the blood was allowed to flow into the churns. The anticoagulant at a final concentration of 7.5% w/v stopped blood clotting. The blood was taken to the laboratory as soon as possible after collection.

Blood Centrifugation

The blood was removed from the churns and filtered through a 1mm nylon mesh sieve to ensure removal of any extraneous material, or any clots which might have formed. The blood from all the churns were mixed together in a polypropylene container fitted with a lid. The container had an outlet at the base and was connected via a peristaltic pump to the top of the separator, an Alfa-Laval type BPB-204A-11 continuous disc centrifuge. Before commencing the work the apparatus was thoroughly cleaned and the discs of the centrifuge autoclaved. The maximum speed of the centrifuge was 8000 r.p.m. and had a capacity of 150 l/hr. When the plasma appeared to contain no haemoglobin it was collected into clean churns and the red cell fraction was discarded.

Ultrafiltration (UF)

Ultrafiltration of plasma was carried out on two systems. For the initial powders the Paterson-Candy International (P.C.I.) reverse osmosis-ultrafiltration unit using a tubular membrane was used, see Figure 7. The membranes used in this system were of cellulose acetate type T5/A and had a nominal 95% cut-off of 20,000 molecular weight. The stated maximum feed pressure was 10.0 $Kg.cm^{-2}$ and the maximum temperature was 50°C. The feed temperature used was 35°C and the feed rate was 13.5 l/min. Further information on running and cleaning the P.C.I. system is given in the thesis of Hurst (1980). The concentration took approximately 4 hours.

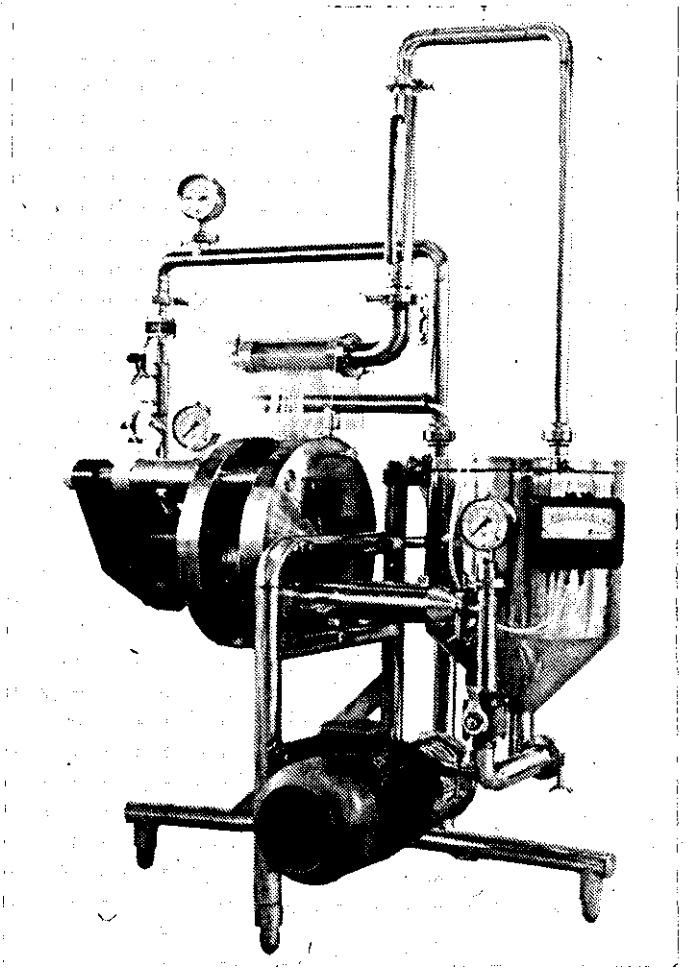
For the later plasma samples the ultrafiltration apparatus was the DDS RO-System, illustrated in Figure 7. The membranes were the laboratory unit UF 36-2-25, with a membrane area of 2.25 m^2. The temperature was maintained at 30°C. To concentrate 50l plasma at 10% solids to 20% solids took approximately 2 hours.

Spray Drying

The spray dryer used (manufactured by Spray Processes Ltd 86 Bunyan Road, Kempston, Beds) was model number 3-8 and is illustrated in Figure 8. The concentrated plasma was pumped into a pneumatic nozzle which atomized the product, in the drying chamber the hot air, temperature approximately 170-190°C, met, with the droplets and the drying occurred. The dried powder was separated from the out going air, at a temperature of approximately 70-80°C, in the cyclone. The dried product collected in a glass or stainless steel vessel.

The regulation of the temperature of input and output was normally done manually , but a computerised program was available so that the operating variables could be adjusted to maintain the temperatures at a given value. Detailed operation of the spray dryer, routine cleaning procedures and unblocking of the nozzle are detailed by Tan and Wallwork (1982).

Figure 7 - Ultrafiltration Equipment



DDS

RO System

PCI System

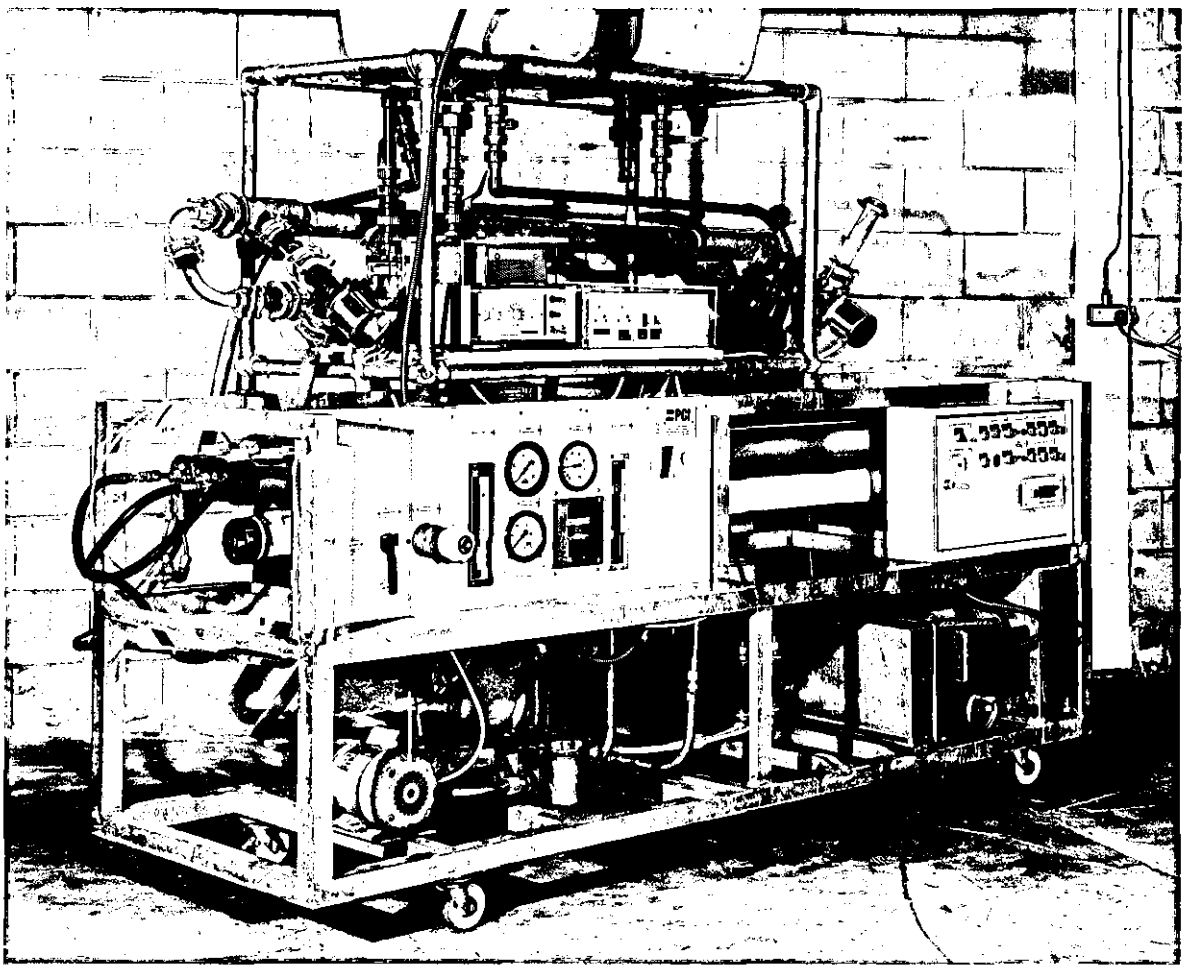
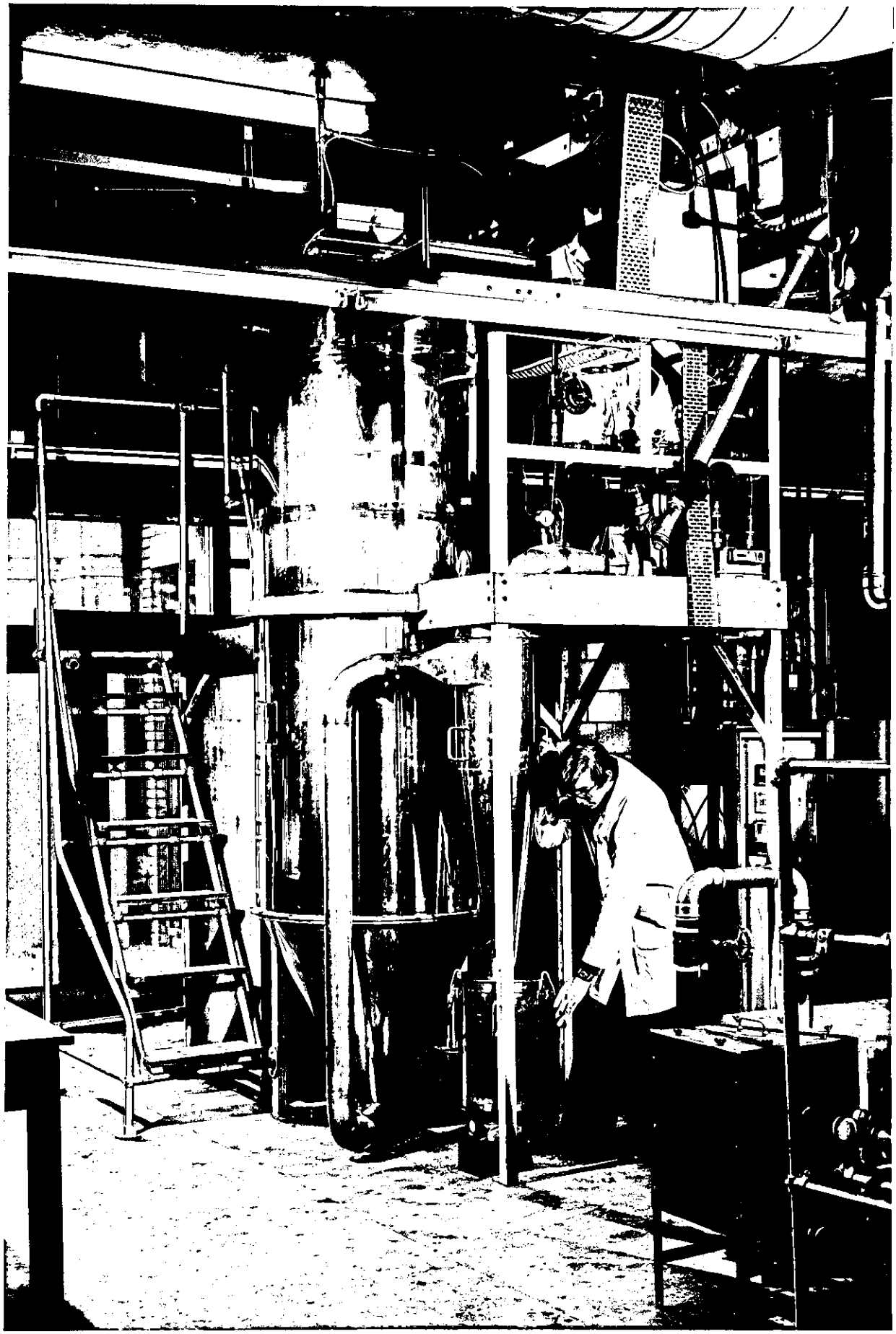


Figure 8 - Spray Dryer



Storage

The product was stored in glass or plastic air tight containers. Storage was at room temperature in the dark.

5.2 Other Protein Powders

Other Plasma or Sera powders produced under the conditions used at Loughborough University of Technology (LUT)

During this study other products have been made, using similar procedures to that described for the production of the plasma powders. The products were:

BP/U.....Bovine plasma frozen at another laboratory and then brought to LUT. The thawed plasma was concentrated using the DDS ultrafiltration system.

BS.....Bovine sera used for powder production, using DDS UF.

BP/M.....Bovine plasma was processed by the Milk Marketing Board under the conditions normally used at LUT.

bP/O.....Blood taken from young cattle fed on barley. The plasma was separated and frozen in another Laboratory before being further processed at LUT using the DDS UF.

PP/O.....Porcine plasma, separated and frozen in another laboratory. Brought to LUT for further processing under normal conditions, using the DDS UF.

PP/F8....Porcine plasma, separated and the blood clotting factor No. VIII was removed by Speywood Laboratories. The plasma was frozen and removed for further processing at LUT, using DDS UF.

Protein Powders associated with blood

RBC.....Spray dried red blood cells. The RBC obtained after the separation of plasma for powder No. 13.

BCHy....Blood cell hydrolysate, obtained from Novo Industries Ltd. 2b Thames Avenue, Windsor, Berks.

AlbFV...Bovine albumin fraction V, by Cohn fractionation. Obtained from Sigma, No. A-4503 lot No. 97C-0184.

AlbFib..Plasma obtained by drying on a bed of spheres produced by Fibrisol Service Limited, Colville Road London W3 8TE.

Proteins obtained from animal waste products

Lcoll..Lencoll, Code 5240-beef RefKL/142, produced from collagen by Lensfield Products Ltd., Maulden Road, Flitwick, Bedford

Lsol...Lensol, Code 4230-beef RefKL/3, produced from a spray dried beef marrow bone stock by Lensfield Products.

Alo....Alomine-042, a spray dried isolate of meat proteins. Produced by Vaessen-Schoemaker, Deventer, Holland.

Alo/P..A 50/50 mix of plasma powder No.8 and the alomine powder (Alo)

Egg products

EggP...Egg albumen powder, obtained from Fisons Scientific Apparatus, Loughborough, Leics. Code A/1280, batch No. 52.

EggF...Egg albumen in a flaked form, obtained from BDH Chemicals Ltd Poole, Dorset. Product No. 33007, batch 611977u.

EggLi..Liquid egg white obtained by the separation of whole fresh eggs. The eggs used were Golden Lay, size 3.

Dairy products

Cas....Casein, soluble light white, obtained from BDH Product No. 44016, batch 251 4780.

Whey...Whey powder, Ref. 1009, obtained from Dairy Crest Creameries, Milk Marketing Board, Mellor , Marchwil, Wrexham, Clwyd

Lact....Lactein 75, obtained from Dairy Crest Whey Division, Milk Marketing Board, Thames Ditton, Surrey.

Soya products

SoyI....Soya Isolate, Ref FP 900, obtained from Lucas Food Ingredients, Bristol, Somerset.

SoyF....Soyolk, a fully cooked soya flour from Soya Foods Ltd Cambridge Road, Barking, Essex.

Other protein isolates

Hap.....A powder obtained from hydrolysed fish, haplochromis. The sample used was UC55 15.12.83, see Ssali (1984)

5.3 Other materials

The oil used for this study was Mazola corn oil from CPK (UK) Ltd. Claygate House, Esher, Surrey. Analysis of the fatty acid content of the oil indicated 57% linoleic, 30% oleic, 2% stearic acid and 11% palmitic.

Chemicals used were of analytical grade unless stated otherwise.

5.4 Analytical Methods

For the assessment of a product during processing and to evaluate its potential use as food ingredient many assays are required. The parameters assessed in this study include the estimation of:-

Haemoglobin....Colour determination in ammonia hydroxide. Evelyn, (1936).
Bilirubin.....Based on method of Jendrassik, (1936).
Boehringer kit Cat. No. 123919.

pH.....Using a Pye Unicam PW 9418 pH meter.

Total protein, Kjeldahl estimation, Pearson (1976).

Protein...Bromocresol green assay, Doumas and Biggs (1972).

Moisture..Drying to constant weight at 85°.

Total lipids. Colour determination with vanillin, using Boehringer kit Cat. No. 124303.

Calcium...Colour determination with o-cresolphthalein complexone, using Boehringer kit Cat. No. 204382.

Salt.....Using Mohr's method with silver nitrate, Bulcher and Nutten, (1960).

Ash.....Heating at 550°C until constant weight, Lees, (1975).

Bulk Density. A tap bulk density, using a cylinder filled with powder, lifted to a height of 3 cm and then allowed to drop, thus causing compaction of the powder. Readings were taken at constant volume.

Specific Gravity. The mass of liquid at a known volume was recorded and the value divided by the weight of water at that volume. Determinations were carried out at 20°C.

Particle Size. Estimation using the Malvern 2200/3300 Particle sizer, lens size normally 100 mm. The standard procedure as used by the Particle Sizing Laboratory at LUT was followed. In the calculation of the data three values (L PS, S PS, and D 50) were recorded.

L PS = largest particle size, 95% by weight of powder having a smaller particle size.

S PS = smallest particle size, 5% by weight of powder having a smaller particle size.

D 50 = average particle size, 50% by weight of powder having a smaller particle size.

Microbiology. Total viable count (TVC), pour plate technique, using dilutions in peptone and agar No. 3 with Lab lemco powder, incubation at 30°C for 3 days.

5.5 Functional Methods

As stated throughout this study one of the greatest difficulties in assessing functionality is the lack of authorized methods. Small alterations in the apparatus or method of assessment can make significant differences to the values obtained, thus making interpretation of results difficult. It is for this reason that the methodology used to assess functionality for this study is given in some detail and procedures have been followed exactly unless stated otherwise. Some of the effects caused by variations to the method are discussed in Chapter 7.

Procedure for making plasma solutions

The correct amount of powder was weighed out and a volume, approximately 75% of the amount of solution required, of distilled water was added. After an initial mix with a spatula the mixture was stirred with a magnetic bar at a rate to cause agitation, but not to form froth. A spatula was used to break up any agglomerates every 20 minutes and complete solution was normally completed after 1 hour. The solution was washed into a measuring cylinder and made up to the mark with distilled water.

Procedure for the estimation of Insoluble Material

APPARATUS

Plastic Universal containers
Centrifuge, B & T MKIV with swing out head of radius 14 cm

METHOD

1. Prepare a 10% (W/V) solution of the product and place a 20 ml aliquot into the Universal, centrifuge at 3000 rpm (1411g) for 5 minutes.
2. Pour off the supernatant and resuspend the pellet in 20 ml distilled water. Respin the sample and repeat the washing procedure.
3. Wash the pellet into a dish and dry to constant weight (see procedure for moisture).

CALCULATION

20 ml of solution produced a pellet of weight P
The % of insoluble product = 50 * P (%)

Procedure for the estimation of Colour of Powder and Solution

APPARATUS

Plastic Universal containers
Colour Chart: Carsons colours (1983) from Carson Hadfields 131 Western Road Mitcham Surrey CR4 3YQ

METHOD

POWDER

A comparison was made between the powder and the colours on the Carsons colour chart. To get the best match the powder was placed on top of the colours which look most suitable for a final decision to be made.

SOLUTIONS

A 10% (W/V) solution of the powder was prepared. An aliquot was put into a plastic Universal bottle that had a clean white label. The solution was then viewed with the label furthest away from the eye. The Universal was held against the chart for a decision on the closest colour match.

CALCULATION

To try and give numerical values to the colours a panel of staff were asked to rank the colours into order. They were asked to place, at position, 1, the colour which was "blandest, lightest and least likely to effect an overall colour of a wide range of food products". The darkest colours, therefore, having the highest values.

Procedure for the estimation of Ability to Disperse

APPARATUS

Orbital shaker, conical flasks 250 ml, centrifuge, drying oven.

METHOD

1. A 100 ml aliquot of water was placed in each conical flask. A 10.00 g sample of the powder to be tested was weighed out into a weighing boat.

2. The powder was gently tapped from the boat onto the surface of the water in the conical flask. The remaining powder, adhering to the boat, was scraped into the flask.

3. Taking care not to agitate the liquid, the flask was placed into the orbital shaker.

4. The flasks were shaken at a speed of 200 rpm, at a temperature of 30°C. After 1 hour a 25 ml aliquot was removed from the flask and centrifuged at 1411g for 5min

5. A 20 ml sample of the supernatant was dried to constant weight.

CALCULATION

Dissolved solids = weight of dried sample * 50%

Procedure for the estimation of Water Absorbance

APPARATUS

10 cm diameter pyrex dishes, chromatography tank with shelf

METHOD

1. Prepare sulphuric acid at a density of 1.20, this will give a relative humidity of 80.5 %

2. Estimation of Water Absorbance

Add approximately 10g of powder to pyrex dish and place dishes into tank and seal lid. After five days remove dish and weigh, return to tank. Repeat weighing daily until constant weights have been achieved.

CALCULATION

Weight of dish	= D
Weight of dish and powder	= D + P
Weight of dish and "wet" powder	= D + (P+W)

Water Absorbance = W/P * 100 ml of water/100 g powder

Procedure for the estimation of Viscosity

APPARATUS

Ostwald viscometer, size C
10% solution, of known Specific Gravity, of test material

METHOD

A 10% solution of the material under test was prepared and used in the viscometer. The viscosity was measured at 20°C.

CALCULATION

Viscosity cp = (Specific gravity * flow time solution)/
(Specific gravity * flow time water)

Procedure for the estimation of Fat Absorbance

APPARATUS

100 ml beakers, internal diameter 49mm
Silica glass test tubes, internal diameter 14mm
Centrifuge B & T mk IV with swing out head of radius 14mm

METHOD

1. The capacity of the test tubes was estimated so that the height in mm could be calculated as a volume.

2. The specific gravity of the corn oil and of the homogenous corn oil/powder mixture was established.

3. Corn oil (20 ml) was added to 2g of powder.

4. The powder and oil were mixed together using a Silverson homogenizer or by mixing with a glass rod.

5. Immediately after homogenizing aliquots of the mixture were removed using a 10 ml glass pipette. The mixture was blown out from the pipette into a glass test tube.

6. The test tubes were then left for a period of between 10 to 20 minutes before they were centrifuged at 1411 g for 5 minutes.

7. The height in mm of each layer was noted. The heights were estimated at four positions around the tube and the values averaged.

CALCULATION

1. By calculating the amount of bound oil in ml and the actual weight of powder in the tube, the amount of oil absorbed by 1 g of powder was estimated. Also, the % of the total volume each layer represented.

2. The fat absorbance was given in some cases as a %. The % was calculated by multiplying the values of ml oil/g sample by 100 * specific gravity of the oil.

Procedure for the estimation of an Emulsion

APPARATUS

Silverson Mixer model L2R serial no. L2R 4297, with 3/4 inch disintegrating head.
250 ml plastic beakers

METHOD

1. A 2% (w/v) solution of bovine plasma was prepared and the specific gravity of the solution was noted.

2. To a 25 ml aliquot of the plasma solution was added 80 ml of corn oil. The addition was achieved by adding the correct weight of oil.

3. The oil and protein solution were mixed together for two minutes using the Silverson at half maximum speed. The mixing probe of the Silverson had to be moved about within the emulsion to ensure homogeneity.

Procedure for the estimation of Emulsion Capacity

APPARATUS

LKB varioperpex peristaltic pump
100 ml plastic beaker

METHOD

1. An emulsion was formed by the method described in the standard procedure for the formation of an emulsion.

2. A 25 g sample of the emulsion was placed in a 100 ml plastic beaker.

3. The Peristaltic pump was set up to deliver oil at the mixing head of the Silverson at a rate of 5 ml per minute.

4. All tubing to deliver the oil to the emulsion was filled with oil. The container, full of oil, which was to be used as the pump feed was weighed.

5. The emulsion was mixed using the Silverson at half maximum speed for half a minute then the pump was started.

6. Moving the mixing probe within the emulsion a close visual examination of the emulsion was made.

7. When the nature of the emulsion changed, as marked by decreased homogeneity and loss of viscosity the pump was stopped.

8. The oil feed container was reweighed and therefore the weight of oil required to break the 25g of emulsion could be calculated.

9. The results were extrapolated to calculate the weight of oil required to break the emulsion formed by 1 g of powder under the above conditions.

Procedure for the estimation of Oil Phase Volume

METHOD

1. The emulsion capacity as estimated in the standard procedure was found.

2. The specific gravity of the oil was noted.

3. The amount of protein and water in each emulsion at the break point was noted.

4. The oil phase volume was calculated from the ratio of oil at the point where the emulsion breaks compared to the total volume of emulsion.

CALCULATION

If the standard procedure was followed the calculation was :--

$$\text{Oil Phase Volume} = (EC/0.916)/(50 + (EC/0.916))$$

where 0.916 = SG of oil

50 = water + powder volume

Procedure for the estimation of Emulsion Stability

APPARATUS

Water bath TECAM TE tempette
Glass Universal bottles (internal diameter 21 mm) with metal screw caps with rubber liners.
Centrifuge, B & T MK IV with swing out head of radius 14cm.

METHOD

1. An emulsion was formed by the standard procedure for the formation of an emulsion.

2. Two separate 10g aliquots of the emulsion were put into glass universals.

3. The universals, well capped, were placed into a water bath at 80°C for one hour.

4. The universals were allowed to stand at ambient temperature. One sample was left for 24 hours, the other for 14 days.

5. At the end of the standing period the universals were centrifuged at 1411 g for 15 minutes.

6. The weight of the free oil which could be removed was noted.

7. The weight of oil released divided by the original weight of oil in the sample was calculated and the results expressed as a % oil released from the emulsion.

Procedure for the estimation of Emulsion Viscosity

APPARATUS

Haake Rotovisco, RV2 with NV measuring system with the following sensor factors.

	500 DMK	50 DMK
A (dyne/cm^2 scale grad)	16.2	1.67
G (cp/scale grad. min)	299	30.9

METHOD

1. An emulsion was formed by the method described in the standard procedure.

2. A portion of the resulting emulsion was used to fill the measuring head of the viscometer. All readings were taken at ambient temperature.

3. The torsion spring was set at 50 or 500 (normally the 500 setting was used).

4. The scale readings were noted for a full range of rpm that gave readings of >29 and less than 100.

5. The slope of the resulting graph of rpm versus scale readings (shear stress) was used to calculate the viscosity.

$$\text{viscosity cp} = \frac{G \times \text{shear stress}}{\text{rpm}}$$

Procedure for the estimation of Emulsion Turbidity and Emulsion Activity Index (EAI), R (an indication of globular size) and N (an indication of number of globules)

APPARATUS

Pye Unicam SP600 spectrophotometer
Kartell 1939 Metacrylate cells (UV), Hot air oven

METHOD

1. The standard procedure was followed to produce an emulsion.

2. A 0.1% (w/v) solution of sodium dodecyl sulphate (SDS) was prepared in distilled water.

3. 1.00 g of emulsion was weighed into a 100 ml volumetric flask and made up to volume with the 0.1% SDS.

4. One ml of the thoroughly mixed 1/100 dilution was added to 4 ml of 0.1% SDS and mixed to form a dilution of 1/500.

5. The absorbance of the 1/500 diluted emulsion was then determined in a 1 cm pathlength cuvette at a wavelength of 500 nm.

6. If the absorbance reading exceeded 0.8 then the sample was diluted further and the dilution factor allowed for in the calculations.

7. The turbidity of the sample =

$$T = 2.303 * \text{Absorbance/pathlength of cuvette}$$

EMULSION ACTIVITIY INDEX

8. The oil volume (OIL.V) was calculated by drying 5 g of emulsion to constant weight at 105°C.

9. Parameters required:

D_o density of oil

D_m density of protein solution

E concentration of solutes; mass per unit mass solvent

C Weight of protein per unit volume of aqueous phase.

W_l loss of weight of emulsion on heating/weight of emulsion

W_d dried weight/weight emulsion

10. Calculation of the oil volume fraction (OIL.V)

$$OIL.V \qquad \frac{W_d - E * (W_l)}{W_d+(W_l)(((1+E)D_o)/D_m) - E)}$$

for normal values $OIL.V = \frac{W_d - 0.0204\ (W_l)}{W_d+(W_l) * 0.9143}$

11. Calculate the EAI

$$EAI = \frac{2 * \text{Turbidity}}{OIL.V. * C}$$

EAI had units of area of interface stabilized per unit weight of protein.

12. Calculation of R

$$R = 3\ OIL.V/2T$$

R is the volume/area mean radius of the dispersed particles.

13. Calculation of N

$$N = 2T^3/(9 * 22/7 * (OIL.V)^2)$$

N is the equivalent number density of particles (the hypothetical number density of particles, all of radius R which have the same T as that observed for the polydisperse system)

REFERENCES

Pearce and Kinsella (1978).

Procedure for the estimation of Foaming Properties

APPARATUS

100 ml glass beaker ID 47.8 mm
100 ml plastic measuring cylinders ID 27.9 mm
Silverson mixer Model L2R Serial No. L2R 4297 with 3/4 inch disintegrating head.

METHOD

1. A 2% (w/v) solution of bovine plasma was prepared.

2. A 50 ml aliquot of solution was placed in a glass beaker. A stopwatch was started and the solution was mixed using the Silverson for 2 minutes. The mixing probe was moved within the solution and foam to allow air to be freely incorporated within the system.

3. As soon as mixing had ceased the foam and solution was carefully transferred into a plastic measuring cylinder.

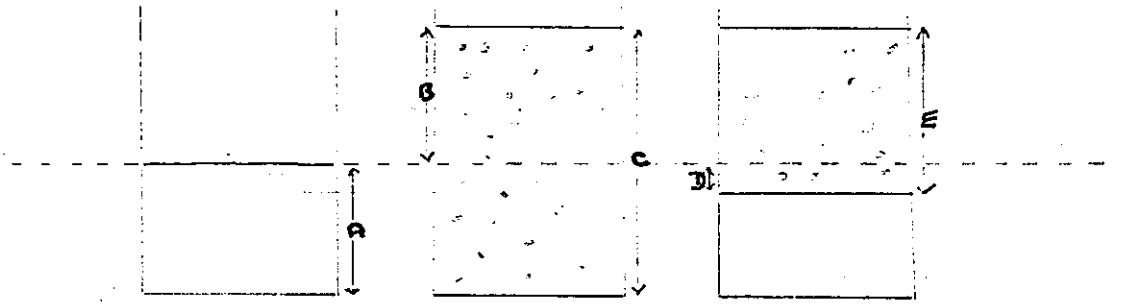
4. At a time reading of 5 minutes, i.e. 5 minutes from the start of mixing and 3 minutes since the end of agitation, the total volume in the measuring cylinder was noted (C) and the volume of foam (E).

5. Readings of foam volume were taken at 25 minutes and 60 minutes after start of mixing.

6. The estimation given in the table could then be made.

7. Often just the foam volumes and the % foam left after 55 minutes were used in evaluating data. The first figure being the indication of volume the second showing stability.

ESTIMATIONS MADE FOR FOAM PARAMETERS



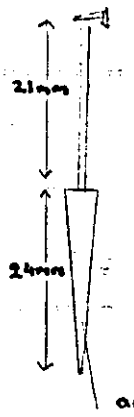
COLUMN NAME	DESCRIPTION	MEASUREMENT CALCULATION
L.VOL.A	Volume of starting liquid (50 ml in standard procedure)	A
F.VOL.E	Volume of foam after 5 minutes	E
25.FVOL	Volume of foam after 25 minutes	E
60.FVOL	Volume of foam after 60 minutes	E
T.VOL.C	Total volume of dispersion at 5 min	C
GAS.B	Volume of gas incorporated into the dispersion	C-A
L.FOAM.D	Volume of liquid in foam	E-B
OVERRUN	The ratio of gas incorporated with the total volume	(B/A)*100%
FOAM.P.V.	Foam phase volume	B/E
GAS.P.V.	Gas emulsion phase volme	B/C
FOAM.VOL	Foam volume or foam expansion	(E/A)*100%
F25/5	% foam left after 20 minutes	$\frac{25.FVOL*100}{F.VOL.E.}$
F60/5	% foam left after 55 minutes	$\frac{60.FVOL*100}{F.VOL.E}$
F 25/60	% Foam left after the last 35 minutes	$\frac{F25/5*100}{F60/5}$

Procedure for the estimation of Gelation

APPARATUS

Glass universal bottles with internal diameter 21mm, with metal screw caps with rubber liners.
Water bath, Tecam TE Templette
A Seta penetrometer by Stanhope Seta Limited, Surrey, England
An aluminium cone (weight 4.19 g) made at LUT

Cone used with the Seta Penetrometer



The cone was manufactured in workshops of LUT

wt. of plunger 47.50 g
wt. of cone 4.19 g

METHOD

GEL FORMATION

1. The dried plasma was dissolved in distilled water to achieve the appropriate concentration. Normally concentrations of 10% and 9% w/v would be used.

2. 20 ml of the plasma solution was poured into glass universal bottles.

3. The bottles were firmly capped.

4. The rack of bottles was put into the water bath preheated to the correct temperature. For the standard estimations this was 75 and 80°C.

5. The level of water in the bath must be sufficient to cover the plasma solutions.

6. After 30 minutes the racks were removed from the bath and left at room temperature for 18-26 hours before measurement.

GEL MEASUREMENT

1. The gels were visually examined to ascertain the opacity and if they were solid enough for the bottle to be inverted without movement of the gel.

2. The aluminium cone was fitted to the penetrometer.

3. The bottle containing the gel was placed on the penetrometer so that the cone and plunger could enter the gel without fouling the bottle.

4. The height of the cone was adjusted so that it had just penetrated the gel. The cone and plunger were released for 8 seconds. The depth, in tenths of mm, the cone had fallen was recorded. If the cone reached the bottom of the gel the reading was recorded as 400.

5. If no gel was formed the arbitrary figure to 600 was assigned to the product. If movement occurred on inversion, the figure 500 was used to define it.

5.6 Statistics

Some statistics for this study have been calculated using Minitab on the Multics computing system. Details for the methods of estimation are given in Ryan, Joiner and Ryan (1981). The term coefficient of variation or relative standard deviation is used to compare the precision of results over a wide range of values. F distributions, as defined by Alder and Roessler (1972), were used to establish variance. The variance of two samples are said to be homogenous if the populations from which they are taken have identical variances.

Mean = arithmetic mean
S.D. = standard deviation
T = Student's T-test value
P = probability
Corr = Correlation coefficient (Pearson product moment)
CV = coefficient of variance = S.D. * 100/Mean %
F = F value = $S.D._a^2/S.D._b^2$

CHAPTER 6

CHAPTER 6

PROCESS EVALUATION

Introduction

To fully utilize plasma as a food ingredient it should be processed to form a dry powder of uniform and predictable quality In this chapter the analysis that took place to establish the quality of powder produced for this study from bovine plasma is discussed.

It was noted early in this project that the microbial content needed controlling if the product produced was to be suitable for human consumption. Results obtained during production of powder and on studies designed to look more closely at the microbial load are summarized in the initial section.

Basic analysis of the constituents of the raw product, plasma, separated from the red blood cells by two different methods, and plasma after concentration was carried out. Methods for assessment of functional properties were developed and their reproducibility using bovine plasma powder established. Ten powders were examined for proximate composition and performance in the functional tests. The variance between functional assay technique and variance when that technique was applied to the ten different powders was also established.

Functional and composition analysis were carried out during spray drying and on storage of the powder for one year to ascertain its uniformity and deterioration. As functional tests are not standardized the assessment of other protein products as indicators of performance is advisable. Results obtained from 23 protein products are discussed. Correlation values between assays done

on BPP and on assays for the other protein products as well as BPP were established.

Many authors advise that solubility is the most important feature when processing a functional protein therefore, a more detailed examination of this property was undertaken and is described in section 6.1.0.

The final section describes the short investigation of the structure of the protein after it had been dried.

6.1 MICROBIAL CONTENT - Appendix 6.1

The amount of microbial contamination of fresh plasma was high, the average count of 163×10^3 being 30 times higher than that acceptable for raw milk. Separation of the plasma from red blood cells (RBC) did not alter the level of contamination. Total viable counts for red blood cell fractions were occasionally very high, but there was no correlation between RBC counts and those obtained from plasma.

Handling the plasma up to the UF stage did not increase the count, but after UF the counts had risen and values between 10^6 to 10^{10} per ml were obtained. If overnight storage occurred the counts again rose by a further 10^2. The process of spray drying did reduce the viable count to about the level obtained before storage, but the microbial load in the powders was far too high. Such powder products need a TVC of less than 10^5 per gram to be suitable as a human food product. The average count obtained for the powders in this study was 20×10^5 per gram. As more powders were produced the amount of contamination increased, until it was considered that after powder No. 11, with a count of 7×10^6, the count must be reduced if meaningful results on the powder were to be obtained.

To investigate the number of bacteria during UF, samples were removed at 30 minute intervals over the four hours of processing. The number of bacteria would be expected to increase as water is removed from the plasma. However, at the end of the run the number of contaminants rose sharply even compared with the protein concentration. To try and reduce the contamination the P.C.I. UF system was dismantled and cleaned very carefully. The difficulties in producing a hygienic environment became clear. The machine had been adapted over a period of time with no consideration for the microbial effects. After the UF apparatus had been cleaned as well as possible powder No. 12 was produced and still the count was exceedingly high, 3×10^6 per g powder. A further powder was prepared as the disturbance of the P.C.I. UF system may have caused the high counts. The counts for powder No 13 produced one week after No. 12 was 4×10^5. At this point all the powders were investigated for contamination by coliforms, staphylococci, salmonella and fungi. Of the nine powders examined 3 indicated coliform contamination, 8 staphylococci and 4 fungi. No salmonella was found in the powders, but there was evidence of contamination in the plasma and concentrated plasma.

The laboratory acquired a new ultrafiltration unit, the DDS system which would concentrate the plasma more rapidly than the P.C.I. equipment. Two samples, plasma BP/U and sera BS, were obtained for processing and were concentrated using the DDS. The microbial counts for the plasma and sera powder were 2×10^4 and 3×10^4 respectively, a marked improvement on the values obtained before. As the studies for the functional and other parameters on plasma powder BP/U did not seem different from results obtained from powders produced when using the P.C.I. it was decided to use the new DDS apparatus for routine production. Plasma was collected once again from Parker's of Leicestershire and a powder produced using the DDS equipment. The powder so produced, No. 16 had counts of less than 10^5 and it was this powder that was considered suitable for the stability trials.

Blood used for this study is not collected under ideal conditions and the production area and apparatus is not dedicated to the production of food stuffs for human consumption. However, with care and ensuring that the processing of the plasma takes less than 14 hours a product with a viable count of less than 10^5 per g can be produced.

6.2 ANALYSIS OF PLASMA

Plasma on arrival

Whole blood arrived in the laboratory in churns. On ten occasions aliquots of blood were taken from the churns for assessment. The whole blood was spun in a B & T MKIV centrifuge, at 1400 g for 5 minutes. The plasma was then assayed for haemoglobin content, bilirubin, pH, total protein and albumin, total solids, lipids and number of bacteria.

Plasma after separation

Plasma samples were collected from the Alfa-Laval separator. Twelve samples were assayed for the same parameters as for the individual churns. In some cases the salt content was also assessed.

Plasma after concentration

Similar assays were carried out on concentrated plasma after the ultra filtration process.

6.2.1 Discussion - Appendix 6.2.1 and Figure 9

Plasma colour

The amount of haemoglobin and bilirubin were estimated in the plasma. Excess of these tetrapyrroles, when processed, produced a dark coloured powder. In all cases the bilirubin levels were less than 0.8% (levels above this value are considered abnormal for bovine samples (BCL, 1983)). The haemoglobin levels were all low, but levels above 1% in the original plasma must be considered too high for the production of a satisfactory powder. No work has been done to establish the exact levels of haemoglobin contamination which will effect the other organoleptic qualities of the powder.

A marked orange colouration was apparent in the bovine plasma on occasions. The colouration was found to be due to β-carotene, isolated and measured according to Machlis and Torrey (1956).

Solids and Proteins

The plasma obtained had a solids content of 8.5% and the protein was 7.8%. During ultrafiltration the solids content was increased to 16.7% with the corresponding increase in protein content. The albumin to globulin ratio was 1:1.04. The albumin in the concentrated plasma could not be estimated by the normal procedure as the viscosity of the plasma was too great. However, no protein was ever detected in the filtrate indicating there was no leakage of the protein through the membrane during ultrafiltration.

Lipids

The average pooled plasma had a lipid level of 339 mg/100 ml. The lipid level in any of the individual plasma samples did not exceed 700 mg/100 ml. These levels are below that in egg albumen samples.

pH

The pH of the plasma was approximately 7.8, there maybe a slight rise in pH during the separation stage. The pH of the concentrated plasma was again in the region of 7.8. The pH of the filtrate was also established and was found to be the same as for the concentrated plasma.

Salt content

The amount of salt was not estimated in all the samples, but plasma had about 0.6 g/100 ml. The same concentration was found in the concentrated plasma, the rest of the salt being lost with the filtrate.

Figure 9

Mean and Standard Deviation for Assays on Plasma before the Separator, after the Separator and at Concentration

	Individual Churns	Plasma after Separator	Concentrat Plasma
No. of samples assayed	10	13	10
Haemoglobin g/100 ml	0.24 ± 0.205	0.16 ± 0.192	0.39 ± 0.5
Bilirubin g/100 ml	0.2 ± 0.07	0.5 ± 0.19	0.7 ± 0.41
Microbial Count 10^3/ml	209 ± 434	110 ± 190	219639 ± 5×10^5
pH	7.6 ± 0.1	7.8 ± 0.34	7.7 ± 0.64
Protein g/100 ml	8.2 ± 0.33	6.9 ± 1.59	16.8 ± 2.5
Albumin g/100 ml	3.8 ± 0.34	3.9 ± 1.35	-
Total Solids %	8.6 ± 0.25	8.5 ± 0.49	16.7 ± 3.0
Lipids mg/100 ml	523 ± 724	339 ± 130	807 ± 240

6.3 METHOD REPRODUCIBILITY FOR POWDERS - Figure 12 page 90

Generally assays and functional tests used for the assessment of BPP were adapted and designed for this purpose. Their accuracy was not determined by cross reference with other methods nor their precision estimated using other powder products. The technical deviation for the estimations on BPP were determined by assaying a well mixed combination of plasma powders at least 7 times on different days using the same procedure. The relative standard deviation of the method was then estimated, results are given in Figure 12. The values for the analytical tests are all below 15% except for the calcium assay at 16%. High CV for total viable counts are common due to the dilution factors involved. It must be remembered that the assays are on powder and some accuracy is lost in the preparation of a solution and/or sampling the heterogeneous material.

The CV for the functional tests are below 15% for most of the assays. There are high variances for foam values as the foam collapses. Difficulties in measuring the height of an aged column of foam occur as collapse is often ragged with large gaps occurring in the foam column. Emulsion Stability also shows great variance, but this is due to the low amounts of oil being freed from the emulsion. The estimations are given in percentage oil released, these values are small and not accurately determined, thus causing high standard deviation. The CV could be reduced if oil retained was quoted instead of that released.

Further studies with BPP in the functional tests of fat absorbance, emulsion formation, foaming and gelation are given in Chapter 7.

6.4 ASSAYS ON BOVINE PLASMA POWDERS

Ten powders were made from bovine plasma during this study. The processing conditions were similar for all the products except that the DDS ultrafiltration system was used, instead of the P.C.I. machine, for powder No. 16.

6.4.1 Discussion - Appendices 6.4.1 and Figures 10 & 12

Powder properties and organoleptic quality

The bovine plasma produced at LUT was a light cream colour, see Figure 10. The powder was not very free flowing, but did not aggregate. Initially the powder had a slight metallic odour, but this quickly disappeared leaving only the slightest smell. Odour would be apparent again if the powder was heated. The flavour of the plasma was not very discernable and not at all unpleasant.

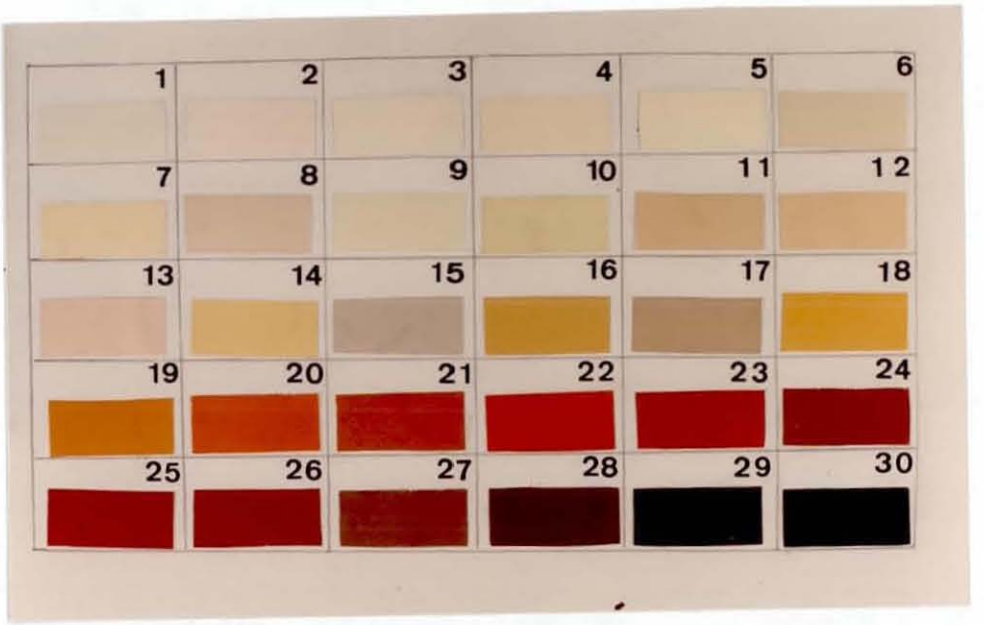
The particle size of powder is considered to be an important feature for its practical use and for its action in some of the functional tests. Work of Cooper (1982) showed that the method of preparation of dried products effected mouthfeel of biscuits. Bulk density of a powder is an important factor for storage and handling properties.

As mentioned for plasma, colour was thought to be related to the amount of haemoglobin or bilirubin. The level of β-carotene was also found to be an important feature. High colour of powder No. 16 was due to the haemoglobin, but the characteristic bright yellow of powders No. 8, 9, 10 and 11 was due to the carotene. These products were produced in July and early August, and reflect the fodder given to the cattle before slaughter. The coloration did fade on standing.

Figure 10.

Colour chart and powder

Chart used for colour coding of powders and solutions



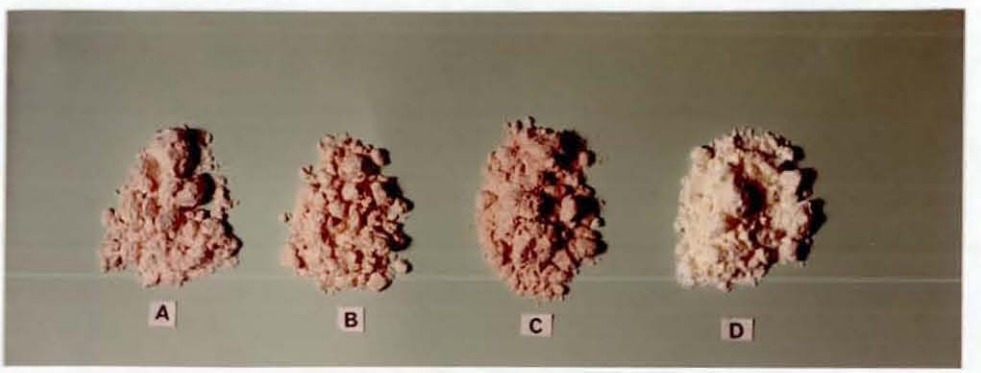
Examples of powders

A BPP No. 1

B BPP No.11

C Porcine powder PP/O

D Egg powder, EggP



Brooks and Ratcliffe (1959) found it impossible to obtain large quantities of plasma that were not turbid and reddish in colour. This haemoglobin contamination must have caused discoloration of the spray dried product. Other workers do not mention the colour of the product they produced. The powder of Brooks and Ratcliffe (1959) was also found to produce a fishy flavour after storage. Masters and Vestergaard (1978) reported that there was no odour problem with spray dried blood as long as it had not deteriorated biologically before drying.

There are many reports of the organoleptic quality of products made incorporating plasma. The acceptability or enhanced flavour when using plasma, either as a fresh liquid or as a dried product, in comminuted meat products is well established (see Bright, 1977). The flavour of confectionery products with plasma replacing a portion of the egg white would appear to be dependent on the product and the source of the plasma. Khan, Rooney and Dill (1979) found that 30% plasma 70% egg could be used to produce Angel cake of acceptable if not superior flavour and odour. Bates, Wu and Murphy (1974) have reported satisfactory flavour and odour in breads containing plasma.

Proximate Composition of BPP

The powders were all dried until no more than 8% of the wet weight was due to moisture. The average solids content for the powders was 94.5% with no powder laying outside the $\pm$ 2SD range. Amount of protein in the sample was estimated at between 75.7 and 81.5% with the average figure being 79.5% and again none lay outside the standard range. Amino acid analysis for the powders is given in Appendix 6.4.1b. Lipid content of the samples was in the range 3.52 to 9.23% and these values are those that could be expected from bovine plasma at this concentration (BCL, 1983). Salt content of the plasma could be very important in the action of some of the functional tests. The total salt content can be high if UF is not used to concentrate the plasma before drying and a salt preparation has been used as the anticoagulant. The percentage salt in BPP

ranged from 4.9 to 2.6%. The amount of calcium associated with a functional protein can be critical, e.g. in gelation. With the sequestration action of the anticoagulant, sodium citrate, the amount of calcium in the powder could be different to that normally occurring in the plasma. Therefore, the amount of this ion was estimated in the powders. The levels quoted for normal bovine plasma are 8-11 mg/100 ml plasma (BCL, 1983) and the range of results obtained for the powders was 55-178 mg/100 g powder.

Microbiology, ash content and water related parameters

Only powder 16 had a TVC of less than 10^5 per gram. The range of counts obtained was 2 - 7220 x 10^3 per gram.

Ash content for the powders made at LUT did not exceed 5.61%. The percentage of insoluble material in the powders was very low and an average value of 0.2% was achieved. Plasma powder No. 16 had a high value of 0.9%, no other powder exceeded 0.33%. As solubility is considered to be such an important property, and as it is said to vary with pH a further study on solubility is given in Section 6.10. Although the figures for solubility showed the powder to be soluble the ability to hydrate, as measured by dispersion was not so good. On average only 62% of the powder is in solution after 1 hour. The ability of the powder to absorb water under the conditions used showed an uptake of about 15 ml of water per 100 g of powder.

Properties of a 10% solution

Some parameters were estimated on a 10% solution, this concentration was used as it represented the type of concentration found in the native plasma.

The colour of the solution was assessed, although the system used did not allow for the cloudiness of some of the samples and this could be an important factor. On the whole the solutions were of similar hue. The colour, a cloudy brown/orange, was darker than anticipated from the light colouration of the powder. It was noticed that the colour was pH dependent.

The pH of the solution was estimated to be about 8.4 and the range was from 8.0 to 9.1. The pH of plasma before processing and after concentration was 7.8. In the body pH of extracellular fluid is normally regulated with great precision and is maintained at just above 7.4 (Whitby, Percy-Robb and Smith; 1975). Brooke and Ratcliffe (1959) found an increase in the pH of spray dried plasma and egg white and thought that the liquids prior to spray drying contained considerable amounts of dissolved carbon dioxide. During drying an equilibrium is reached with a very small pressure of carbon dioxide in the air (about 0.0003 atm.) and the subsequential loss of carbon dioxide from the drying product increases the pH value.

The specific gravity of a 10% solution was estimated to be between 1.02 and 1.03, with the average value being 1.027. The viscosity of the solutions as measured with the Ostwald Viscometer showed that most solutions had a value of 2.4 cp with no value lying outside the normal range.

Fat absorption

The most noticeable effect when assaying for fat absorption was the formation of two layers for the powder/oil mixture. One occurring at the top of the tube, the other at the base. The oil in between was relatively clear. No mention of the two layers has been found in any of the references dealing with fat absorption. The average amount of fat absorbed was 1.36 g of oil per g of powder. The percentage volume of the top layer was 12.2% and that for the base was 12.9%, none of the results lie outside the $\pm$ 2SD range. In detailed studies significant differences between the powders were established, but intrabatch differences could also be detected.

Emulsion Values

The values quoted in this part of the study relating to the ability of the plasma powders to emulsify fat are the emulsion

capacity, emulsion stability after 24 hours and after 14 days, the viscosity of an emulsion and the oil phase volume.

The emulsion formed using 25 ml of 2% solution of plasma powder with 80 ml of oil was light cream in colour, had a creamy texture, was not shiny and was stiff enough to form peaks. The values quoted for viscosity are not necessary correct as a viscometer with a helical path was not available at the outset of the study and the emulsion did not flow back into the path of the cup on the Haake Viscometer. Using a Brookfield synchroelectric viscometer, model RVT, speed 5 RPM and spindle A the viscosity of the emulsion was found to be 144000 cp and according to the scale of McWatters and Cherry (1977) can be classified as "very thick, mayonnaise like".

The mean value for EC was 287 g of oil per g of protein as assessed by the standard procedure. The lowest value was 260 and the highest 305. The results indicate the high stability of the emulsion. The OPV was over 82% for all the powders.

Foam formation

Foam formation was achieved using a 2% (w/v) solution of the plasma powder in distilled water. The foam was of large bubble size and slightly dark in colour. The average value for the amount of foam formed under the standard procedure was 61 ml, this corresponds to a foam expansion figure of 122%. On average over 50% of the foam produced was present 60 minutes after mixing. The standard deviation on the results showed a variance of 15% for the initial foam volumes and a high deviation of 30% for the stability figure. The high variations also occurred in the replicate assays for BPP. None of the powder samples produced values which lay outside $\pm$ 2SD of the mean.

To test the foaming ability of BPP the utility test of preparing an Angel cake was conducted, see Figure 11. As flour was added to the whisked BPP foam it decreased in volume, on cooking the cake mix rises to a great height, but this is not maintained and sinking occurs. The odour of the cooking cake was not pleasant. The final product had a satisfactory taste and smell, but was sticky and had a gummy mouthfeel. The cake formed using half egg white and half BPP did not have the qualities of the full egg product.

Gelation

Gels strong enough to support the penetrometer cone were achieved for all the plasma powders at strengths and temperatures stated in the standard procedure. The gels formed were clear and light brown in colour. Heated at temperatures in excess of 100°C the gels will liquefy and reset on cooling, but their strength was not so great as the original gel.

The gels were harder with increasing temperature and powder strength. The range for the gel total score was 864 to 1201 1/10 mm penetration. The values as agar equivalents for the combinations of concentration and temperature are G10%80 = 1.05, G9%80 = 0.60, G10%75 = 0.75 and G9%75 = 0.55% agar.

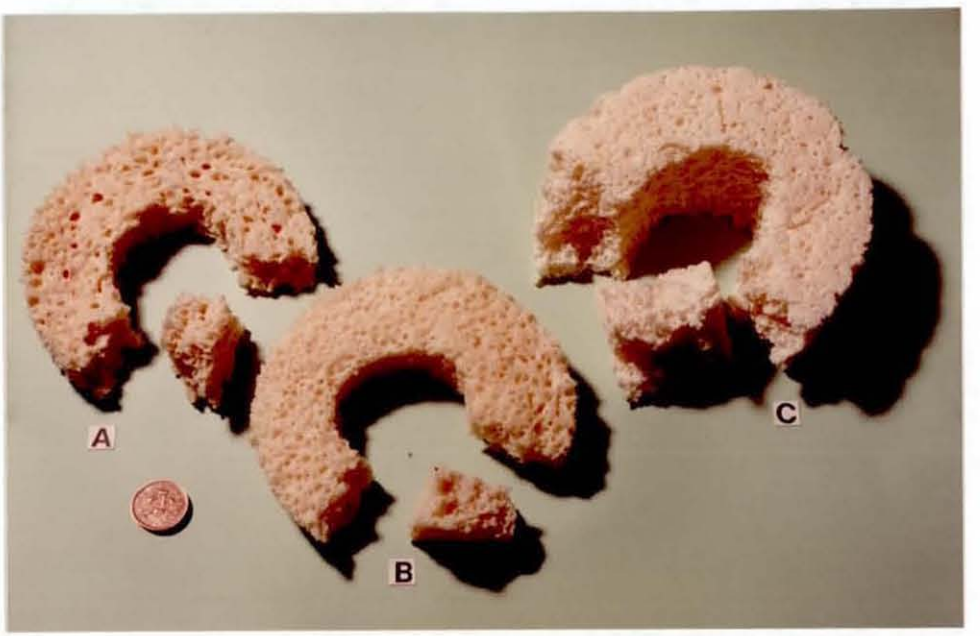
6.5 VARIANCE OF RESULTS Figure 12

The variances of two samples are said to be homogeneous if the populations from which the two samples are taken have identical variances. If the variance of the assay procedure and that of BPP samples are not different, it would indicate that the deviation in assaying different BPP was not greater than that obtained for the technical deviation of the assay procedure. F values were calculated to establish if the variances of the assay procedures and the BPP could be considered homogenous.

Statistically the variances of particle size, amount of solids, calcium, ash, insoluble material, dispersion values, and total viable count were not homogeneous at the 5% level. Viscosity, fat absorbance, emulsion stability and some of the gelation values had standard variations which indicated differences between the technical deviation and that obtained for the powder at the 2.5% level. The foam and EC values showed no increased variation with the different powders compared with the technical variation for those assay procedures.

Some assays indicated that the variation in the results for powders was higher than that due to technical deviation. In other assay procedures there is just as much error in estimating one powder as estimating different powders. On the whole the powders made were similar and it would be difficult to detect changes in the raw material or slight differences in the processing using the analysis discussed.

Figure 11 Angel Cake



A = 100% BPP

B = 50% BPP/50% Egg Albumen (EggP)

C = 100% EggP

FIGURE 12 - Assay on Bovine Plasma Powder and Coefficients of Variation of Assays

CODE	ASSAY	UNITS	Mean	SD	CV for method	CV for powder
B.DEN	Bulk density of the powder	g/ml	0.404	0.0262	12	6
LPS	Largest particle size	μ	55.5	18.2	8	33
SPS	Smallest particle size	μ	2.6	0.3	4	12
D 50 PS	Average particle size	μ	15.9	3.6	6	23
Pdr Col	Powder Colour, code value	-	5.5	4.1	-	-
Solids	Amount of solids	%	94.5	1.65	1	2
Protein	Protein by Kjeldahl	%	79.5	1.57	5	2
Lipids	Amount of lipid	%	5.99	2.18	12	36
Sod. Cl	Amount of salt	%	3.3	0.82	11	25
Calc	Amount of calcium	mg/100g	16.5	23.0	16	139
Micro	No of viable counts	103/g	2004	2545	65	127
Ash	Amount of ash	%	4.31	1.08	7	25
In Sol	Insoluble material	%	0.20	0.26	7	130
Disp	Amount of powder going into sol.	%	6.16	1.13	7	18
Wat Abs	Amount of water absorbed by powder	ml/100 g	15.30	1.72	12	11
Sol Col	Solution colour	-	27	-	-	-
pH	pH of 10% solution	-	8.4	0.31	2	4
Sp.G.	Specific gravity of 10% solution	g/ml	1.0268	0.0033	0.5	0.3
Visc.	Viscosity of 10% solution	cp	2.413	0.161	3	7
Fat	ml of oil absorbed per g powder	m/g	1.356	0.230	8	17
Fat B	% of the base layer	%	12.85	2.34	12	18
Fat T	% of the top layer	%	12.25	3.28	5	27
Em Cap	Emulsion capacity	g.oil/g	287	12.3	5	4
Em S1	Amount of oil released from emulsion after 24 hours	%	10.4	3.8	117	37
EM S14	Amount of oil released from emulsion after 14 days	%	4.5	2.1	71	46
OPV	Oil phase	%	83.7	-	-	-
Foam 5M	Volume of foam after 5 min	ml	61	9.2	14	15
Foam 25M	Volume of foam after 25 min	ml	45	8.9	31	20
Foam 60M	Volume of foam after 60 min	ml	32	12.9	48	40
F60/5	60 min vol/5 min vol x 100	%	50.8	15.1	27	30
G 10% 80	depth of penetration in gel formed at 10% @ 80	1/10 mm	190	13.8	17	7
G 9% 80	depth of penetration in gel formed at 9% @ 80	1/10 mm	282	56.9	16	20
G 10% 75	depth of penetration in gel formed at 10% @ 75	1/10 mm	223	19.7	13	6
G 9% 75	depth of penetration in gel formed at 9% @ 75	1/10 mm	341	54.3	13	16
Tot. Gel	Total penetration score	1/10 mm	935	304.0	10	33

6.6 POWDER UNIFORMITY DURING SPRAY DRYING

If a powder is to be used routinely in the food industry the powder provided must be of a consistent quality. To ensure that the properties of BPP did not alter during spray drying samples of product were removed at intervals during processing. A record of the inlet and outlet temperature was obtained at four minute intervals. The computerized control was set at an inlet temperature of 180°C and outlet temperature of 75°C. After the required time interval the sides of the spray dryer were rapped and the container in which the powder had accumulated was removed. A clean container was immediately substituted and powder collection continued. The powder in the bin was mixed well and an aliquot removed for assay. Powder samples were obtained after 20, 40, 60, 80 100, 120, 150 and 180 minutes after the start of the spray drying process. The total duration for the drying was 200 minutes.

When the 8 aliquots had been removed the rest of the powder was transferred into a mixer with a screw type blade. The powder was gently mixed for 30 minutes. Five samples were taken from this homogeneous mixture and were used as controls for the other samples.

6.6.1 Discussion See Appendices 6.6

During the spray drying process the atomiser on the spray dryer became blocked on two occasions. This resulted in a sudden lowering of the inlet temperature and a corresponding rise at the outlet. The samples that would be effected by this were samples taken after 120 and 150 minutes. Correlations between sampling time and assay results were attempted. As a range of temperatures were obtained at the inlet and outlet these were also correlated against the assay results.

Powder properties and organoleptic quality

There was no strong correlation between the time of sampling, the inlet temperature and the outlet temperature for the particle size estimations. A correlation of 0.77♦ was found between the outlet temperature and the bulk density, but a graphical portrayal (Appendix 6.6.b, 6.6.c) of the data indicates the small range of temperature variation that occurred at most time points. All the powders were cream in colour and no differences in hue could be detected. The samples all tasted and smelt the same.

Proximate composition of the plasma powders

There was no indication that the levels of moisture, protein, lipid, sodium chloride and calcium were dependent of the length of time of spray drying. The inlet and outlet temperature also did not effect these values, except that there was a correlation between the temperature and the calcium levels (Appendix 6.6.c). Most noticeable was the very high (249 mg/100 g) level of calcium in the initial sample. No statistical difference between the means of the samples taken at various times during spray drying and the control samples was found.

Microbiology, ash content and water related parameters

The assays for ash, dispersion and water absorbance were not done in this study. No variation in number of bacteria nor amount of insoluble material was found with time, inlet and outlet temperature. It was noted that the amount of insoluble material obtained with this preparation was greater than for other BPP. No statistical differences were found between the samples taken at different time points and the sample taken from the homogenous batch.

Properties of a 10% solution

The colour, pH, specific gravity and viscosity of the 10% solutions prepared from the different samples were all similar in nature.

Fat absorption

There was little detectable difference between the data obtained from the samples taken during the drying and those taken from the homogenous batch. No correlation was obtained for fat absorbance with duration or temperature of spray drying.

Emulsion values

In the study of emulsion formation no indication of the emulsion capacity or 14 day stability could be related to the time the samples were taken, or to the corresponding inlet or outlet temperatures. However, there was a statistical difference, $p < 0.05$, between the mean EC values obtained for the samples obtained from the bulk sampling and those collected at specific times during the run.

Foam formation

No relationship was established between the foam values, time of sampling, or outlet or inlet temperature.

Gelation

The gels made at 9% concentration at 80°C and those made at 10% concentration at 75°C, for the samples taken at the specific time points, were found to be significantly different from the results obtained for the samples taken from the bulk powder. In both cases the gels were less hard. For the gel total there was no significant difference. The graph of total gel against time of sampling (see Appendix 6.6c) does seem to indicate an increase in gel strength over the time 40 to 120 minutes.

6.7 STABILITY OF BPP

For a powder to be useful in the manufacturing of food products the behaviour of the product must be predictable. A powder's characteristic qualities must be stable with time. Whether the functional properties changed with the age of the powder was also important for the further investigation of methods for functionality.

The powder used for the stability trial was No. 16 and was that produced for the study of powder uniformity, Section 6.6. When the powder had been mixed, samples were removed and placed in glass jars which were stored in the dark. At intervals over a one year period a jar would be removed from storage and the contents examined for the range of properties normally assessed for BPP. The five samples initially taken from the homogeneous batch were used to establish the first time point. After one year in storage five samples were removed and assays performed on all five samples. The differences between the initial values and those obtained after one year were then examined.

6.7.1 Discussion - Appendices 6.7

Altogether 15 time points, plus the initial and one year assays, were carried out on powder No. 16. From week 0 to week 6 the assays were carried out weekly, then two weekly until week 10, then at no longer time intervals than 4 weeks until the powder was 30 weeks old. A time interval of 6 weeks was then used until the powder was one year old. The assessment of the powders over such a time span also was an indication of how well the assay procedures could be undertaken routinely and the precision of the methods over a period of time. The results indicated that the methods were practical for routine use and assays were reproducible over a one year period.

The powder appeared to look, taste and smell the same at the end of the study as it did at the start. There was a significant difference between the bulk density of the powders at the start and end of the study, but no indication that there was an alteration during the one year period. There was an indication of a reduction in average particle size for the year old samples, but no trend was seen during the year (see Appendix 6.7.b).

The data do not indicate that the constituents of the powder altered. There was no indication that the microbial quality of the powder altered. A reduction in the amount of insoluble material was found in the final samples, but no general change with time was observed. The viscosity of a 10% solution of BPP was higher at the end of the study than that at the start. Stability of the foam may have been slightly improved with the ageing powder. Samples at the start and end of the study differed from each other in respect to values for EC, G9%80 and G9%75, but samples taken during the study indicate no correlation with time.

6.8 PROPERTIES OF PROTEIN PRODUCTS IN COMPARISON WITH BPP

To give an indication of the properties of BPP other protein products were also assessed. The list of proteins is given in section 5.2. Solutions of the proteins were made according to the weight of powder, with no allowance for the varying amounts of protein in the product. Solutions were all made in distilled water and no adjustment to pH was made. For the fresh egg white the gently mixed albumen was used for the 10% solution and this was diluted, one part egg white plus four parts water for the use in assays using a 2% solution.

The results for the 23 protein products tested were ranked so the values higher and lower than the mean $\pm$ 2 SD for the BPP were indicated, see Figure 13.

6.8.1 Discussion - See Appendices 6.8 and Figure 13

The procedures used were those developed for use with bovine plasma and may not have given optimum values for the other protein products. The studies in Chapter 7 indicate that values for functional properties can not be simply calculated to give the results for equivalent amounts of protein, the assays have to be repeated using the correct amount of powder to achieve the same amount of protein as that occurring in the BPP samples.

Powder properties and organoleptic quality

The physical properties of the protein products differed greatly. No product tested had smaller particle size than the BPP, but most of the commercially available products had larger average size. The flaked egg albumen was of far greater size than the other products. The bulk density of most of the products was greater than the BPP. Most products fell within the normal range for the powder colour, except that the egg flake and most noticeably the red blood cell product had higher colour values (see Figure 10).

Proximate composition of the powders

The moisture content of all the solid products was low, but the amount of protein they contained ranged from 98.6% for lensol to 12.6% for whey. The amino acid analysis of some of the powders is given in Appendix 6.4.1.b. A range of lipid and salt contents occurred in the protein products, some of the salt contents being particularly high in the hydrolysed samples.

Microbiology, ash content and water related parameters

The range for the total viable count for the BPP was very great with the higher values being unacceptable for a food product. The protein products were mostly free from gross contamination except the plasma sample of Fibrisol which had 1.46×10^6 viable organisms per gram powder. The ash content of the powders ranged from lower to higher than that of the BPP. High ash values for the plasma dried by Fibrisol, the blood cell hydrolysate and whey were obtained. The solubility of a protein product is very important and the lack of insoluble protein in the BPP is good in comparison with some of the other protein products. The plasma from Fibrisol had 1.3% of the solids insoluble, but values of over 5% were obtained for casein, soy isolate and lencoll. The dispersion of the powders in water and their subsequent solubilization showed that most commercial products were better than the BPP, but the soy products and lencoll were not as good. The range of values obtained for the uptake of water vapour by the BPP was large, but although the powders were obviously damp the hygroscopic effect was never sufficient for the powders to be deliquescent as occurred for the Fibrisol sample and casein. There was apparently no water uptake by the egg flake and the albumin Fraction V.

Figure 13 - Ranked data for the protein powders compared with BPP

B.DEN	D5OPS	PDR COL	SOLIDS	PROTEIN	LIPID	SOD. CL	CALC	MICRO	ASH	SOL.COL	pH	
AlbFib												LARGEST NUMERICA VALUES
Lcoll	EggF											
Whey	AlbFV											Values larger than mean + 2SD for BPP
SoyF	Coll											
Cas	Whey											
Hap	AlbFib											
EggP	SoyI			Lsol								
EggF	Lcoll			RBC								
BCIly	Cas			Alo		Hap						
RBC	Lact			EggP		BCIly				RBC		
LSol	BCIly			Alo/P		BP/M				bp/O		
SoyI	RBC			BS	Cas	SoyI			AlbFib	PP/U	BP/M	
Coll	SoyF	RBC		AlbFV	Soy I	SoyF	bP/O		BCIly	PP/F8	AlbFib	
BP/U	EggP	EggF	LColl	BP/M	Whey	AlbFib	Whey	AlbFib	Whey	BS	EggLi	
VALUES OF MEAN ± 2SD FOR THE 10 BOVINE PLASMA POWDERS MADE AT LUT												
AlbFV			AlbFV	PP/O	BP/M	Lact			Lsol	Lcoll	SoyI	Values smaller than mean ± 2SD for BPP
bP/O			EggLi	AlbFib	Egg F	Lsol			BP/U	Hap	Alo/P	
				Lact	Alo	Cas			Lact	BCIly	Cas	
				SoyF	BCIly				BS	SoyI	EggP	
				Hap	AlbFV				PP/F8	EggF	Lact	
				Whey	EggLi				AlbFV	AlbFV	Hap	
				EggLi	Lcoll				RBC	Alo/P	SoyF	
					Lsol					EggLi	Alo	
										Whey	Whey	
										Alo	BCIly	
										EggP	EggF	
										Cas	AlbFV	
										SoyF	Lcoll	
										Lact		
												SMALLEST NUMERIC VALUE

IN SOL	DISP	WAT ABS	SP.G.	FAT	FAT T	EM CAP	EMS 14	FOAM 5M	FIN% FOAM	GEL TOT
							BCHy			BCHy
							Lact			SoyF
							Alo			Coll
	Hap						SoyF			Lcoll
	Alo						Lsol			Hap
	Alo/P						Hap			Lsol
Lcoll	bP/O		Hap				Whey			Cas
SoyI	BCHy		EggLi				SoyI			SoyI.
Cas	Whey		Whey				Lcoll			Whey
Hap	AlbFV		AlbFib				Cas			Lact
SoyF	EggF		BP/M				AlbFib			BS
AlbFib	Lsol	Cas	BCHy			Cas	BS			Coll/P
Alo	Lact	AlbFib	Alo/P	AlbFib		BS	BP/u	AlbFV	SoyI	AlbFib
VALUES OF MEAN ± 2SD FOR THE 10 BOVINE PLASMA POWDERS MADE AT LUT										
	SoyF	BP/M	RBC	Lcoll	Lsol	bP/O		Alo	Alo	PP/o
	SoyI	PP/F8	SoyI	SoyF	EggP	pp/O		BS	Cas	EggLi
	Lcoll	RBC		Hap	BCHy	EggF		Soy I	BS	
		EggF		PP/F8	AlbFV	SoyI		Hap	BCHy	
		AlbFV		RBC	EggF	EggP		Whey	Lact	
				AlbFib	SoyF	Alo/P		SoyF		
				Alo/P	Hap	Egg Li		Lact		
				Alo	AlbFib	BP/M		Lcoll		
				Whey	Alo	Whey		BCHy		
					Whey	Soy F				
					Lcoll	Lcoll				
						Hap				
						Lact				
						Alo				
						BCHy				

Largest numerical value

Smallest numerical

Properties of a 10% solution

The colour of the commercial protein products was lighter than for the solution of BPP. The pH values obtained for the BPP ranged from 7.8 to 9.1 and most of the commercial products had values below this. The dried egg powder and flake had pH values of 6.7 and 5.2 respectively, but the fresh egg white value was 9.1 and therefore the egg products span the pH range for the BPP.

The specific gravity and viscosity of the fresh egg white was greater than the other products tested. The viscosity of some of the products was not assessed as the insoluble material hindered assay with the viscometer.

Fat absorption

All products have some capacity to absorb oil, even inert glass beads (Jasmin, 1983). The two oil/powder layers formed with the BPP did not occur for some other powders. No top layer was seen for the flaked egg, whey, soy flour and the fish product. The only product tested that had higher absorbance than the BPP was the albumin fraction V, this did not form any top layer. The plasma product from Fibrisol also did not form the light density layer, the total amount of oil absorbed by this product was less than that expected for BPP (see Figure 18). Most products held less oil than the BPP, the only powders made at LUT with less absorbance were the porcine plasma without factor VIII and the red cell powder.

Emulsion values

The results show that, under the conditions used, bovine plasma powder had very good emulsifying properties when compared to other protein products. Similar conclusions have been drawn by other workers (Sattelee, Free and Levin, 1973; Pearce and Kinsella, 1978).

De Vuono et al (1979) found that whole plasma gave very good results and was better than the globulin and albumin assayed separately. Howell (1981) found albumin the best protein for emulsification. The results obtained for the bovine albumin in this study were in the same range as the results for whole plasma, although it was below the range when adjusted for the higher protein levels. Appendix 6.8.b

The only products which had the same as or better emulsion capacity than BPP were the other blood products, the albumin, the red cells, lensol and casein. The product lensol is a spray dried beef marrow bone stock. It gave high EC values, but the emulsions formed were unstable. Casein produced oil-in-water emulsions of higher capacity than those normally achieved with BPP, but again the stability was not so good.

The only product which was more stable than the blood products was the fresh egg white, but that was at the 24 hour time point, after 14 days the results were equable. The EC values obtained for the three egg products were all lower than the range established for BPP. It is known that egg albumen is readily denatured at surfaces and there can be a marked depletion of this protein from the solution during the formation of an emulsion from egg albumen (Pearce and Kinsella, 1978).

β-Lactoglobin is normally considered to be a good emulsifying agent, but α-lactoglobin is easily denatured and a mixture of these two proteins occur in whey. The whey powder was low in protein and gave low EC values and the emulsion formed was unstable. In the assay where the level of protein was adjusted the EC was marginally higher. In the product Lactein 75 the powder contained more protein than whey, but no higher EC values were obtained.

Foam formation

Some products hardly formed a foam. Blood cell hydrolysate, soya flour, lencol, whey and lactein did not produce 10ml of foam. Other products that did not perform as well as the bovine plasma powder were haplochromis fish hydrolysate and soya isolate. The only product that was better than the BPP was the albumin. The stability of the albumin was not markedly good.

The product most used in the confectionary trade for foam production is egg white. In other studies done on comparing the foaming properties of BPP with egg, the plasma has been found superior (Thompson, 1983; West and Wier, 1979). In this study, under the conditions used, the values obtained for the egg products fell within those obtained for the BPP, however, some bovine plasmas did not perform as well as the dried egg white. On average the three egg white products produced a more stable foam than for BPP and the volume formed with egg was slightly higher than that normally achieved by the plasma. The egg globulins including lysozyme are considered to produce foam of large volume with small bubbles. Proteins form a complex mixture in egg white and may play different roles in foam production and stability. Ovomucin is not a good foamer, however within egg white it is this protein which stabilizes the foam after short whipping times. This may be due to the rapid loss of solubility of the ovomucin at the air/liquid interface (MacDonnell et al, 1955).

The albumin isolated from plasma by Cohn fractionation produced more foam than the total plasma. This also occurred in the study of De Vuono et al (1979). Buckingham (1970) found that albumin which was extremely soluble produced weak, short persistence foams unless the solubility of the protein was deliberately reduced. The foaming properties of the plasma shows the net effect of all the different protein fractions.

Gelation

Some of the protein products did not form gels:- lencoll, lensol, alomine, casein, soy flour and haplochromis. Other products formed gels which could not retard the penetrometer cone:- alomine mixed with BPP, whey, lactein and soy isolate. Gels formed with egg albumen and bovine albumin were as strong as the bovine plasma samples at 10 and 9% and stronger at 8% heated to 75°C. However, the gels formed by the egg and albumin were opaque and there was marked syneresis from these gels. These findings are similar to that of Hickson et al (1980) when he compared egg albumen with plasma proteins using viscosity index determinations. The results of Howell and Lawrie (1984, a and b) indicated that the gels formed with plasma were stronger than those formed by egg albumen.

6.9 CORRELATION BETWEEN ASSAYS

Many assays were performed on the protein products and it was hoped that the data might be used to establish mathematical models for the functional properties. Such methods have been described by Holmes (1981). However the relation between the constitutents of the powder and its behaviour are very complex and the reduction of the data to fit mathematical models was considered inappropriate.

A list of correlation values for assays on BPP and other protein is given in Appendix 6.9. Only one value exceeded 0.8, that was for EC against the average particle size.

6.10 SOLUBILITY OF BPP - Appendices 6.10

As described in Section 2.3 the solubility of a protein can be very important for the overall value of its functional use. A more detailed study of the solubility properties of one of the powders was therefore undertaken.

6.10.1 Titration curve and solubility at different pH values

The powder used throughout this section of work was No. 11. A solution containing 100 g of powder was made up to 1000 ml with distilled water. The solution was then divided into two. The amount of 1M hydrochloric acid required to change the pH of the solution by half a pH unit was noted and a 1 ml aliquot removed. This procedure was carried out until the pH 3 was reached. The same procedure was used to increase the pH of the plasma solution from the naturally occurring 8.0 to that of pH 12 using 1M sodium hydroxide. The aliquots taken at each stage, after standing for

at least three hours, were centrifuged in the bench centrifuge at 1400 g for 5 minutes. The supernatant was assayed to estimate the amount of protein in solution. This whole procedure was carried out at least three times.

The results are shown in Figures 14 and 15 and the values are in Appendix 6.10. In the titration curve it can be seen that no true plateau occurs. The results for the solubility at various pH values denotes a statistically significant difference between the amount of protein in solution at pH 4.5 and 5 compared with that at pH 8.0. No other significant differences were observed between the solubility at the altered pH and that of plasma powder when dissolved in fresh distilled water. The decrease in protein solubility from a maximum of 91.9% to the minimum value was 17%. The actual amount of soluble material for powder 11 was estimated at 99.79% at the natural pH by the standard method for measuring insoluble powder.

The solubility of processed plasma has been investigated by several workers. Tybor, Dill and Landmann (1973) spray dried plasma and then investigated the solubility profile. They found only slight dependence on the pH as indicated by the 7% decrease in protein at the point of minimum solubility, pH 4.8. In their 1975 study they found that the plasma proteins dried at inlet temperatures of 160°C and 193°C had the same solubility, the outlet temperature was the same in both cases. The minimum solubility again occurred at 4.8 and was 74% of the total protein, whilst levels of 90% were achieved for pH values below 4.0 and above 6.0. The incorporation of lactose prior to drying demonstrated a protective effect on protein solubility.

The study by Delaney, Donnelly and Bender (1975) investigated solubility of a powder they had produced by ultrafiltration then spray drying and an unnamed commercial product, which was stated to be "one of the best on the market". The powder they produced

Figure 14 - Titration curve for BPP

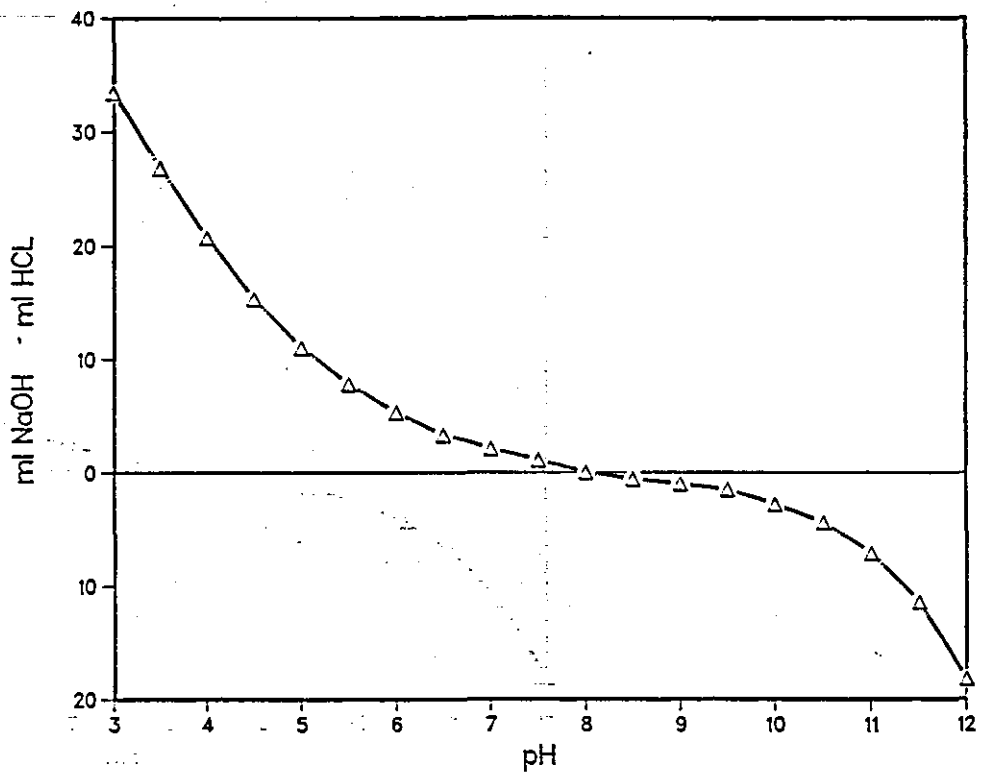
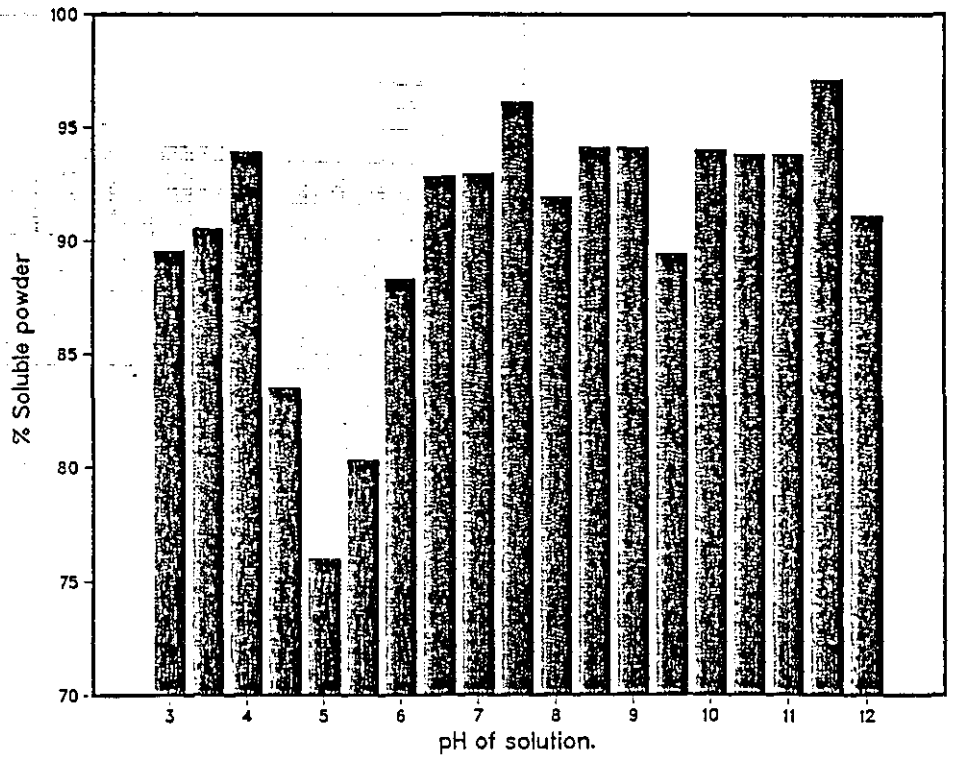


Figure 15 - Solubility of BPP at different pH values



had excellent solubility over the range pH 2-10 with a minimum value of 93% at around pH 4. The overall solubility of the commercial product was considerably lower, the maximum values not exceeding 80%. The minimum value for solubility was again exhibited at pH 4, but the value was as low as 65%.

The small differences between solubility at the maximum and minimum points was found by De Vuono et al (1979) for plasma produced by freeze drying. The minimum (70%) occurred at pH 5, whilst the maximum solubility was nearly 100%. The plasma studied by Hermansson and Tornberg (1978) was soluble to a high degree, the minimum solubility being 70%.

6.10.2 Solubility at different concentrations

It was considered that the concentration of powder in the solution could effect the amount of protein dissolved. To investigate this a range of four concentrations of powder were prepared; 2.5, 5.0, 7.5 and 10.0%. Three different pH values were investigated; pH 5, 8 and 10. All the solutions were allowed to stabilise for four hours then the solutions were centrifuged at 1400 g in the bench centrifuge for 5 minutes. The supernatant was used to assay for protein by the Biuret method.

The results are tabulated in Appendix 6.10.b and are given as the % of the protein, as measured by Biuret, in solution compared with the total weight of powder used to prepare the solution. Powder No. 11 contained 80.0% protein,(Kjeldahl estimation). Using the Student's T test significant differences were seen between the results obtained for the solubility for the concentration of 2.5% and concentrations of 7.5 and 10% at pH 5. Concentration differences were not detected for pH 8.0, but there was a difference between the 2.5% and the 10% level at pH 10. There was a significant difference for the results obtained at each concentration between pH 5 and 8 also with pH 5 and 10.

6.10.3 Solubility with sodium chloride - Appendix 6.10.b

To establish if the amount of salt present was important in the solubility of the plasma powders, a range of powder concentrations was made in a solution of 1M sodium chloride. The pH of the solutions were then altered if necessary to pH 5, 8 and 10. The amount of protein in solution was then estimated as previously stated.

There was no significant differences between the various concentration levels of sodium chloride at the same pH value. The only difference in protein levels that could be differentiated between the different pH levels for the same powder concentration was at the 7.5% concentration at pH 5 and 8. Hermansson and Tornberg (1978) also found that the solubility of the protein improved at pH 5 in the presence of salt at levels over 0.3M. They found that the solubility was independent of salt concentration up to 0.6M at pH 7.0.

6.10.4 Solubility with sucrose - Appendix 6.10.b

The method used for the salt was applied to this study except that 1M sucrose instead of the 1M sodium chloride was used.

The inclusion of this level of sugar did seem to negate the effects noticed when only the powder was present. All the concentrations of powder produced the same percentage of protein in solution and the variation in pH did not statistically effect the solubility. The only assay that showed an apparent effect was that at pH 10, concentration 2.5%. The values obtained for these samples were low and statistical differences were noted between pH 10 concentrations 2.5 and 5% and between the 5% concentrations at pH 8 and 10.

6.11 INVESTIGATION OF THE PROTEIN STRUCTURE AFTER DRYING - Appendix 6.11

The assessment of changes in the tertiary and quaternary structure of a protein can be difficult to assess. Functional assays may not be sensitive enough to give an accurate assessment of the damage the protein may have undergone during processing.

6.11.1 Electrophoresis

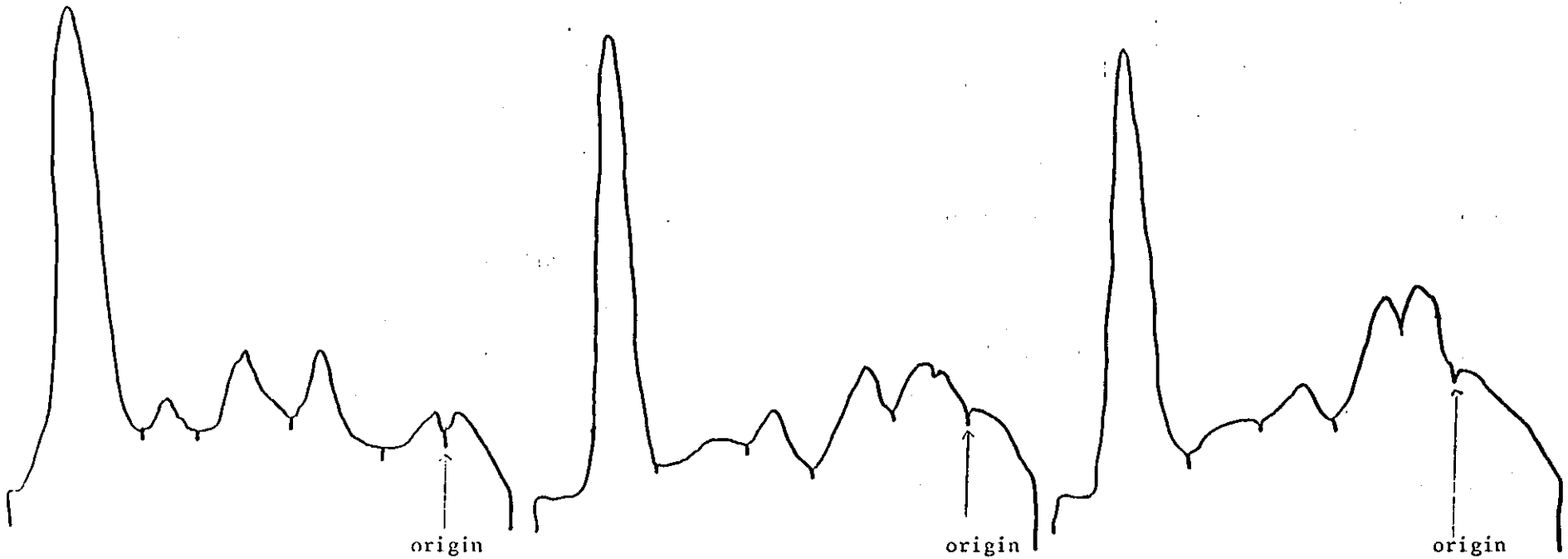
Powder and frozen plasma samples were investigated by electrophoresis, using agarose gel with a tris buffer on a Shandon system that had been developed for the estimation of proteins in animal plasma (Toxicol, 1983). The powder sample was prepared at a concentration of 10% w/v in distilled water. The 10% solution and the thawed plasma were centrifuged and the supernatant used in the assay. A human control plasma, supplied by Boehringer, named Precinorm U was also assayed at the same time as the bovine plasma. The patterns obtained from scanning the stained and dried gels is given in Figure 16.

The protein fractions in the bovine samples have very similar motility in an electric field as the human plasma. There was no identifiable difference between the dried and frozen plasma samples.

6.11.2 Lactate Dehydrogenase (LD) Assay

To identify if the drying of plasma alters the protein composition and structure it was decided to investigate if processed samples still retained enzyme activity. The enzyme chosen for assessment was lactate dehydrogenase EC1.1.1.27. This enzyme has a high molecule weight (135000) and its tertiary and quaternary structure is of great importance as it consists of four polypeptide chains (Whitby, Percy-Robb and Smith, 1975). High LD levels can occur in cattle plasma without a pathological cause (BCL, 1983).

Figure 16 - Densitometric Scans of Electrophoretic Strips



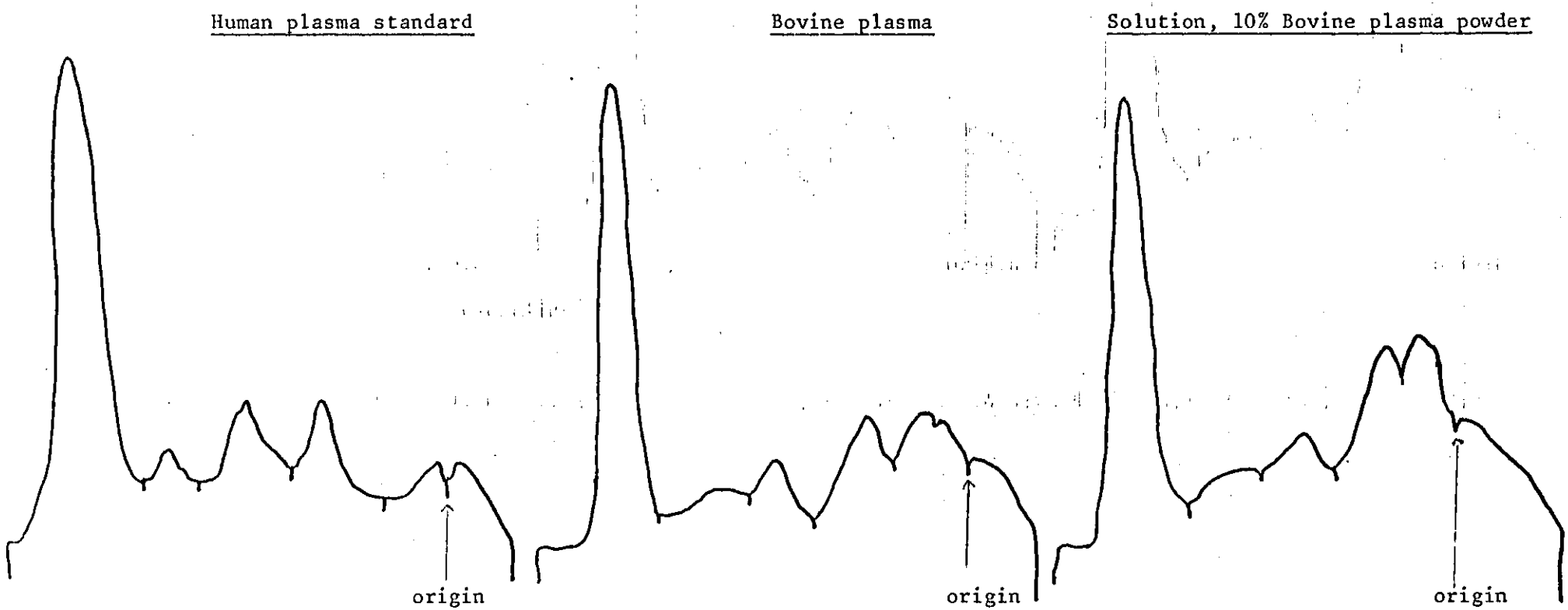
	Albumen	α_1	α_2	B	α
Ratio	55.7 :	6.4 :	15.0 :	11.6 :	11.3

Globulins

Bovine plasma: 39.9: 7.6: 9.5: 14.5:28.5

Solution, 10% Bovine plasma powder: 35.3: 7.9: 10.7: 15.8: 30.3

Figure 16 - Densitometric Scans of Electrophoretic Strips



	Albumen	Globulins α_1	α_2	B	α
Ratio	55.7 :	6.4 :	15.0 :	11.6 :	11.3
	39.9:	7.6:	9.5:	14.5:	28.5
	35.3:	7.9:	10.7:	15.8:	30.3

Method

The samples used in the initial study were those collected over a period of time. The samples were plasma, plasma concentrated by ultrafiltration and 10% solutions of plasma powder. The concentrated plasma and the plasma samples had been stored frozen at 20°C for periods of up to 2 years. They may have been through several freeze thaw cycles. The samples used were chosen at random and were not matched for animal, date or protein content.

In a second study samples of plasma, plasma after concentration and the final powdered product were taken from one production run.

A colorimetric kit obtained from Boehringer Diagnostica was used according to the instructions for the assay of LD in the samples . The results were calculated as International Units (U) per litre of sample and, because of the widely varying amounts of protein in the sample types, as U per 100 g protein.

Results

Initial Study

It is normal for the level of LD in cattle plasma to vary over a large range. The resulting large standard deviation in the results hinders comparisons as no pairing had taken place.

The standard deviation was found to be large especially for the concentrated plasma. The range of results for the plasma and concentrated plasma is not seen in the powders. This may well have indicated that, although some action remains, the high levels of LD activity have been eradicated.

Second Experiment

The results show high levels of the enzyme in the plasma, 92 U/100 g protein, less in the concentrated plasma, 69 U/100 g protein, and a great reduction in the amount after spray drying,

21 U/100 g protein. The three sets of results are significantly different from each other.

Discussion

This brief study does indicate that changes are taking place in the protein, with UF changing the protein to a limited extent and the spray drying causing further inhibition of enzyme activity.

CHAPTER 7

CHAPTER 7

METHODOLOGY FOR FUNCTIONAL PROPERTIES

The lack of standard techniques to establish values for functional properties made it essential to develop a methodology suitable for BPP. During the development of each technique it was discovered that many variables would alter the values obtained. Some of those factors for fat absorbance (FA), emulsification (EM), foaming (FOAM) and gelation (GEL) are discussed in this chapter.

The paragraphs have been coded to indicate the variable under discussion. The table below, Figure 17, shows which variables have been studied for the four functional properties.

Figure 17 Methodology for Functional Properties

Code	Variable	Functional Property			
		FA	EM	FOAM	GEL
A	Establishing a method	✓	✓	✓	✓
B	Method of mixing	✓	✓	✓	x
b	Duration of mixing	✓	✓	✓	x
C	Variation in temperature	✓	x	✓	✓
c	Duration of heating	x	x	x	✓
D	Powder concentration	✓	✓	✓	✓
E	Change in water volume	✓	✓	✓	x
e	Change in oil volume	✓	x	x	x
F	Salt levels	x	✓	✓	✓
G	Sugar levels	x	✓	✓	✓
H	pH	✓	✓	✓	✓

ADDITIONAL ASSAYS

PARAMETER	ASSAY	PARAGRAPH CODE
FA	Variation in the oil	I
	Contents of oil powder mix	J
	Fine and coarse particles	K
	Heating BPP	L
	Measurement of fractions of BPP	M
EM	Rate of oil addition	I
	Viscosity	J
	Establishing globular size	K
FOAM	Presence of lipid	I
GEL	Mechanism of gelation	I

7.1 FAT ABSORPTION

Figure 18

Fat absorption

A = BPP
B = whey powder
C = Fibrisol powder

7.1.A Establishing a Method

Quoted methods for the measurement of fat absorption have varied but do not mention problems with the mixture adhering to the mixing rod and to the sides of the tube, which occurred when methods were tried with the BPP. To overcome these problems larger amounts of the powder and oil were used in the standard procedure and the mixing carried out using a Silverson mixer. The amount of homogeneous mixture adhering to the Silverson probe was ignored and an aliquot of the mixture transferred from the mixing vessel to a test tube. Although this amount was removed with a 10 ml pipette the volume transferred was not always the same as over a ml of mixture adhered to the pipette. This varying amount of mixture made a calculation necessary to establish the theoretical amount of oil and powder in the volume of mixture and hence the amount of bound oil to the actual amount of plasma powder The calculation was based on the specific gravities of the oil and the oil/powder mixture.

One major difficulty of the standard method was in estimating the various levels. Boundary layers were clear, but not level after centrifugation. To try and eliminate any errors the measurements were taken at four points around the tube.

The method produced three distinct layers, which have been named base (B), middle (M) and top (T) throughout this study. The base and top layer were cream in colour, the T being lighter than B. They were of a thick consistency. If the centrifugation time was increased or the samples left to stand for long periods, the middle layer became clearer and the base layer increased slightly in volume. No difference in T was observed. The two distinct layers formed by the binding of oil to the powder occurred with all BPP tested.

7.1.B Variation in Mixing Procedures Appendix 7.1.B

Method

The oil and powder were mixed together for 1 minute using a glass rod, care was taken to stir gently and not to entrap air into the mixture. The results were compared with a series assayed the same day using the normal mixing method, i.e. Silverson for 1 minute.

In a second experiment instead of centrifuging the mixture after 1 hour, the mixture was allowed to stand for up to 24 hours before remixing and then separating the layers.

Results and Discussion

Gentle mixing with a rod achieved the same values as with the homogenizer, thus showing that the powder will absorb oil as long as the powder particles are dispersed within the oil. The amounts of the two layers did not vary and were not effected by air entrapment within the mixture. The standard method could be revised so that the mixing would be done using a glass rod.

Wolf and Cowan (1971) reported that maximum absorption occurred in 15 to 20 minutes. In this study all the oil was bound to the BPP during the process of mixing and immediately afterwards.

No further binding occurs in the next 24 hours. There was a statistically different amount of the top layer, a reduction of 16.4 to 14%, when the mixture had been allowed to stand for 24 hours, the amount of top layer decreased with time, but the amount of oil did not vary (Appendix 7.1.B.b).

7.1.b Variation in Duration of Mixing - Appendix 7.1.b

Method

The standard procedure for fat absorption estimations was used except that the length of time the powder/oil mixture was stirred by the Silverson varied between 0.5 and 4 minutes.

Results and Discussion

The length of time the oil and power were mixed did not affect the quantity of oil absorbed by the powder. As FA has been mainly attributed to the physical entrapment of oil (Kinsella, 1976) long thorough mixing would have seemed a prerequisite for high absorption values. However, the close association of the powder particles with the fat would appear to be achieved after short mixing times.

7.1.C. Variation in Temperature - Appendix 7.1.C

Method

The standard method for the estimation of fat absorbance was used except that the 20 ml of oil was held at various temperatures before addition of the powder. The oil/powder mix was held at the relevant temperature before centrifugation and was maintained at the appropriate temperature during measurement of the three layers. The specific gravities at each temperature were also determined.

Results and Discussion

The results were calculated in ml and as % values. The correlation between the mean values for fat absorbance and temperature gave coefficients of -0.896 (ml/g) and -0.945 (%), Figure 19, indicating a decreased absorbance with temperature. There was no significant differences between the values obtained compared to those at 4°C, but at this temperature the results varied a great deal causing a high standard deviation. The amount of oil absorbed as a %((ml of oil absorbed/g powder) * 100 * SG of oil) at 20°C was statistically different from the values obtained at 40, 60 and 80°C. There were no differences if the values in ml/g powder were estimated.

In the work by Hutton and Campbell (1977) various soy compounds were assayed for FA at 4, 20 and 90°C. The FA for each product varied with temperature, but each response depended on the product. Not all products (measured as % sample weight, as is basis) had the highest absorbance at 4°C, some absorbances were increased from 20 to 90°C, while others decreased. However, Hutton and Campbell (1981) concluded that, overall, as temperature decreased from 90 to 4°C fat absorption increased. Schachtel (1981) compared FA at 4 and 70°C for single cell protein, soya protein and sodium caseinate. He found a statistically insignificant lowering effect for FA at the higher temperature.

7.1.D. Variation in Amount of Powder - Appendix 7.1.D

Method

The standard method for fat absorption was used, but the amount of powder used was 0.5 g (2.47% w/w), 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 g (18.4% w/w).

Results and Discussion

Correlations between the various amounts of the layers and the amount of powder were all numerically greater than 0.9. The top and bottom levels formed by the method were dependent on the amount of powder used and increased in direct proportion to the quantity. Figure 20 shows high amounts of oil being absorbed when the powder levels were below 2g; at levels of 2g and above the amount of oil per g of powder appears to be constant. This was further demonstrated by comparing the oil values obtained for 2, 3. 5, 4 and 4.5 g of BPP, as no significant differences between the values were obtained.

7.1.E Variation in Volume of Water - Appendix 7.1.E

Method

The normal method was used except 50 µl of water was added to the 2 g sample of powder before oil additon.

Results and Discussion

The results show that the addition of water to the powder does not effect its ability to bind fat.

7.1.e Variation in Volume of Oil - Appendix 7.1.e

Method

The standard method for fat absorption was used but , the amount of oil varied between 10 and 30 ml. For comparison 8 assays were done using 40 ml of oil with 4 g of powder and 60 ml of oil with 4 g of powder.

Results and Discussion

At powder concentrations of 17 to 6% w/w, achieved by varying the oil volume, the amount of oil absorbed per gram of powder did not alter.

7.1.H Variation in pH - Appendix 7.1.H

Method

The normal method to estimate FA was used except that 50 µl of 1N sulphuric acid was added to the oil before mixing with the 2g sample of powder.

Results and Discussion

The hydrogen ion concentration does not appear to be a critical factor as the results are not dissimilar to those obtained for the controls.

7.1.I Variation in the Type of Oil - Appendix 7.1.I

Method

The standard procedure was used to estimate the amount of fat absorbed. The solid fats (Pura vegetable oil and Pura lard, best before end of May, 1984) were heated and kept liquid throughout the experiment.

Results and Discussion

Very little difference was seen for FA when using lard and a vegetable oil which was solid at room temperature compared with the corn oil. There was slightly more of the top layer when using the lard and the amount of oil absorbed was just significantly higher than the results obtained when using corn oil.

Figure 19 - Fat absorbance of BPP at different temperatures

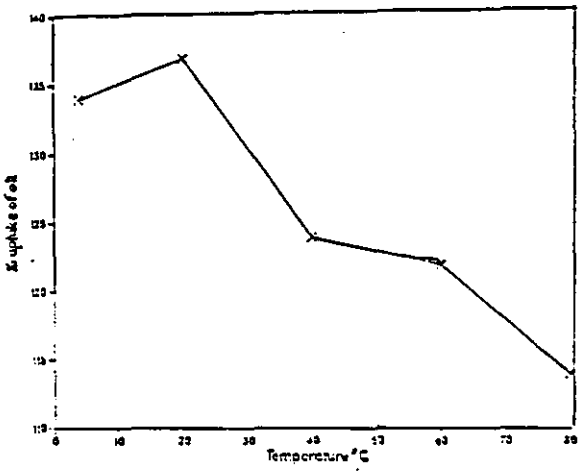


Figure 20 - Amount of oil taken up by varying amounts of powder

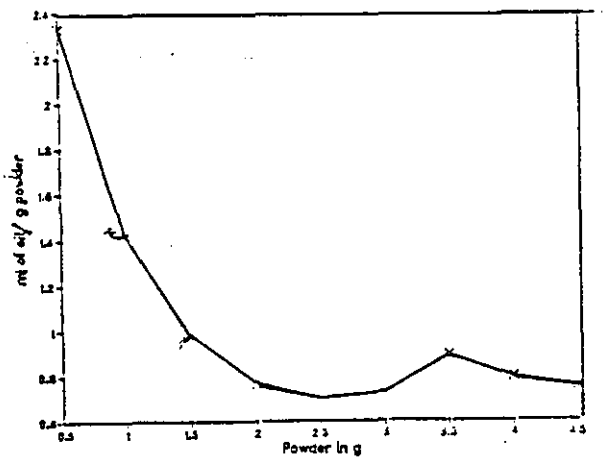
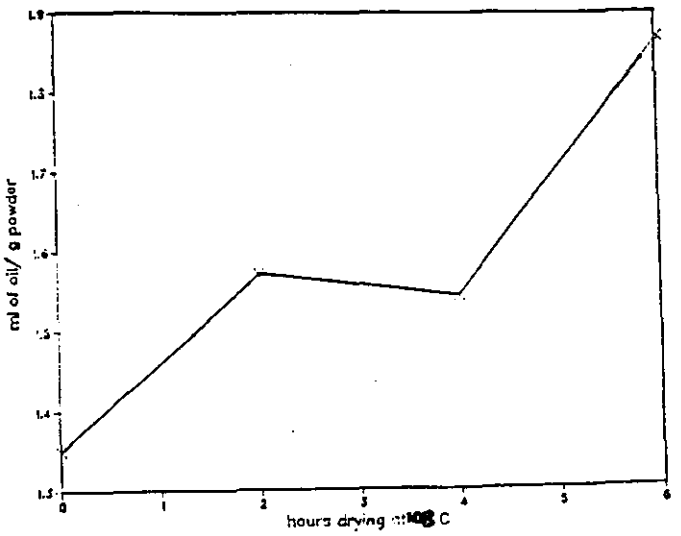


Figure 21 - Amount of oil taken up by BPP when heated at 108°C for different times



7.1.J. Content of the T and B Layers - Appendix 7.1.J

The formation of two layers, of higher and lower specific gravity than corn oil, for mixtures of BPP with corn oil made further analysis of these mixtures necessary for a better understanding of the procedure.

Method

The two powder/oil layers were formed in the usual manner except the amounts were increased to 40 g of powder mixed with 200 ml of oil. Samples of the top and bottom layers were taken and respun to ensure uncontaminated samples of the two layers could be taken. Approximately 5.0 g of each layer was extracted with ether, some water had to be added to break the emulsions if they occurred. The ether extract was evaporated and the weight of residue recorded. Protein content of the top and bottom layers was estimated using standard Kjeldahl techniques.

The results were calculated as % weight for the powder and oil components and as % volume, with the assumption that the specific gravity (0.916 g/ml) for oil had not changed.

Results and Discussion

Results indicated that the differences between the top and bottom layers was not so much a change in weight of the two constitutents, but in the volume occupied by BPP. The ratio of oil to powder by weight showed that slightly less powder was present in the bottom layer. A marked difference in the layers could be seen from the ratios for the respective volumes of oil and powder. The top layer having powder in twice the volume

compared with the base layer. This may indicate particles of a less dense nature, perhaps with air trapped inside the particles during spray drying.

T and B may not be homogenous, there could be a range of densities for the powder/oil mixtures formed above and below the free corn oil. Particle size has been reported as an important factor in fat absorbance (Hutton and Campbell, 1977; Jasmin, 1983) but this study would indicate the more important factor would be shape and bulk density of the individual particles. Good correlations between fat absorption and bulk density were established by Wang and Kinsella (1976) and Schactel (1981). The accurate regulation of the pressure control in the spray drying of the plasma could be essential for particles of standard shape and size which would therefore give reproducible fat absorption values.

7.1.K. Fat Absorption with Fine and Coarse Particles - Appendix 7.1.K

The relationship between the high and low density powder/oil mixtures required further investigation in respect to the powder particle size. A sample of fine and coarse particle size was obtained from the same batch of powder and assayed.

Method

A sample from a well mixed batch of bovine plasma powder was taken and kept for assay. The powder was then passed through an Augsberg Alpine Classifier at a speed just high enough to collect a sample of powder of low particle size. The residue powder was passed once again through the Classifier to ensure a removal of the particles of small size. A sample of powder of low particle size

was kept. The residue powder was again passed through the Alpine and this time the speed was adjusted so that coarse particles could be collected. Samples of high, low and middle range particle size powders were assayed for fat absorption in the normal manner.

Results and Discussion

The most noticeable difference in the results was the reduction of fat absorption after the powder had been put through the classifier. The amount of top layer formed by any of the products was less than that of the original powder. This indicates that alterations had occurred within the classifier, perhaps heat changes or mechanical disruption of fragile particles. The particles could have contained air sacs and if broken they would create particles of lower size, but of higher density. The fine particles had a higher fat absorption and also formed more top layer than the coarse particles. However, some top layer was formed even with the powder of large particle size.

7.1.L Variation when BPP was heated for Various Times - Appendix 7.1.L

The amount of fat absorbed is normally attributed to the protein. Several authors have investigated the relationship between the amount of soluble protein, as distinct from the total protein, and fat absorption, but no correlation has been found (Lin, Humbert and Sosulski, 1974; Schachtel, 1981). When a protein is subjected to heat, in the presence of moisture, denaturation of the protein is likely to occur (Beckel, Bull and Hopper, 1942). This study was done to see if heating the powder would effect the FA.

Method

The normal method for FA was used. One powder sample was placed in an oven for 5 hours at 85°C and this powder used in the estimation. BPP samples were put in an oven for 2,4,5 hours at a temperature of 108°C the powders were allowed to cool and then, with an unheated control, assayed for fat absorption. Values were calculated to allow for the moisture content.

Results and Discussion

No differences in FA were detected between the control and the powder dried at 85°C for 5 hours. The tendency for samples dried at 108°C to take up more oil increased the longer they had been heated at that temperature. The correlation between FA mean values and the heating time was 0.922 , Figure 21 .. If the weight of powder was calculated to allow for the moisture content the correlation was 0.860. The T and B layers both tended to increase after prolonged heating.

This work would indicate prolonged heating of powder actually encourages the uptake of oil. This could be due to the moisture inhibiting the uptake in non heated samples or to the heating process altering the particles or protein to encourage absorbance. Section 7.1.E indicates no alteration in FA when small quantities of water was added to the powder. No action was taken to establish the actual amount of denaturation caused by the heating regimes. The decrease in the uptake in oil with increasing temperature, as described in Section 7.1.C was not due to the powder being heated.

7.1.M. Fat Absorbance with Various Protein Fractions of Bovine Plasma - Appendix 7.1.M

The functional property of fat absorbance is dependent on the protein and on the physical properties of the powder.

To establish if the manufacturing process was important in the FA a variety of powders, all from plasma, were investigated.

Method

Solutions of BPP were made and then redried using a freeze drying process. A fresh plasma sample and a sample of BPP No.11 were taken through the freeze drying process to act as controls.

Products obtained from plasma, by other methods, were also assessed. The following products were used to determine fat absorption:-

Code	Product
2	Powder No.2 Manufactured at LUT from bovine plasma concentrated by UF, spray dried on 25.4.83
A	Bovine Albumin Supplied by Sigma, lot 123f-0634 No. A-706. Described as 98-99% albumin, remainder mostly globulins, 1.3% water. A crystalline cream flaked material prepared from pasteurized bovine serum and low temperature solvent precipitation, charcoal treatment and extensive dialysis to reduce the low molecular weight substances.
G	Bovine Globulins Supplied by Sigma, lot 93f-9360 No. G-2263. Described as Cohn fraction II and III, protein 75% rest mostly sodium chloride. A flaked white/cream powder which was not shiny.
FV	Bovine Albumin Fraction V Supplied by Sigma, lot 97C-0184 No. A-4503. Described as Cohn fraction V, nitrogen content 15.4%, 2.4% water. A fine cream powder which was light reflective.

P fd	Bovine Plasma freeze dried
10%fd	10% Solution Powder No.11 10% solution freeze dried
20%fd	20% Solution Powder No.11 20% solution freeze dried
SD fd	Powder No 11 Powder No. 11 put through the freeze drying process
11	Powder No.11 Normal powder No.11 made from bovine plasma, manufactured at LUT by UF and spray drying.

Results and Discussion

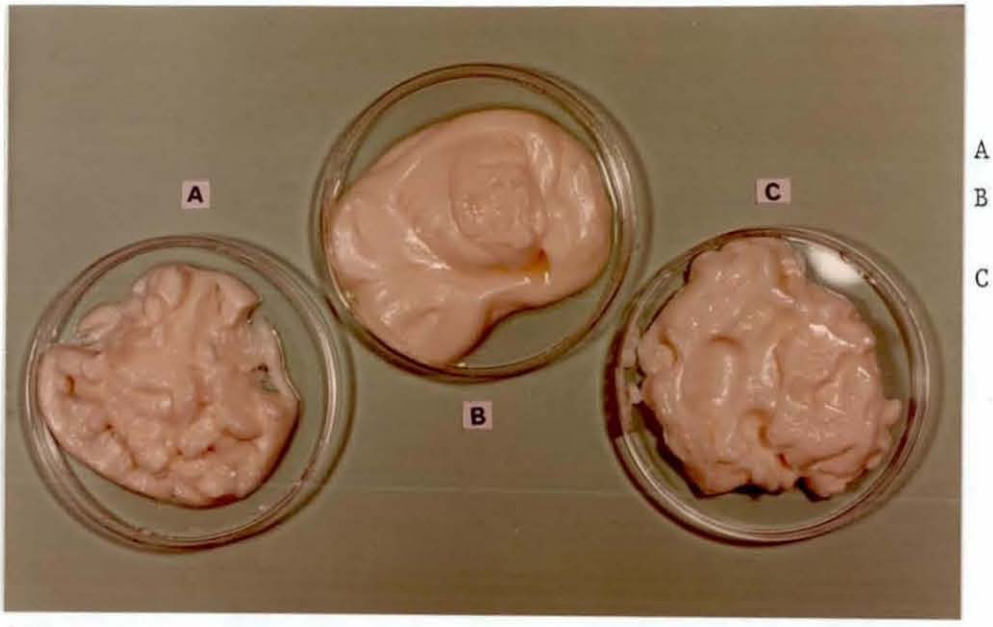
Only the powder formed by spray drying produced the top layer of powder/oil mix, the freeze drying process did not alter, in any way, the already dried powder. The use of powder produced from the freeze dried solutions of powders formed by spray drying did not produce any top layer, proving that the phenomenon of low specific gravity oil/powder layers is dependent on the physical properties of the powder. The freeze dried material did have a higher fat absorbance value, as calculated by the standard procedure, than the powders formed by spray drying. The freeze drying of the protein solutions produced powders of larger particle size and of less spherical shape than the spray dried products.

The various plasma fractions gave differing values for fat absorbance. The two albumin fractions gave very different values, although chemically the fractions should be similar. The fat absorbance value for globulin was very great at 6.35 ml oil/g powder, nearly 5 times greater than that of spray dried whole plasma. The particles of the plasma fraction powders were not very spherical in nature and of greater size compared with the spray dried plasma, average size for bovine albumin (A) was 188 μ and for powder No. 2 it was 17μ.

The size and shape of the particles are most important in fat absorption. The formation of the top layer only occurs with small spherical particles produced by spray drying. Even when the particle size is approximately the same there can be marked differences between powders produced in different ways, but which are chemically similar, i.e., the powders A and FV. The globulin fraction would appear to absorb more oil than an albumin fraction made by the same process and formed of particles of similar size.

7.2 EMULSIFICATION

Figure 22 - Emulsions made from 1% powder, 24% water and 75% oil



A = BPP

B = BPP after 14 days

C = Egg Albumen

7.2A Establishing a Method

In general the process and the equipment used in making food emulsions, particularly very viscous emulsions, exert a major influence on the properties of the emulsion. The criteria used to estimate an emulsion are various. In the procedures used in this work emulsion capacity is the most important, with the stability and the viscosity also being noted. In a further investigation the changes noted for EC under various conditions were examined in respect to the alteration in the globule size of the dispersed phase.

7.2.B Variation in Mixing Procedures Appendix 7.2.B

Method

A 2% solution of BPP in 25 ml water was added to 80 ml of corn oil. The mixture was mixed for two minutes using the standard Silverson 18788 fitted with one of the six heads being tested. The EC was determined, the same Silverson head being used.

Results and Discussion

Tornberg (1980) stated that the emulsifying conditions were the dominant factor in emulsion formation and the protein was of only minor importance. This was found to be the case in this study. No emulsions were formed with the large (1.25 inch) screens, even though the mixing appeared to be vigorous and complete. In contrast the smaller heads for the Silverson, even with apertures of the same type and shape as the large heads, all formed emulsions.

The head producing the emulsion of highest capacity was the 0.75 inch emulsifying head, next was the head used in the standard procedure the 0.75 inch disintegrating head and then the microunit. No action was taken to preclude the introduction of air into the system. The incorporation of a gaseous phase into an emulsion has been reported to reduce the emulsifying properties (Pearce and Kinsella, 1978).

7.2.b Variation in Duration of Mixing Appendix 7.2.b

Method

The standard methods were employed except the initial mixing time was altered.

Results and Discussion

Many studies on emulsions have reported that the length of time the emulsion had been mixed was relevant (Tornberg and Hermansson, 1977; Hayes, Stranaghan and Dunkerley, 1979; Tornberg, 1980; Waniska, Shetty and Kinsella, 1981). Under the conditions used no change in ES was apparent. The viscosity data, although known not to be numerically correct (Section 6.4.1), indicate an increase to 2minutes and then remains constant at that level. Huffman, Lee and Burnes (1975) found that viscosities increased with increased mixer

speeds. The increased oil - water emulsion viscosities with increasing rate of shear are associated with decreased oil particle size (Becher, 1966).

The EC appears to increase to a maximum at 2 minutes and then to decrease, see Figure 23. The same findings of increasing shear forces decreasing the EC has been reported by many workers. Some studied duration of mixing, or the number of times an emulsion had passed through an homogenizer (Hayes, Stranaghan and Dunkerley, 1979) or the speed of mixing (Ramanathan and Ran, 1978; Betchart, Fong and Hanamoto, 1979). The stability rating has also been reported to be dependent on the shear forces, prolonged emulsification will give a creaming stability up to a certain limit where no further increase in mixing enhances the stability (Tornberg and Hermansson, 1977). Waniska, Shetty and Kinsella (1981) investigated the kinetic energy being imparted to an emulsion system by temperature. Again the finding of increased emulsifying properties followed by a decrease was obtained as the temperature increased.

Carpenter and Saffle (1964) stated that differences in droplet size were related to the change in EC with blender speed. Ivey, Webb and Jones (1970) suggested that the oil is emulsified until the continuous phase is spread too thinly to prevent the agglomeration of the oil. The phenomenon of the decreasing capacity can be explained if excessive unfolding of the protein occurs. The denaturation of the protein will destabilize the film at the oil-water interface and coalescence of the fat droplets will occur.

7.2.D Variation in Amount of Powder Appendix 7.2.D

Method

A 5% solution of bovine plasma powder was prepared and diluted with distilled water to form a range of concentrations of 0.015 to 1.25 g of powder per 25 ml of solution. The 25 ml aliquots were used in the standard procedure to produce values for emulsion capacity, stability, viscosity and emulsion turbidity.

Figure 23 - Effects of Duration of Mixing on Emulsion Properties of BPP

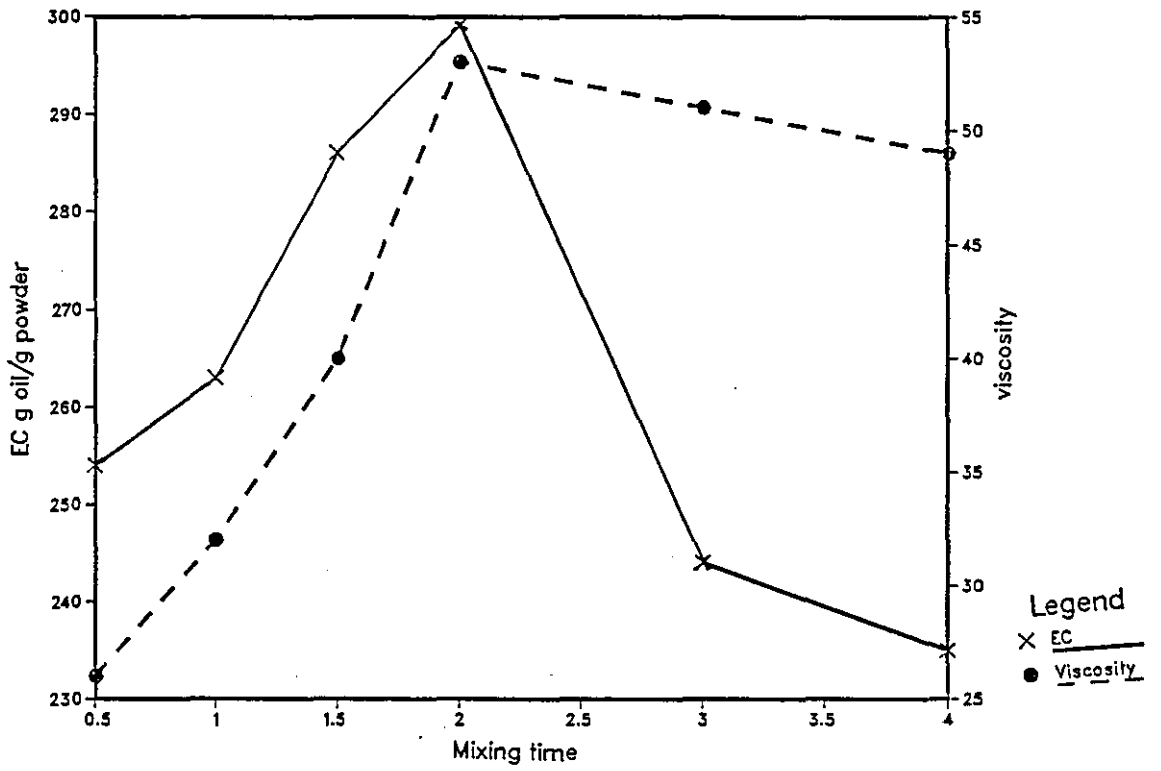
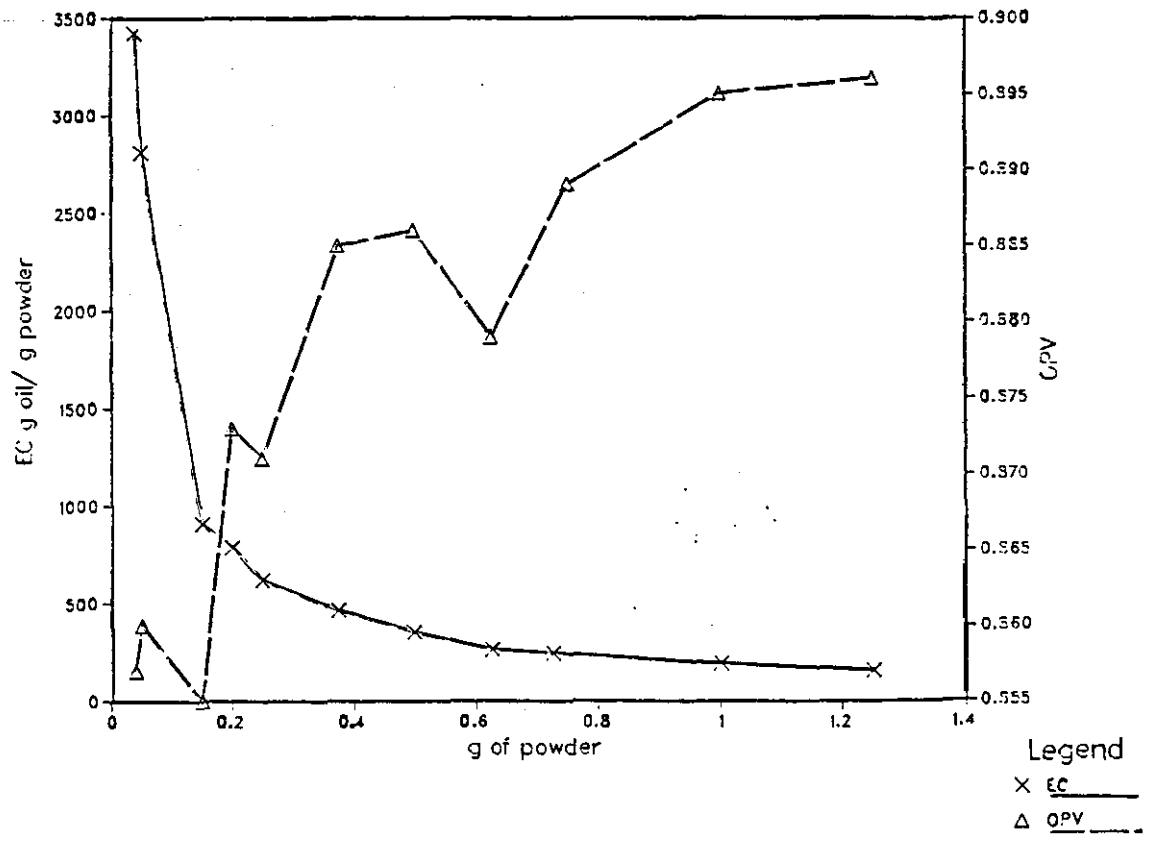


Figure 24 - Emulsion Properties with Variation in the Amount of Powder



Results and Discussion

The actual volume of oil added to the sample increased as the protein concentration increased. However, if the values were calculated as g of oil per g of protein there is a hyperbolic relationship with lower EC values with higher protein concentrations See Figure 24. The curve will approximate to a straight line if values of 1/EC are used, see Appendix 7.2.E.c. This type of curve has been obtained with other protein products:- groundnut protein (Ramanatham and Ran, 1978), peanut flour (McWatters and Holmes, 1979a), muscle protein (Acton and Saffle, 1972).

Oil Phase Volume (OPV)

Acton and Saffle (1972) determined that a phase volume factor exerted some control over the maximum level of oil incorporated into emulsions. The results obtained in this study were used to determine the phase volume of oil at which the emulsion collapse occurred. The percentage variation between EC values for 0.0005g and 1.25 g powder was 94% while for OPV the difference was only 5%. This type of result was obtained by Acton and Saffle (1972). In the study of Crenwelge et al (1974) the OPV obtained using varying concentrations of protein altered by more than 40% for the globin, soy, cottonseed and nonfat dried milk.

Tybor, Dill and Landmann (1973) investigated the OPV in relation to spray dried and unprocessed serum (control). The OPV values for the processed serum were all less than 50% and there was a straight line relationship between the protein concentration and the OPV, while the control values gave a hyperbolic curve with OPV values of 60 to 90%. All the OPV values in this study with the BPP were above 85%. Different types of curve were obtained for the serum processed by Tybor, Dill and Landmann (1973) at different pH values. The hyperbolic curve for the control serum was obtained at all pH values investigated.

In a later study Tybor, Dill and Landmann (1975) investigated the effect of spray drying at different temperatures on the OPV at different protein concentrations. It was found that drying either at 160 or at 193°C, did not effect the hyperbolic curve for OPV and the maximum response to protein concentration occurred at about 0.5 g protein/100 ml.

Viscosity

The values obtained for viscosity in this study are not to be relied on, due to the system of measurement. The results indicate very low values at protein concentrations of less than 1.5%, then a steady level from 1.5 to 3.0%, the levels are then increased again. In the study of Acton and Saffle (1970) there was no great difference in emulsion viscosity up to 0.25% protein, but, at 0.5 to 0.75% protein the emulsion viscosity increased considerably.

Stability

The stability values only indicate the samples which had broken and those which had not. As the protein concentration was increased, more protein was available for film formation. The amount of oil is constant for all the samples in the ES study, therefore the total surface area of the emulsified oil was constant. The increased protein will therefore increase the thickness and strength of the film and enhance emulsion stability as was found by Wang and Kinsella (1976). Evidence of increased protein layer thickness around the fat droplets with increased protein concentration was observed microscopically by Ivey, Webb and Jones (1970).

Turbidity

There was a general increase in turbidity with protein concentration. Pearce and Kinsella (1978) found that protein levels greater than 0.1% caused a progressive increase in turbidity.

Turbidity can be interpreted as emulsifying activity (EA). Waniska, Shetty and Kinsella (1981) found that EA progressively increased with protein concentration although not linearly. As the amount of protein in the continuous phase increases the total interfacial area does not increase as rapidly. It has been reported that <8 mg of protein could provide cover for $1M^2$ of oil water interface. Protein levels greater than that required to significant alter the total surface area will thicken and strengthen the film. Waniska, Shetty and Kinsella (1981) found that emulsions made with high protein concentrations were less affected by prolonged homogenization.

7.2.E. Variation in Volume of Water - Appendix 7.2.E.

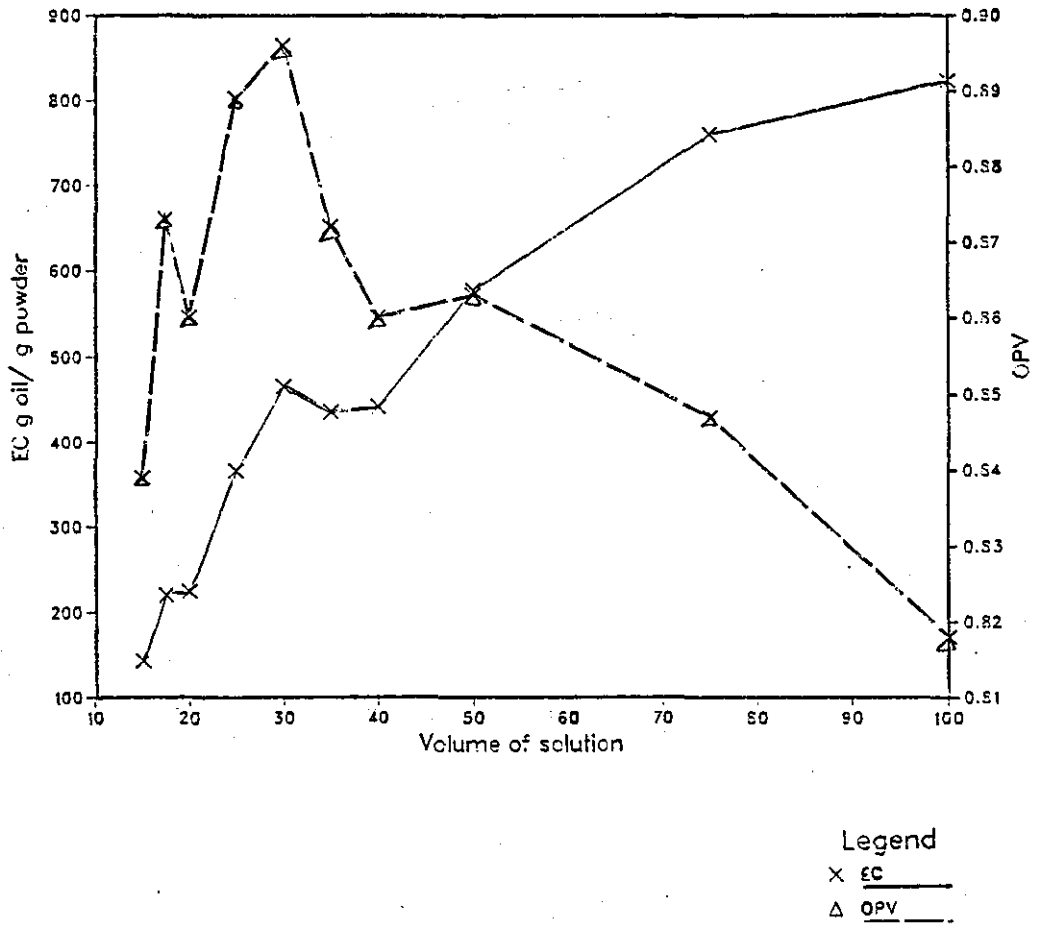
A 10% solution of BPP was prepared. A 5 ml sample of the 10% solution was added to each test plus a volume of water to achieve volumes of 5 to 100 ml. These samples were used in the standard procedure to study the emulsifying properties.

Results and Discussion

The increase in the water volume means that the continuous phase is greater, but the protein is less concentrated within the water phase. Figure 25 shows the increased EC as the water volume increases. A hyperbolic effect was obtained over the range studied. If even more water was added to the system there was marked creaming with the emulsion floating to the top leaving the continuous phase below.

The OPV appears to reach a maximum level and then is reduced. The highest value obtained was 0.89, with a water volume of 30 ml which meant the protein level of 1.3% was present in the solution.

Figure 25. Emulsion Properties with Variation in Amount of Water in the Sample



If the results are plotted as EC against powder g/100 ml the hyperbolic curve is similar to that obtained for the powder variation, Appendix 7.2.E.b. A plot of 1/EC values when altering the concentration of powder produced a near linear relationship, $r > 0.95$. Plots of log EC and similar graphs for variation in the continuous phase volume were hyperbolic, Appendix 7.2.E.c. The effect of continuous phase alteration on the EC value is apparently equivalent to the volume remaining constant and the protein level changing, the protein concentration therefore being the important variable. The values obtained for the OPV are not similar in both experiments.

7.2.F Variation in the Amount of Salt - Appendix 7.2.F

Method

A range of concentrations for sodium chloride were prepared by making up a 200 mM stock solution and diluting this to achieve a 2% W/v protein solution containing 6, 12, 25, 50, 100, 200 mM sodium chloride. Samples of 5% (856 mM) and 10% (1712 mM) sodium chloride were also made. Aliquots of 25 ml were used in the standard procedure to produce emulsion values.

Results and Discussion

Powder No. 12 already consisted of 2.6% salt and therefore the solution already was approximately 9 mM. The values stated are for the additional salt levels and do not include that concomitant with the protein source.

Most sodium chloride levels seem to enhance the EC of the bovine plasma, only 10% salt being worse than no sodium chloride addition. The increase of 6 mM much elevated the EC while 12 mM was not so good as 25 mM. There then seems to be a decline in EC as the sodium chloride concentration increases, with a level of 1712 mM (10%) being less than before the salt addition. Working with BSA and using EA

as a criterion for emulsifying properties Waniska, Shetty and Kinsella (1981) reported the same sort of findings. It is reported that sodium chloride levels < 20 mM failed to give optimum EA, levels of 100 - 600 mM enhanced EA and levels above 750 mM decreased activity.

Salt appears to effect the emulsifying properties of a variety of proteins. Peanut flour is affected at levels of 100 mM and 1000 mM sodium chloride with the EC being reduced and the emulsions totally inhibited at some pH levels (McWatters and Holmes, 1979a). There was also an association between pH and salt with soy flour, levels of 100 mM at certain pH values stopped emulsions while levels of 1000 mM enhanced EC (McWatters and Holmes, 1979b). Nath and Narasinga (1981) also studied soy protein and found an increase in EC up to 900 mM sodium chloride and a decrease at higher values. Similar results were found for Guar protein and protein from Candida Utilis.

In the study of Tornberg and Jonsson (1981) the interfacial behaviour of blood plasma was investigated and it was found that 0.2M sodium chloride enhanced the interfacial activity over a range of protein concentrations. Even when the plasma already contained salt an additional amount had a large influence on the surface activity. When no salt or very low ionic strengths were used the electrical double layer surrounding the protein was not at its strongest, causing the electrostatic forces during adsorption and between the molecules already adsorbed to be high. The protein will therefore not pack densely at the interface. This would be related to low interfacial activity (Tornberg and Jonsson, 1981) and the lower EA of blood plasma (Waniska, Shetty and Kinsella, 1981). Over a range of salt levels the surface changes of the protein at the interface are probably neutralized, causing reduced repulsion and therefore increased rate of protein adsorption. The interfacial concentration and the strength of the film are then at an optimum to achieve high EC values.

At higher ranges of ionic strength it is possible that the salt enhances the solubility of the protein (salting in), encouraging it to stay in the continuous phase rather than its transfer to the oil/water interface.

The effect of salt on an emulsion system can be complicated. Not only is the level of salt important, but also the pH and concentration of the protein. The results of these studies would indicate that sodium chloride levels of up to an additional 100 mM would enhance EC of blood plasma. It should be noted that all salts may not have the same effect as they vary in their action on the conformational stability of the protein. Also the diverse reports on the level and consequences of sodium chloride on EC of other protein substances could be due to the variance in protein structure and therefore activity of the molecule at the oil/water interface. The salting-in effect of addition of salt must be considered for products not very soluble in water.

7.2.G Variation in Amount of Glucose - Appendix 7.2.G

Method

The same range of concentrations as prepared for the sodium chloride were used. The 5% glucose was equivalent to 252 mM and the 10% was 504 mM.

Results and Discussion

The values obtained for the ES were higher than normal for some concentrations of glucose. The EC was higher with low additions (6-50 mM) of glucose the level then drops below that achieved when no additional sugar was added. Wang and Kinsella (1976) working with alfalfa leaf protein, found that the addition of 50% sucrose produced inconsistent effects and that in the EC test the inversion point was undetectable under the standard conditions.

High levels of glucose could encourage protein away from the interfacial film into the bulk phase as glucose is a polyhydroxyl compound. Low levels of the glucose could reduce the electrostatic forces at the interfacial film, thus forming denser films and higher emulsion capacities.

7.2.H Variation in pH - Appendices 7.2.H.

Initial Study

Method

A solution containing 2% bovine plasma No. 12 was prepared. In study A the pH of 25 ml aliquots of solution was altered by the addition of 1M hydrochloric acid or 1M sodium hydroxide. In the other studies the bulk solution was divided into two aliquots. To one portion was added 1M Sodium Hydroxide and the pH of the solution carefully monitored. When the pH was at a value required a 25 ml aliquot was taken for use with the standard procedure to establish the emulsion properties. The second portion of 2% BPP was used to obtain the acidic range of samples, by the addition of 1M Hydrochloric acid. This procedure was repeated and the studies have been called B and C. In the study C the standard Silverson 18788 was used.

The pH of the emulsion after formation was also noted. The data for the solubility of the protein at different pH values were used to calculate the emulsion data in terms of the amount of soluble protein in each solution for study C.

Results and Discussion

No consideration for the dilution effect, nor the increase in salt effect, which may have occurred in the samples of altered pH, was made. The natural pH of the samples was 8.4. To ensure that the oil did not have any marked effect on the pH of the system it was tested and was found to have an acid value of only 0.19 mg KOH/g.

Results were different in each experiment and not reproducible. It was noted that during the first experiment that some emulsions looked different. All the emulsions were similar at the mixing of 80 ml oil with the 25 ml of 2% solution. However, when the 25 g aliquot was taken and additional oil added some of the emulsions became thick and viscous while others became thinner, but with no defined breakpoint. This type of result had not been seen when working with emulsions at their natural pH, normally between 8.1 - 8.5. Oil could be initially added with or without homogenization in the early stages of EC estimations without the results being different. Therefore, no careful check on homogenization speed was made during the initial few ml of oil addition to the 25 ml aliquot. The emulsions, at various pH values, must have oil added in an exactly specified manner for the procedure to be reproduced giving the same emulsion structure and therefore EC value. This would indicate that the emulsions formed at some pH values are not so stable as others and are therefore more affected by the shear forces.

Second Study

Experiment 1

Method

Samples were prepared by weighing out 0.5 g of BPP No.12 and adding 25 ml of water. The resulting solution was adjusted to pH 9.5. This was done five times. A bulk solution containing 200 ml of water and 4 g of BPP was prepared and adjusted from 8.1 to a pH of 9.5 using 0.1M sodium hydroxide. Five 25 ml aliquots were taken. The 10 samples were then assayed using the standard procedure to ascertain the emulsion capacity and the EAI values.

Results and Discussion

The results are given in Appendix 7.2.H.b. There were no significant differences in the two types of sample analysed by using the turbidity data. The assay for EC did show differences in the mean values, but the standard deviation were similar in both sets of results. The results of McWatters and Cherry (1977) indicated

that two step pH adjustment of a protein would effect the values for emulsification.

This study does indicate the complex nature of emulsion formation. The bulk and single sample were identical in all respects except in the nature of pH adjustment yet a significant difference between the two sample types was found for the EC values.

Experiment II

Method

Solutions were obtained over a range of pH values as for B and C. Emulsion capacity and EAI values were estimated. The study was repeated.

Results and Discussion

The rate and time of the shear was carefully monitored for all the samples and in this experiment the replicate samples showed good correlation, see Figure 26 .

General Discussion

Emulsion capacity

The results for emulsion capacity indicate a low value at pH 5, this corresponds to the pH where the solubility is least. It has been suggested that pH influences the emulsion capacity in an indirect manner by affecting the solubility of the protein. Swift, Lockett and Fryar (1961) reported that only the soluble protein acted as the emulsifier. However, although at certain pH values the BPP is less soluble the reduction is only 15%. The study on powder concentration Section 7.2.D. indicates that the change of protein from 0.5 g to 0.425g is only equivalent to $\sim$ 3 ml less oil emulsified by the sample and hence, if the same protein weight is assumed, a difference in EC of only 4%, not the changes of 20% seen in this work.

The bovine powder is a combination of proteins and if they have different abilities in the stabilization of the emulsion and are precipitated at variable levels, a marked decrease in EC could be recorded without much change in the overall level of protein in solution.

The lowest EC values are recorded at the pI region for the bovine plasma. This has been found with other proteins, sunflower meal (Huffman, Lee and Burns, 1975), guar protein (Nath and Narasinga, 1981), globin, cottonseed and non fat dry milk (Crenwelge et al, 1974), peanut flour (McWatters and Holmes, 1979a), groundnut protein (Ramantham and Ran, 1978).

Emulsion Viscosity

The viscosities of the emulsions were measured in the first two studies, but with no conclusions. McWatters and Holmes (1979a) found that the viscosity of emulsions made with peanut flour was least at the isoelectric point, while the viscosity of whey protein was found to be highest at its isoelectric point (Yamauchi et al 1980).

Turbidity Measurements

The results for the turbidity and EAI estimations from this study do not give a clear indication of the effect of pH. The lowest values do appear to occur at the isoelectric point, but other low points also occur. In the study using whey proteins it was found that the turbidity increased with pH for values above 5.5, but was constant below this value (Pearce and Kinsella, 1978). The pI of β-lactoglobin, the major protein component of whey protein, is approximately 5.5. Waniska, Shetty and Kinsella (1981) working with BSA noted a marked decrease in EA at pH 9, but steady levels from pH 4 to 8.

Figure 26 - Emulsion capacity of BPP at different pH values

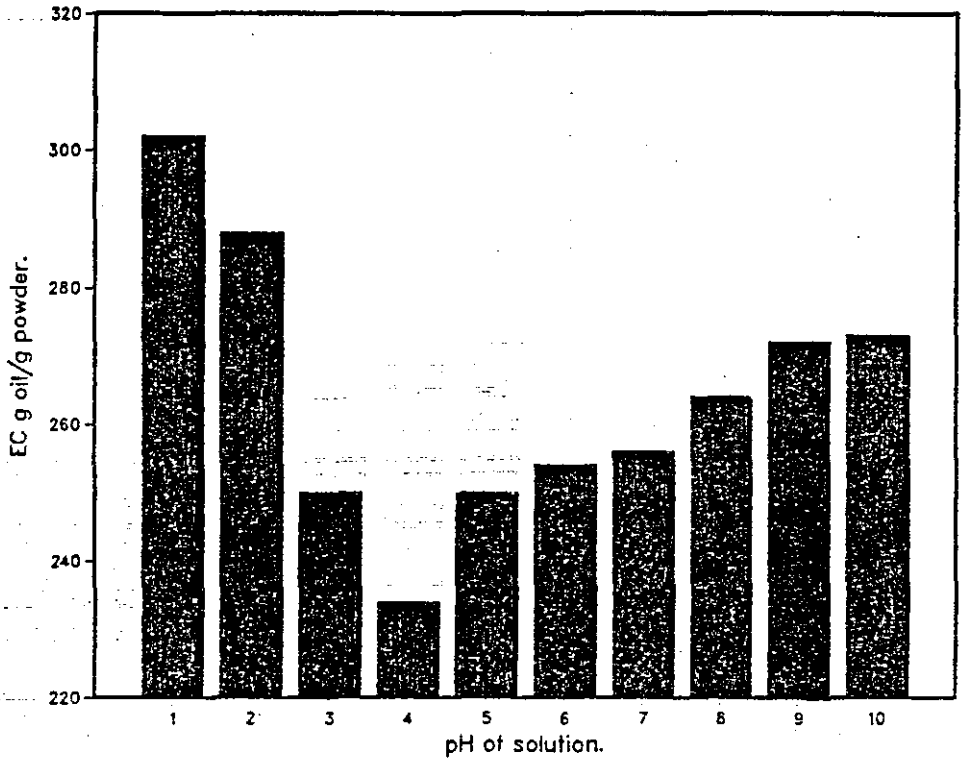
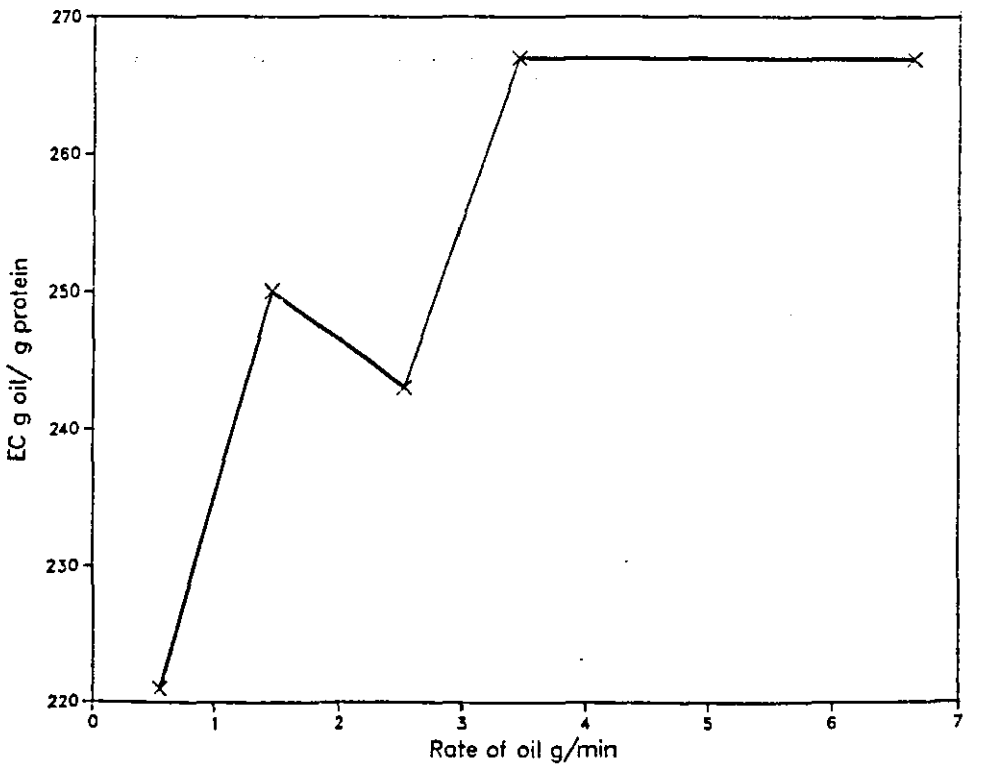


Figure 27 - Emulsion capacity of BPP when oil is added to the Emulsion at different rates



Emulsion Stability

The method used to establish stability in this study only measured the gross changes. From the results obtained the emulsions were less stable at the acid range of pH values and in the range pH 11-12. A water layer was also noted in some samples, denoting creaming was taking place. With whey protein Yamauchi et al (1980) found that the stability was minimal at the pI. However, there were other findings by different workers. In general stability seems to be minimal near the pI which suggests that surface rigidity is important in resisting coalescence. However, the studies indicating the opposite effect could show a role for electrostatic or steric repulsion between the surface films. This could be particularly true at low protein concentrations

7.2.I Effect of Rate of Oil addition on EC - Appendix 7.2.I.

Not only has the amount of shear been found important in the formation of emulsions but, also the rate at which the oil is added.

Methods

An emulsion formed by the standard method was divided into 25 g aliquots. Each aliquot was mixed with oil being added at a known rate, until the emulsion broke. The EC for the sample was calculated.

Results and Discussion

The capacity apparently increased as the oil addition rate increased, up to 3 g per minute, the levels were then steady. It was shown in Section 7.2.b that the duration of mixing affects EC A decline in EC occurs with increased time of mixing as the rate of oil being added to the emulsion gets less , Figure 27. Similar

results were found by Swift, Lockett and Fryar, (1961) and Huffman Lee and Barnes (1975). Swift, Lockett and Fryar (1961) suggested that the rate at which protein membranes are formed is almost instantaneous at high oil addition rates. Carpenter and Saffle (1964) had concluded that varying the rate of oil addition had no effect on EC. Crenwelge et al (1974) found that the rate of addition had an effect on certain proteins while others were unaffected.

7.2.J. Viscosity of Emulsion at Various Times after Mixing - Appendix 7.2.J

Method

Emulsions were made according to the standard procedure. The viscosity of the emulsion was estimated immediately and then at intervals. The emulsion was kept at room temperature. After the 24 hour reading the emulsion was remixed and the viscosity determined again.

Results and Discussion

The initial high viscosity obtained for the emulsion drops after 20 minutes and remains very steady over the next 6 hours. A high value was recorded after the overnight stand, but this was lowered after remixing.

7.2.K. Estimation of Particle Size to establish Emulsification Properties - Appendices 7.2.K.

There were three methods to estimate particle size. Turbidity of emulsions can be used to determine size and EAI values (see Section 2.8.4 and 5.5) The Coulter Counter TA11 with population accessory (Coulter Electronics Ltd, Norwell Drive, Luton, Beds) could be used following the methods of the Particle Sizing Laboratory, LUT. The solution supplied for size estimation consisted of 1 g of the standard emulsion made up to 100 ml with 0.1% SDS. The orifice tube used for the estimation was 200 μ. The particle

distribution between 80.64 and 4 μ was calculated. An example of the print out is given in Appendix 7.2.K.a. The results were calculated from a graph of cumulative % weight against the log 10 size in microns. The values used were the low size cut off point CC.S (cumulative weight 5%), the large size cut off point CC.L (cumulative weight 95%) and the size at 50%, CC.D50.

The other method employed the Malvern particle sizer and the method was the standard procedure, but the emulsion was suspended in 0.1% SDS. The Malvern was fitted with 100 μ lens and analysed the particles within the range 1.9 to 188.0 μ. A copy of the print out is given in Appendix 7.2.K.b.

Some photographs of the emulsion, stabilized in agar, were taken. A Leitz Orthoplan optical microscope fitted with a camera containing Ilford FP4 film was used.

Particle Size

There was a most noticeable difference in the numerical values obtained for the size distribution for the Malvern and Coulter Counter. Those obtained for the Coulter always being greater. However, the correlation between the results was good, r = 0.868 for BPP (Appendix 7.2.K.c). This difference between the two methods of estimation has been noted for many different particles (Clifton, 1985) and is not due to the particles being formed by oil globules.

The value R calculated from the turbidity data is meant to give an indication of particle size. The correlation between the Coulter size and R was 0.776 for BPP while the M.D50 figure with R had a correlation of 0.933.

Data on Various Mixing Times

In the original study EC increased up to 2 minutes mixing and then decreased again. The particle sizing indicated higher values for 0.5 minutes mixing compared to 2 minutes, but lower values were achieved at 4.0 minutes. This indicated that the globular size decreased with mixing. The EAI values showed no significant difference between the EAI values at 0.5 compared with 2 minutes, however the value 2 minutes was higher. At 4 minutes the EAI was greater than at 2 minutes and was significantly different. This indicated an inverse correlation between EAI and particle size. The results for particle size and EAI did not follow the same pattern as achieved for EC when examining various mixing times.

Data on the Oil Variation

The particle size of the globules and the EAI values were examined for oil volumes of 40, 80 and 100 ml. There were no significant differences for the EAI values even though the "oil. v." was of course much affected. Hardly any significant differences in particle size was found, but there may have been a tendency for decreased particle size as the oil volume increased.

Data on Emulsions with Variation in Protein Content

With the protein concentration there were great differences in EC values as EC is based on protein content. The OPV curve rose slightly with increased powder concentration. The hyperbolic curve for EC showed a marked decrease in capacity with increasing concentration of protein.

With the particle sizing no significant differences were seen although a lowering of size was noted at 5% powder and an increase at 0.5% compared with the 2% powder concentration.

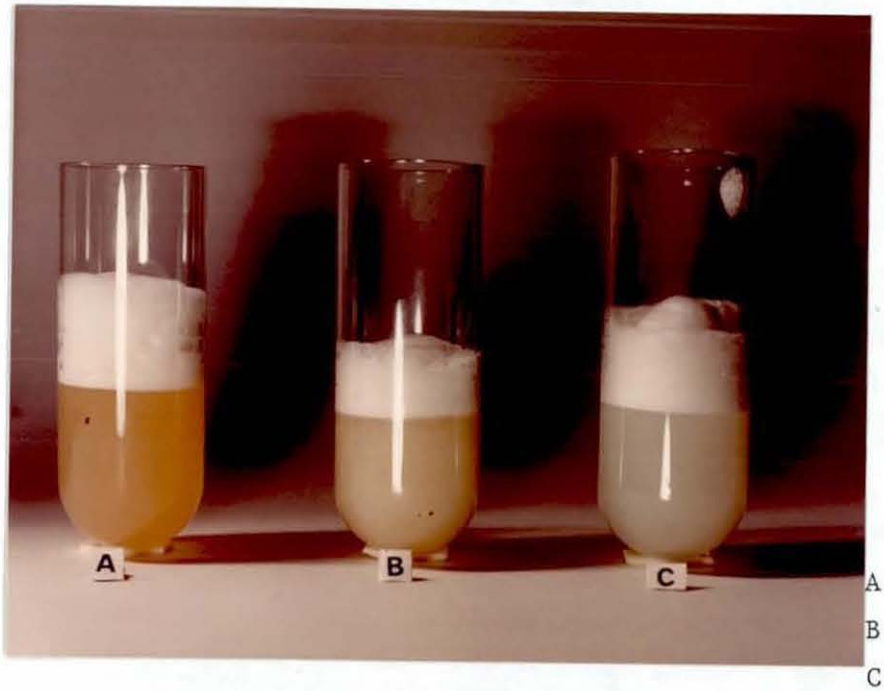
Data for Variation in pH

Difficulties had been experienced in obtaining satisfactory EC values with varying pH values. However, it was thought that a minimum EC value was obtained at pH5.

The particle size was found to be different at pH 5 and 9 than at pH 8.1, the size being larger at the naturally occurring pH value. The EAI values were greater for pH5 and 9 than that occurring at pH 8.1 and was significantly so at pH 9.0. It should be noted that these results do not correspond to those achieved in the original pH study.

7.3 FOAM FORMATION

Figure 28 - Foam Formation



A = Foam:BPP at pH 8.4
B = Foam:BPP at pH 5.0
C = Foam: Egg Albumen Powder

7.3.A Establishing a Method

To produce a foam a gas has to be introduced into the system. The bubble size in a foam can vary enormously and is affected by the conditions of formation. It was found that aeration of a solution of 2% BPP would produce a foam volume of 100 times more than that produced under the standard procedure. The method of formation must therefore be carefully defined in comparing foam volume data.

7.3.B Variation in Mixing Procedures - Appendices 7.3.B

Method

Solutions of 2% w/v BPP and 2% w/v egg albumen were prepared. Each of these solutions, 50 ml, were mixed by a Silverson mixer or within a Waring commercial blender cup. The foam was transferred to measuring cylinders and the total volume and amount of foam recorded at 5, 25 and 60 minutesfrom the start of mixing.

Results and Discussion

The study was only brief to give an indication of the preferable method. The longer the mixing time the more foam for egg in the

Waring blender, but less for the plasma. Higher foam volumes were achieved with the Silverson for the egg, and only slightly less for the bovine plasma compared with the blender. It was necessary to get the foam out of the mixing vessel quickly without disturbing the foam too much when transferring to the measuring cylinders. This was difficult to do with the vessels from the Waring blender. The Silverson was chosen for the standard procedure.

Variation in the size of the Mixing Vessel

Method

The standard procedure using the Silverson mixer was followed in all respects except that the foams were produced in vessels of various internal diameters (ID), a list of vessels used is given in Appendix 7.3.B.b. The ID of the vessels was noted. After the foam had been produced it was transferred to the normal plastic cylinders.

Results and Discussion

The results indicate that the volume of protein stabilized foam increased when the vessel in which it was mixed was of large diameter, see Figure 29. Expansion was increased throughout the range 35.6 to 76.7 mm ID at the three time points studied. However, there was some tailing off of volume increase at diameters greater than 65.4 mm at time points 25 and 60 minutes indicating some loss of stability.

Variation in size of the Holding Vessel

Method

The ID of a range of vessels were estimated, a list of the vessels used and the results are given in Appendix 7.3.B.d and 7.3.B.e. The volume of material held in each vessel was also calculated from a measurement of the height of product. The

standard procedure was carried out for the manufacture of a foam, except that after production the foam was transferred into the appropriate vessel instead of the normal 100 ml measuring cylinder. The total volume of material and the height of foam was estimated at 5 minutes, but at 25 and 60 minutes just the height of foam was recorded.

Results and Discussion

The results indicate that the vessel holding the foam, for a time interval of 58 minutes does not dramatically alter the amount of foam. The matrix of the foam is therefore not dependent on the surface area of the walls supporting the gas/liquid system, or on the height of foam. Similar findings were found by Halling (1981).

7.3.b Variation in the duration of Mixing Appendices 7.3.b

Method

The standard methods were employed except the initial mixing time was altered between 0.5 to 5 minutes duration.

Results and Discussion

The length of mixing time has been stated as being important to the volume and the stability of a protein stabilized foam (Baldwin and Sinthavalai, 1974; Miller, Herman and Gronninger, 1976; Halling, 1981). Authors report that too much mixing results in foam breakdown (MacDonnel et al, 1955; Kinsella, 1979; Charley,1982). Under the conditions used in this study the volume of foam stabilized by BPP continued to increase as the mixing time lengthened, Figure 30. The initial foam volume increased as did the foam volume remaining after 25 and 60 minutes. There was some loss of stability (E25/60) with length of agitation. This effect was not apparent for the stability at 60 minutes. Baldwin and Sinthavalai (1974) found fish protein foams to be more stable with longer mixing times.

Figure 29 - Foam expansion when 2% solution of BPP was mixed in different sized containers

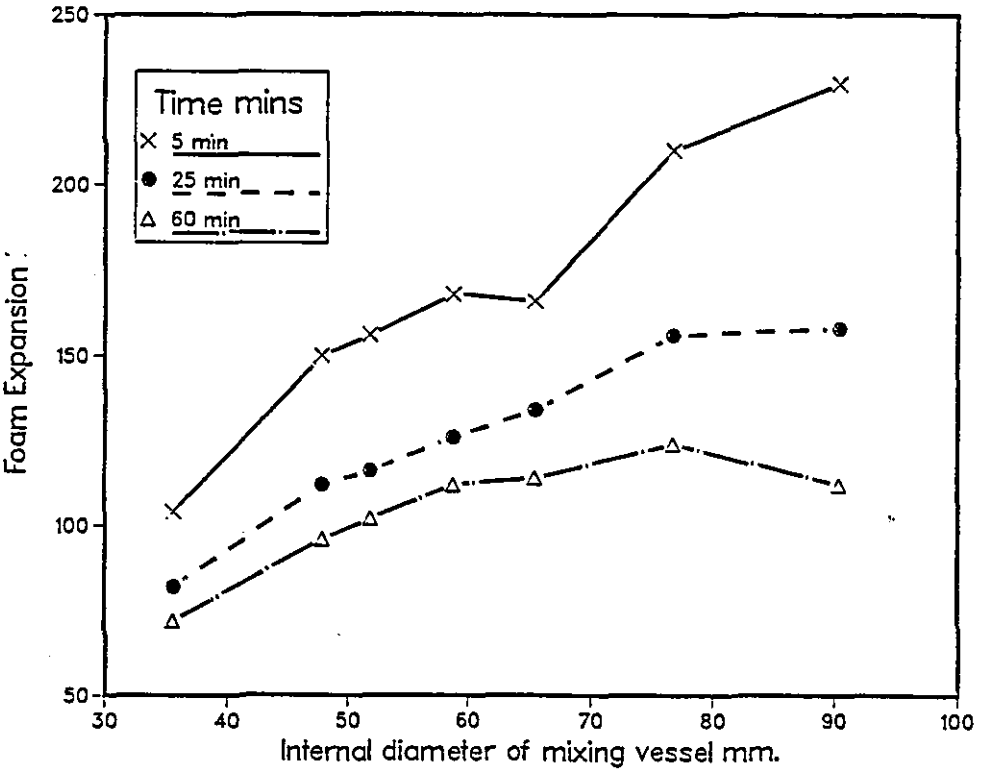
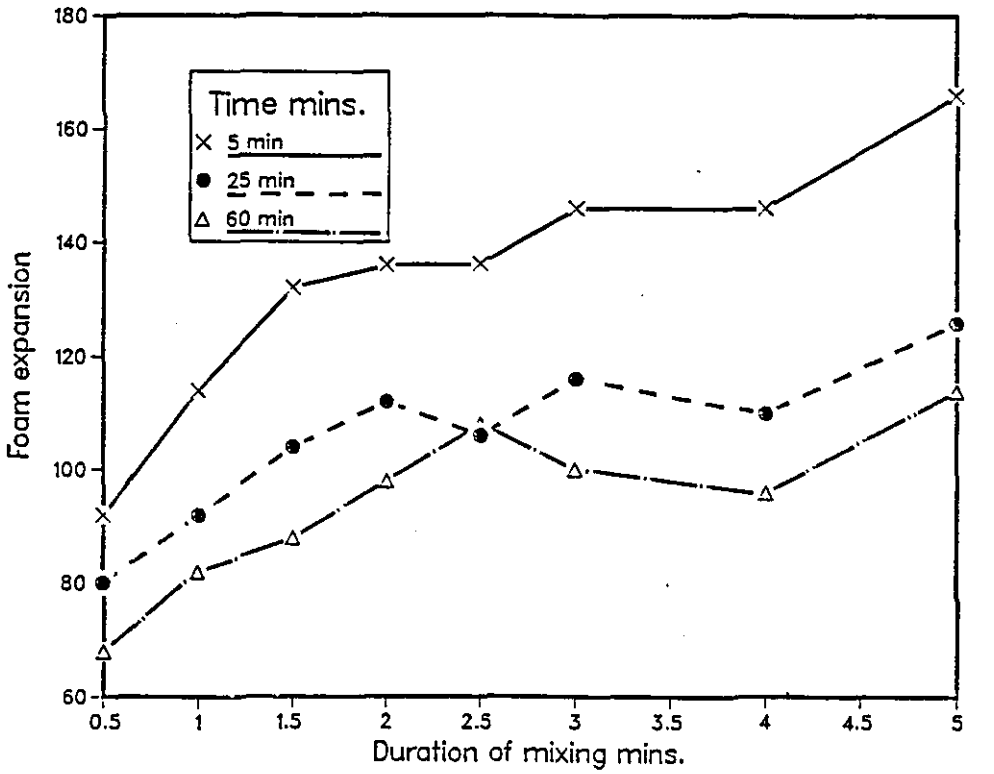


Figure 30 - Foam expansion when a 2% solution of BPP was mixed for different time periods



As a protein is beaten the foam gets thicker, finer, whiter and stiffer. The stiffening of the foam is attributed to the surface denatured protein in the film being rendered insoluble and the film around the air cell no longer being elastic (Charley, 1982).

7.3.C Variation in the Temperature - Appendices 7.3.C

Method

The standard method for foam production was used. The protein solution was allowed to equilibrate at the temperature under test for 15 minutes before aeration. The foam produced was transferred into a measuring cylinder at the temperature under test and stored at that temperature.

Results and Discussion

The results in Figure 31 indicated low foam expansion at 4°C rising to maximum between 20 and 30 and then a slight decline in volume as the temperature increased to 80°C. This pattern was also shown for total volume, volume of gas and liquid incorporated into the dispersion, the overrun values, foam expansion and the gas phase volume. A 'U' shaped curve, the inverse of the other parameters occurred for foam phase volume against temperature.

The pattern for stability was hard to define after 25 minutes, but after an hour there may have been a decline in stability as temperature increased from 4 to 70°C, although the results indicated more stability at 80°C. The holding of a 2% solution of BPP at 80°C for 15 minutes could have denatured the protein to a certain degree.

Cumper (1954) reported that, as long as it is not heat coagulated (when it will not form a foam), heat denatured protein forms a more stable foam. However, other authors report a decline

in foam persistence at the higher temperatures or no change.

7.3.D Variation in the amount of Powder - Appendices 7.3.D

Method

A 12% w/v solution of BPP was prepared. Portions of this solution were diluted with distilled water to form aliquots of 50 ml with a range of concentrations from 0.1 to 12%. These aliquots were used in the standard procedure to produce data on the foaming properties.

Results and Discussion

Tybor, Dill and Landmann (1975) found the response to foam volume of globin, plasma and egg albumen, using the whipping test, to be hyperbolic with the maximum foaming values occurring at 1.7 g protein/100 ml for plasma. Powder No. 12, used throughout these studies had a protein content of 79.8%. The powder concentration of 2% was therefore equivalent to a protein level of 1.6%.

The results and Figure 32 show the hyperbolic shape expected for foam volume, however the curve does not level off until powder concentrations of 6% have been reached. The differing results could be due to the rate of foam production by the equipment used. Many authors have reported the increase in foam volume and strength as protein concentration increases and some have reported a decline if even higher concentrations were used. At very low protein levels a coarse, weak foam of low density was obtained. Cumper (1954) stated that at the higher protein concentrations the foam consisted of smaller bubbles and therefore the total interface was greater. He thought that the total air/water interface in the foam may well have been directly proportional to the weight of the protein. Buckingham (1970) thought that the plateau effect on foam volume and concentration represented a saturation point for the system, where the protein packed at the air/liquid interface to a maximum level.

Figure 31 - Foam expansion when 2% solution of BPP was aerated at different temperatures

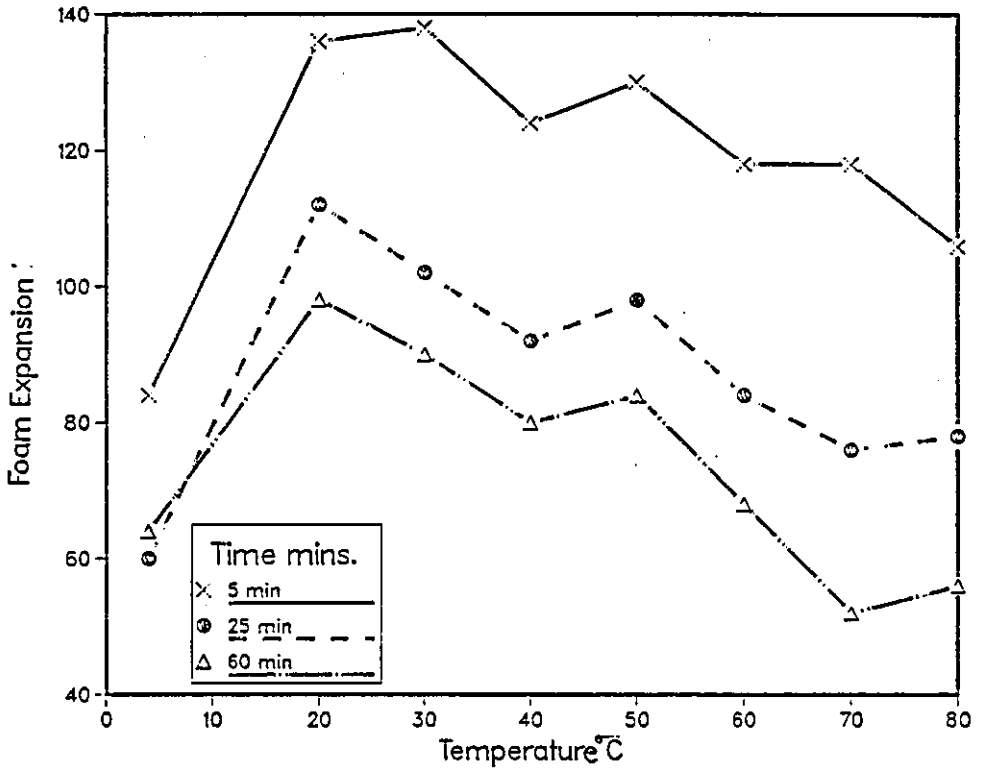
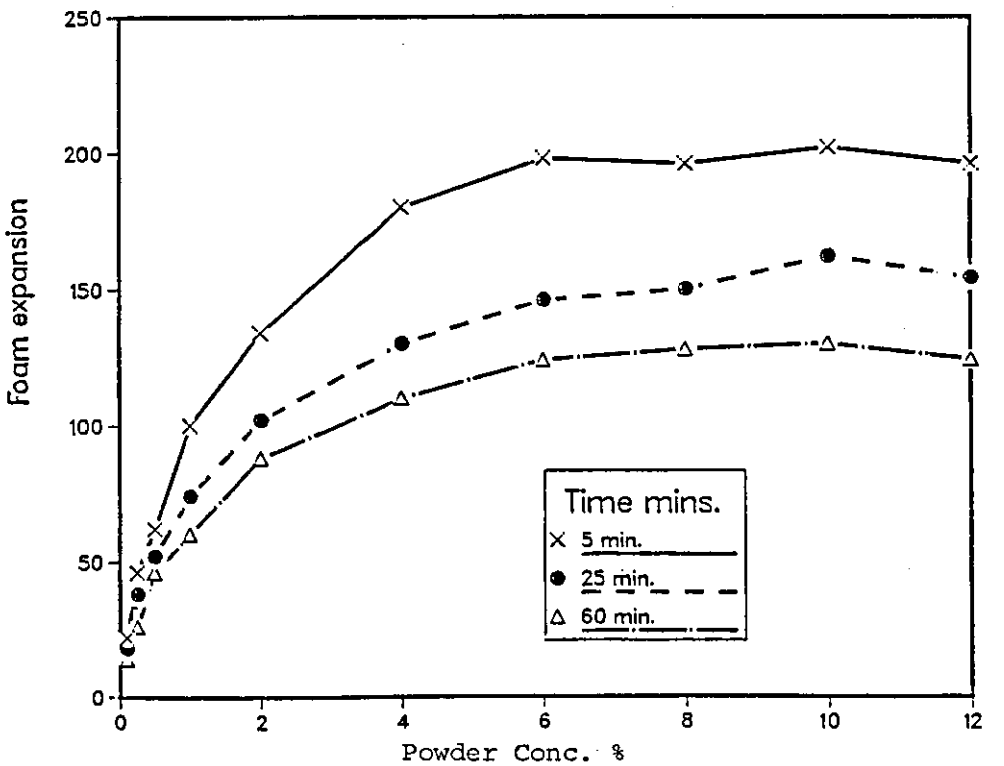


Figure 32 - Foam expansion when solutions of various concentrations of BPP are aerated



The literature is not in agreement with the effects of concentration on the foam stability. Halling (1981) maintained that there was little change in foam stability at low protein concentrations, then there was a steady increase up to high concentrations as the viscosity in the liquid increases. In this study there was no correlation between concentration and stability.

7.3.E Variation in the Volume of Water - Appendices 7.3.E

Method

A bulk solution of 2% BPP was prepared. Aliquots (25 to 150 ml) of this solution were used in the standard procedure. The powder concentration range was 0.125 to 0.75 g per assay compared with 0.5 g in the standard procedure.

Results and Discussion

With variation in the amount of liquid used to make a foam the results, after 5 minutes, show a rapid increase in foam volume with the rate decreasing with the large volumes. Results at 25 minutes and 60 minutes indicated hyperbolic curves, Figure 33. These curves are very similar to those obtained in Figure 32 in which the volume remained constant, but the protein concentration varied. This work is in agreement with Halling (1981) in which he states that the volume of foam is dependent on the total amount and not the concentration of the protein in solution.

Values for foam formation differ from emulsions in the units normally used to describe functionality. With emulsions concentration is an important factor as results are expressed per unit weight. This is not normal for foaming where the actual volume of foam or the volume of foam over the original volume of starting liquid,

Figure 33 - Foam volume with various volumes of continuous phase

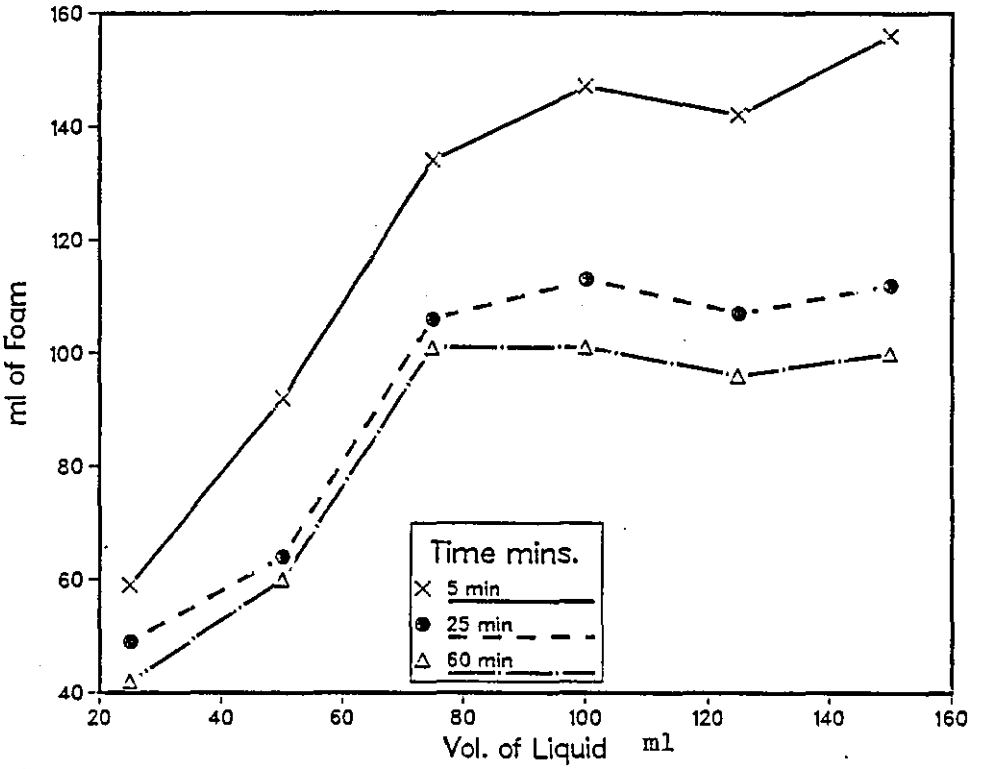
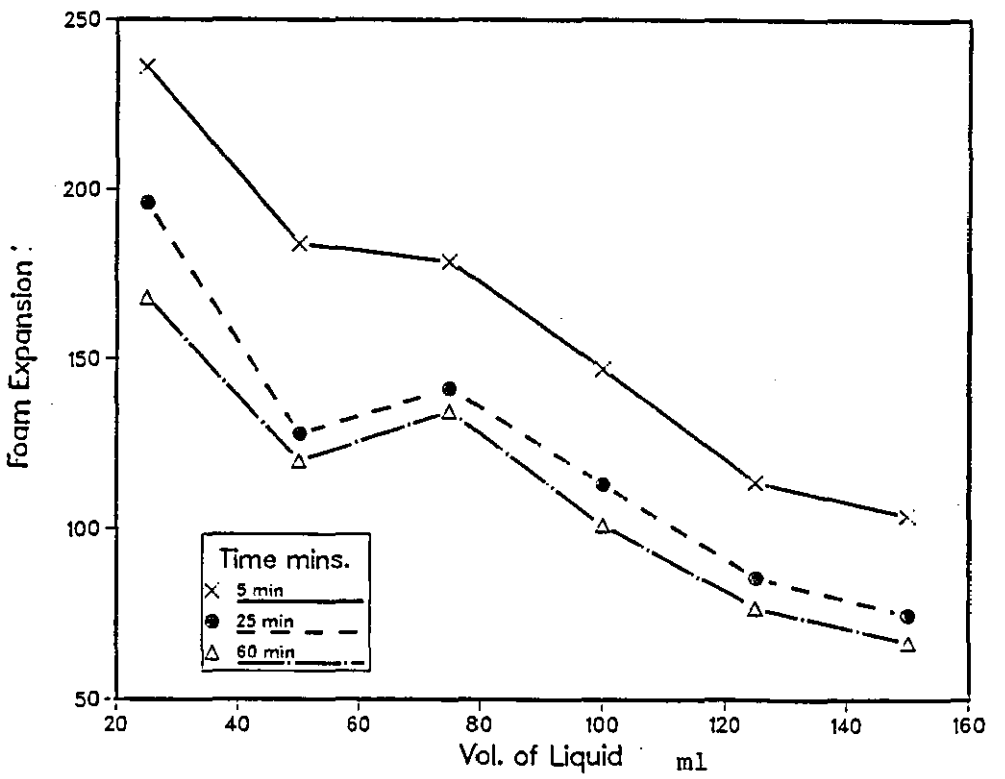


Figure 34 - Foam expansion with various volumes of continuous phase



foam expansion, are quoted. In all other experiments reported in this study the two values of ml of foam and foam expansion have been proportional as the continuous phase was constant. However, in this case the two methods for expressing the results are not comparable. Figure 34 gives the results for foam expansion against volume of liquid in the initial solution. This curve shows a near linear decrease in foam expansion with increased volume of solution.

As with the study on protein concentrations no conclusions could be drawn on the effects of change in volume of the protein solutions on the stability of the foam.

7.3.F. Variation in the amount of Salt - Appendices 7.3.F

Method

A range of concentrations for sodium chloride were prepared by making up a 200 mM stock solution and diluting this to achieve a 2% protein solution containing 6, 12, 25, 50, 100 and 200 mM sodium chloride. Samples of 5% (856 mM) and 10% (1712 mM) sodium chloride were also prepared. These solutions were used in the standard procedure.

Results and Discussion

There are contradictory statements in the literature about the effect on the foaming properties of proteins with the addition of sodium chloride. The variation in results were probably due to the different protein solutions studied. The pH of the solution is also important when studying changes in ionic strength (Bikerman, et al, 1953).

In this study the addition of low levels of sodium chloride to the plasma solution, already containing approximately 9 mM salt, did not radically alter foam stability or expansion. At levels of 5 and 10% perhaps there was an increase in foam volume.

Cumper (1954) thought that increased salt levels lead to higher volume foams due to the greater ionic strength reducing the forces between the polypeptide chains. This reduces the surface viscosity and rigidity of adsorbed protein films. A more compact protein conformation at the air/liquid interface is achieved where the rate of coagulation is reduced, thereby allowing more foam to be formed.

7.3.G Variation in the amount of Glucose - Appendices 7.3.G

Method

A range of concentrations similar to those prepared in section 7.3.F. were prepared, but glucose was used. Levels of 5 and 10% glucose corresponded to 252 and 504 mM.

Results and Discussion

The results indicate that the amount of glucose added to the plasma solution did not radically alter the foaming volume or stability. A small increase in the glucose content to levels of 100 mM did appear to increase the volume of foam formed by up to 10%. These higher volumes were achieved for glucose concentrations of 100 to 500 mM. West and Weir (1979) and Waniska and Kinsella (1979) also found that sucrose had little effect on foam expansion and stability. At levels as high as 30 to 60% the sucrose tended to intially increase stability for some protein stabilized foams (Miller, Herman and Gronninger, 1976; Waniska and Kinsella, 1979).

7.3.H Variation in pH - Appendices 7.3.H.

Method

A bulk solution of 2% w/v BPP No. 12 was prepared and divided into two. To one portion was added 1M sodium hydroxide and the pH of the solution carefully monitored. When a required pH was reached

a 50 ml aliquot was removed and further alkali added to the remaining solution. Samples ranging from pH 8.5 to 9.5 were collected and used in the standard procedures for foaming properties. The second portion of the bulk 2% solution was changed in pH by the addition of 1M hydrochloric acid to obtain samples from pH 8.0 to 4.5.

In another piece of work 12 solutions, each of 50 ml and containing 1g of powder No. 2 were prepared. The pH of six of these solutions at pH 8.4 was lowered to 6.6. The foaming properties of all the solutions were then established using the standard procedure.

Results and Discussion

As the character of the protein is altered at different pH values there may be many routes by which the alteration of pH will affect the foaming properties of BPP. For example the solubility of the protein may be affected, the strength and/or thickness of the interfacial film may be altered, the protein may be more or less able to unfold at the interface. The exact behaviour of BPP in solution at different pH values is difficult to define as it is a mixture of proteins. Also as Glazer and Dogan (1953) pointed out the pK values at the interface (for BSA: carboxyl group at pH 5.0 and the amino and hydroxyl group at 11.5) may not be the same as the values in the bulk phase. Halling (1981) reviewed the effect of pH on the foaming properties of proteins and concluded that most foams are stable and maximum in volume near the pI, however he mentioned several exceptions. It is thought that the solubility of the protein at different pH values is the dominant effect. BPP is very soluble throughout all the pH range studied. Plasma is a complex mixture of proteins which all have different pI values and will contribute to differing extents to the foaming properties. The foam expansion curve in Figure 35 indicates two peaks of foam formation occurring at pH 6.0 and 8.5. The pI of albumin is normally quoted at pH 5.0 and

that of globin at pH 6-8 however, this does depend on the salt concentration.

The pH at which the greatest amount of foam was achieved also exhibited the best stability. An exception was at pH 9.5 when the foam was stable over the first 25 minutes but less so after 60 minutes.

The results for the foam volume and stability comparing solutions of Powder No. 2 at pH 6.6 and pH 8.4 showed significant differences. The volume and the stability were higher for the lower pH value. Results obtained for powder No. 12 over the range of pH values indicated that the pH of 8.4 and 6.6 both occur in the peak regions of foam volumes and look to be similar in size. More significant changes may have been apparent if the pH values at the maxima and minima had been investigated. A pH of 6.6 was chosen as this was near the pH of dried egg albumen (code EggP) which was being used as a standard for foam formation.

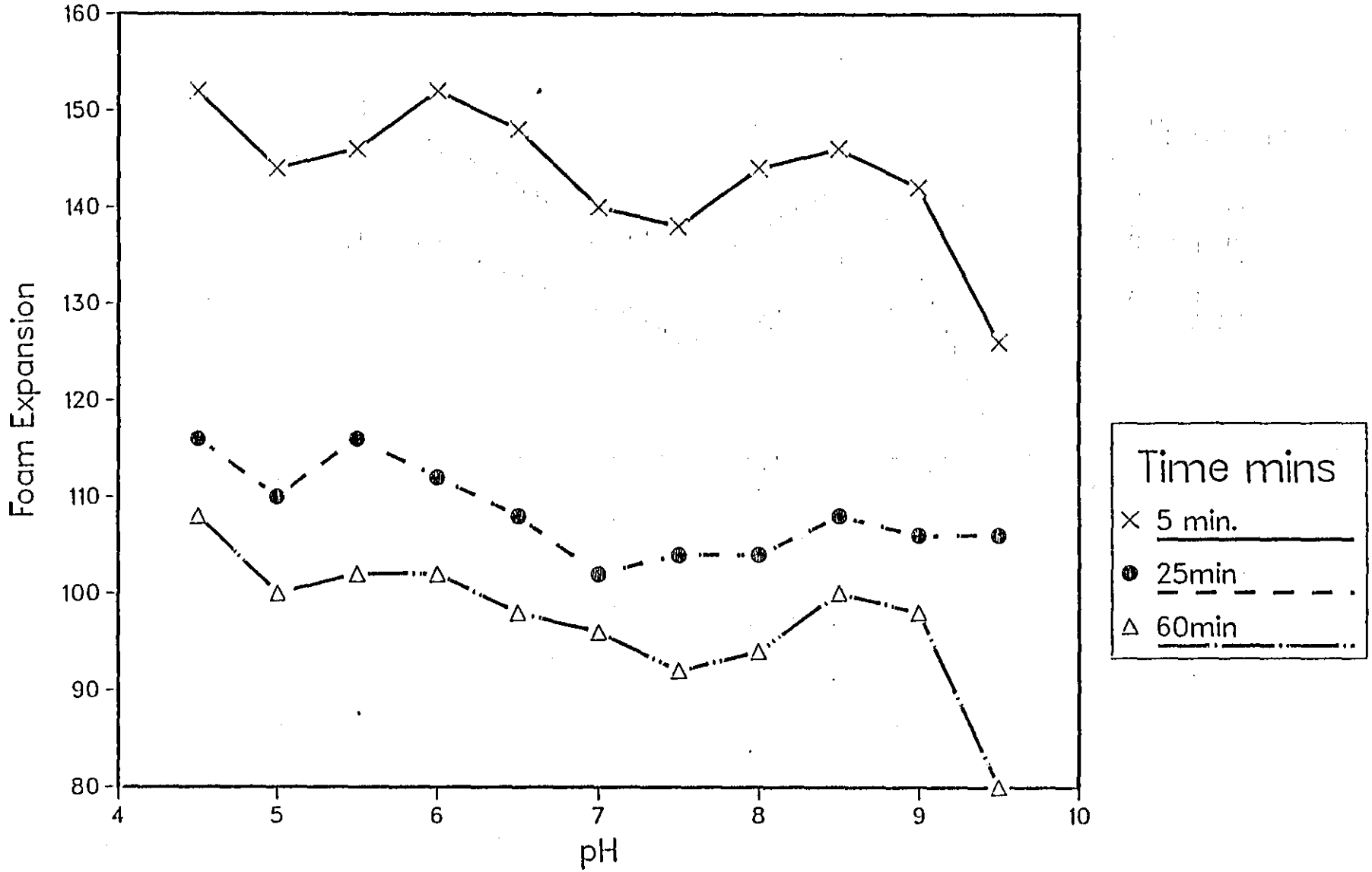
Tybor, Dill and Landmann (1975) prepared spray dried plasma and investigated its foaming properties. Their plasma exhibited a linear response to pH with a decline from pH 4.0 to 9.6. In the study of De Vuono et al (1979) with whole plasma an increase in volume and stability was found at pH 8 compared to that at 4 and 6.

The results in this study show that pH does effect the foaming properties of BPP. Published results for foaming properties of plasma at different pH values are not in agreement with this study. However, it should be noted that the authors do not agree about the effects.

7.3.I <u>The Presence of Lipids in a Foam System</u> - Appendix 7.3.I

The depletion of foam volume and the cessation of foam stability in the presence of surface active materials is well

Figure 35 Foam expansion of BPP at different pH values



documented. The difficulties in producing protein stabilized foams in the presence of small quantities of lipids, present often as a contaminant, is often experienced in the baking industry. To investigate the behaviour of BPP when whipped in the presence of lipids the following brief study was done.

Method

A 2% solution of BPP No. 3 was prepared and divided into 50 ml aliquots. Olive oil was added to 3 aliquots to achieve a concentration of 2000 ppm. Whole pasteurised milk was added to other aliquots at concentrations of 2000, 100, 20 ppm for triplicate analysis at each level. The plasma solutions with and without the added lipid were then tested using the standard procedure for the estimation of foaming.

Results and Discussion

Olive oil at a level of 2000 ppm did not suppress the foaming properties of the plasma. At 2000 ppm of added milk, but containing much less lipid, there was a marked decrease in the total volume of foam at all three time points. There was also a decrease in the stability of the foam that was produced in the presence of the milk, however the decrease was only significant at the 6% level. The amount of foam present at 25 minutes was noted at milk concentrations of 100 and 20 ppm, the lower level does not apparently have any effects. There was a decrease at the 100 ppm level but it was not significant ($t = 2.889$, $p = 0.0631$).

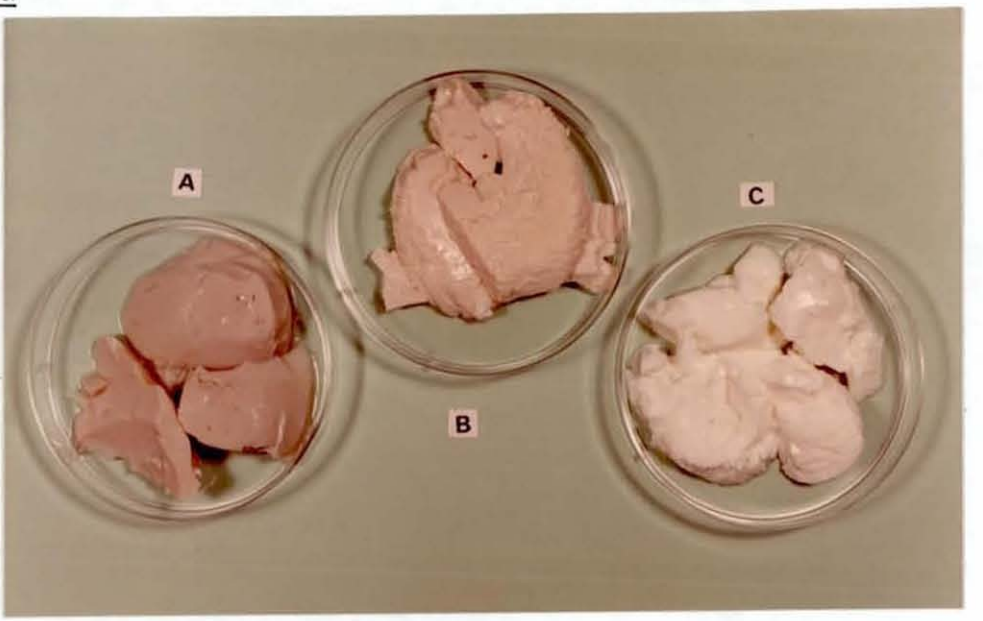
Halling (1981) reported that pure non polar fats and oils could often be added in large quantities without serious effects to foam systems. It was the lipids bound tightly to proteins and oil droplets containing solid crystals which dramatically reduced the foaming abilities of protein.

BPP foaming properties seem susceptible to low levels of milk but not to be affected by olive oil at 2000 ppm. Care must therefore be taken to ensure that the glassware used in the analysis of foaming is free from contamination.

7.4 GELATION

Figure 36

Gels



A = Gel, 10% w/v solution is of BPP at pH 8.4

B = Gel, 10% w/v solution of BPP at pH 5.0

C = Gell, 10% w/v egg albumen at pH 6.4

7.4. A Establishing a Method - Appendix 7.4. A

The penetrometer is often employed for the investigation of gels, see Section 2. 9.2. However, the cone shape and size used with the penetrometer varies and for this study the apparatus was especially made. To try and establish a standard so that the values for the penetration of the cone into the plasma gel could be related to a standard type of gel would help the results to be meaningful. The use of such standards is common practice in establishing the strengths of such commodities as table jellys (Pearson, 1976).

Agar was chosen as the standard as it is widely available, obtained in a pure form and would make gels of the required hardness. Although the gelation mechanism for the bovine plasma gels and agar are not the same the graphs produced for hardness versus concentration are similar in shape.

Method

A 4% solution of the agar was produced by adding 10 g of 'lab M' agar No. 2 Code MC6 lot No. 751217 (London Analytical and Bacteriological Media Ltd., 50 Mark Lane, London) to 240 ml distilled water. The mixture was then heated until the agar had completely dissolved. The beaker with the agar was placed in a water bath at 60°C to reach equilibrium. Also, in the water bath were racks containing various amounts of distilled water so that by the addition of the 4% agar a range of concentrations 0.5 to 4% could be achieved in the final volumes of 20 ml. On each addition of agar to a bottle it was capped and thoroughly mixed and left in the water bath. When all the concentrations had been made the contents of each bottle was again mixed and left at room temperature for 18 hours.

The standard procedure was used to estimate the hardness of the agar gels. After an initial study, further concentrations of agar were used to achieve a suitable curve.

Results and Discussion

A sufficiently hard gel was obtained over the concentration range 0.4 to 4% for the Universals containing the gels to be inverted without disturbance of the gels. Gels below 0.5% concentration could not retard the fall of the penetrometer cone. All the gels formed were very clear and light brown in colour. The hardness, as measured by the penetrometer, of agar gels at concentrations 4 to 0.4% were similar to the hardness of gels obtained with bovine plasma powder at concentrations of 15 to 8%.

The Figure 37 shows the penetration values at each concentration, it was this curve which was used to convert mm penetration into agar equivalents for the protein solutions. Figure 38 is a graph of agar concentration against the log of the penetration depth.

Figure 37 - Depth of penetration into gels made with different concentrations of agar

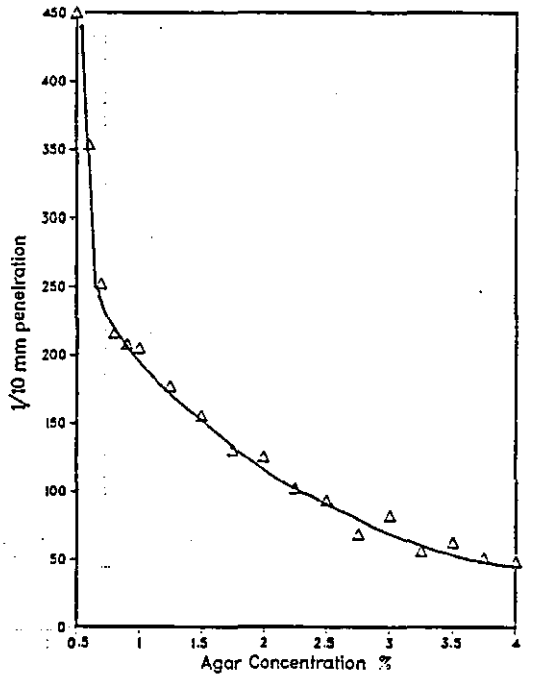
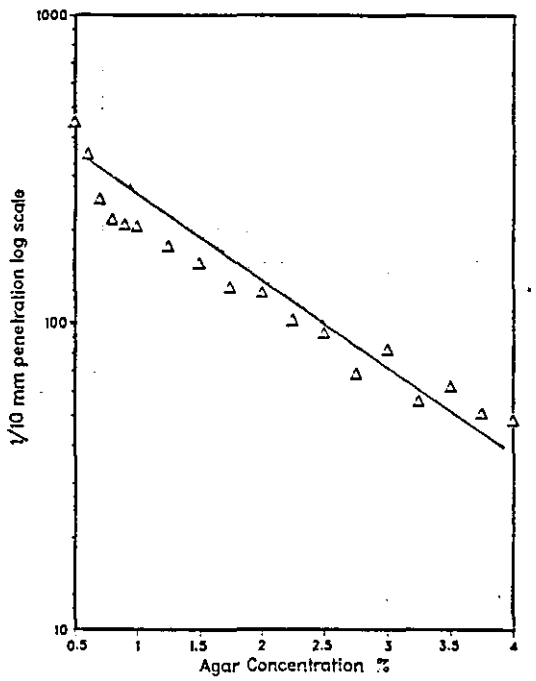


Figure 38 - $\log_{10}$ of penetration into gels made with different concentrations of agar



correlation = 0.952

The graphs show that the agar gels increase with hardness with increasing concentration and the relationship over the range studied was almost linear for concentration versus $\log_{10}$ penetration depth. These results are similar to those of Ferry (1948) who found the relationship between concentration and melting of gels was a log factor.

7.4.C Variation in temperature - Appendix 7.4 C.

The temperature at which gelation will occur can effect the use of a functional protein in a variety of products. Hermansson and Luciasan (1982) reported that a temperature of 72°C was required to form plasma gels and that there was increased water loss from gels heated above 82°C. Heating above an optimum temperature causes an increased tendency towards protein-protein interaction and partial disruption of the protein network due to local aggregation phenomena (Hermansson, 1982b).

The temperature at which gelation took place was therefore studied. Kalab and Emmons (1974) noted that the temperature at which the penetration took place was also important, but this was not investigated in this study, all gels being measured at room temperature.

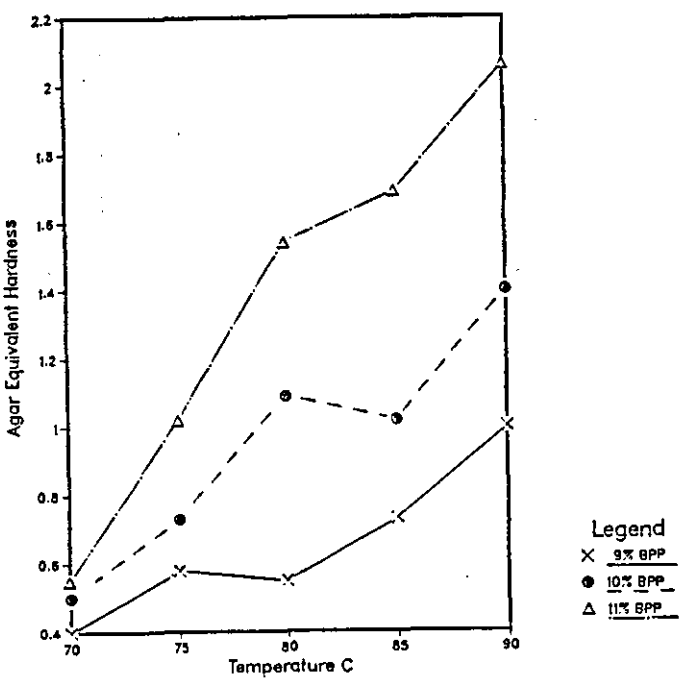
Method

The standard procedure was used to form the gels except the temperature of the water bath was varied. Temperatures of 75, 80, 85, 90 and 70°C were used in that order. The gels were measured by the standard procedure. The mean values for the penetration were interpreted as agar equivalents using the standard curve as demonstrated in Figure 37.

Results and Discussion

All the gels formed were sufficiently hard not to move on inversion, they were all clear and light brown in colour. The results, as seen in Figure 39, show that the gels formed at higher temperatures are harder than the gels formed at the lower temperatures. The relationship between the hardness as agar equivalents and temperature of gel formation (70 to 90°C) is nearly linear. Kalab, Emmons and Voisey (1971) reported that milk gels increased in firmness above 80°C almost linearly to a maximum at 100°C.

Figure 39 - Gel hardness with increasing temperature



7.4.c Duration of heating

Many workers (Kalab, 1971 ; Catsimpoolas and Meyer, 1971; Harper et al, 1978) have studied the relationship between the temperature and time for gelation. In these studies no variation in time was investigated, but to ensure a sufficient time period was being used to allow any changes due to temperature to have occurred the temperature inside the gelation container was investigated.

Method

A 10% solution of BPP was prepared. To the glass Universal bottle was added 20 ml of solution and a Digitron thermocouple positioned in the centre of the container. The universal was then placed into a preheated water bath (80°C reading for the mercury thermometer, 80.8°C reading for the thermocouple). The thermocouple reading was noted at regular intervals.

Results and Discussion

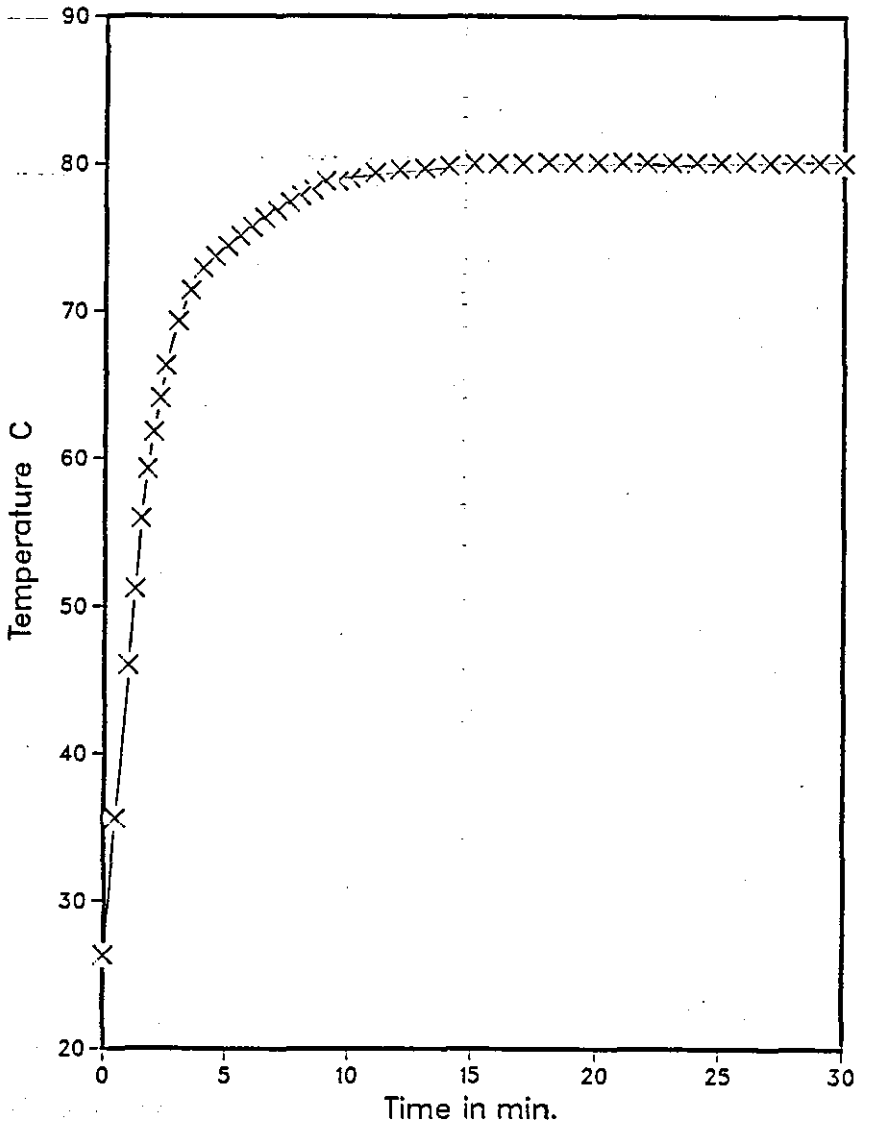
The results of the increase in temperature with time are given in Figure 40. A steady temperature was achieved after 15 minutes, but gel formation could be detected after 5 minutes. As a steady temperature occurred after 15 minutes it was decided that a further 15 minutes should be sufficient to ensure complete gelation at the set temperature.

7.4.D Variation in Amount of Powder - Appendix 7.4.D

Method

The standard procedure for gel formation and measurement was followed. A range of concentrations from 8 to 15% were prepared for BPP No. 8. When several concentrations were used at the same time the highest concentration was diluted to achieve the solutions containing lower amounts of the plasma. The results were obtained from several studies over a two month period.

Figure 40 - Measurement of heat transfer into 20 ml aliquot of a 10% w/v solution of BPP as it gelled.



Results and Discussion

Below a minimum concentration of protein gels cannot form although aggregation and increase in viscosity can occur (Catsimpoolas and Meyer, 1970). The concentration of protein used in this study was sufficient to form gels strong enough to withstand inversion. Few of the gels formed at 8% concentration were sufficiently hard to prevent the penetrometer cone from travelling right through the gel. At high concentrations the cone just pierced the gel. Figure 41 indicates a near linear relationship ($r = 0.964$) between the log of depth of penetration and concentration over the range 8 to 15%. The data is similar to that found by other workers. Hickson et al (1982) found an asymptotic maximum with concentration and Hermannsson (1982b) reported penetration was less with increasing concentration. Kalab (1971) found that for milk gels a hyperbolic function fitted the data better than the exponential function. In this study the correlation for 1 divided by the depth of penetration was 0.987.

7.3.F Variation in the amount of Salt - Appendices 7.3.F

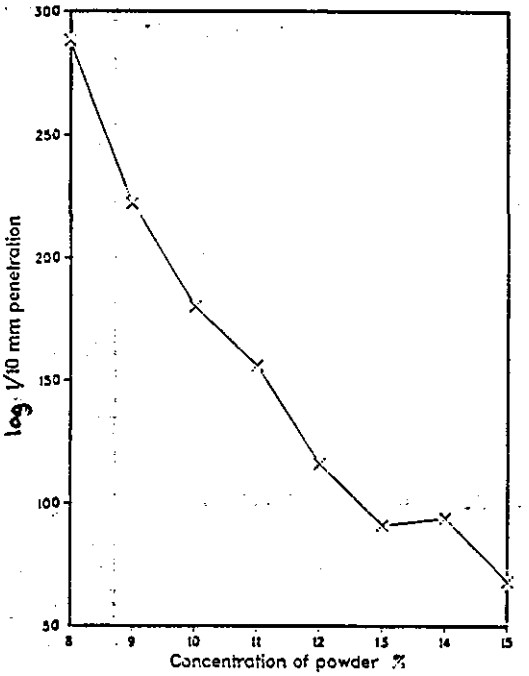
Method

Solutions formed by dissolving BPP at concentrations of 8, 9 and 10% were made. To these were added the appropriate amount of one of the following:- sodium chloride, sodium citrate, calcium chloride, calcium acetate. High concentrations of 5 and 10% were used for the sodium chloride, as well as the lower concentrations of 2,4,6 and 16mM. A further study was carried out using a concentration range of 0 to 50 mM sodium or calcium chloride in the plasma solution. All gels were made and measured according to the standard procedure.

Results and Discussion

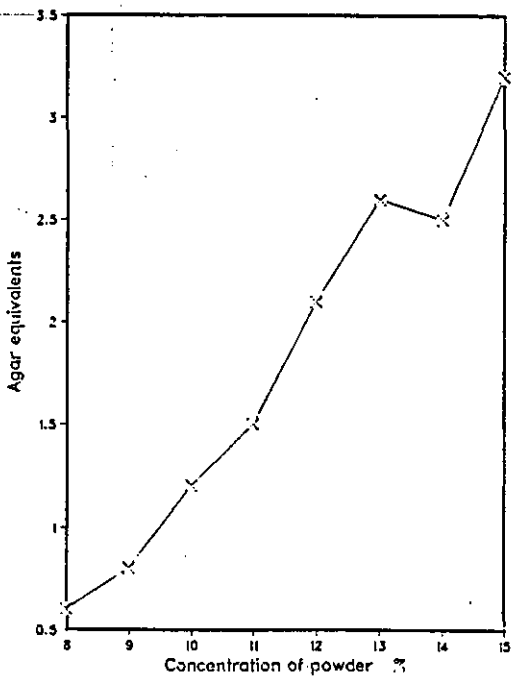
At all plasma concentrations the effect of the sodium chloride was to harden the gel, the results are given in Appendix 7.3.F

Figure 41 - $\log_{10}$ of penetration into gels made with different concentrations of BPP



correlation = 0.964

Figure 42 - Concentration of plasma vs agar equivalents for gel hardness



The decrease in the depth of penetration of the cone was highly significant at the 8 and 9% plasma concentration with the addition of 5 and 10% sodium chloride. The decrease was not so marked at the 10% plasma concentration although it was statistically significant with the 10% salt solution. With the 8% plasma concentration the hardest gel formed was with the 5% salt. At 9% plasma there was no significant difference between the 5 and 10% sodium chloride. This was also found for the 10% plasma gels.

Gels were formed using low concentrations of various salts. It was noted that the gels formed at 8 and 16 mM calcium chloride and calcium acetate were more opaque than the other gels. Figure 43 shows depth of penetration at the various concentrations of the salts used. Very low levels of sodium and calcium salts do seem to dramatically effect the hardness of the gel, Appendix 7.3.F.b In the study using a range of concentrations for the sodium and calcium chloride, (Appendix 7.3. F.c. and Figure 44) the gels formed with calcium chloride at 10 to 50 mM were "milky" in appearance. Some of the results had a wide standard deviation. This was often due to "aeration" of the gel at the surface and consequently it was difficult to place the penetrometer cone at the surface of the gel. The results indicate a significant increase in hardness of the gel which reaches a maximum, at about 15 mM salt concentration over the range studied. The actual gel hardness not being so great with the sodium chloride as the calcium salt. The gel hardening effect appears to be getting less at 50 mM concentration for both chloride salts. A significant hardening of the gel occurred again at 10% (1712mM) sodium chloride concentration, but no increase in hardness was detected at 5% (856mM) sodium chloride.

Ferry (1948) considered that the ionic effects on gelation followed the Hofmeister series, with highly hydrated cations such as lithium, calcium and magnesium and large non polar or large highly polarizable anions having the most effect.

Figure 43 - Gel hardness after addition of salt solution

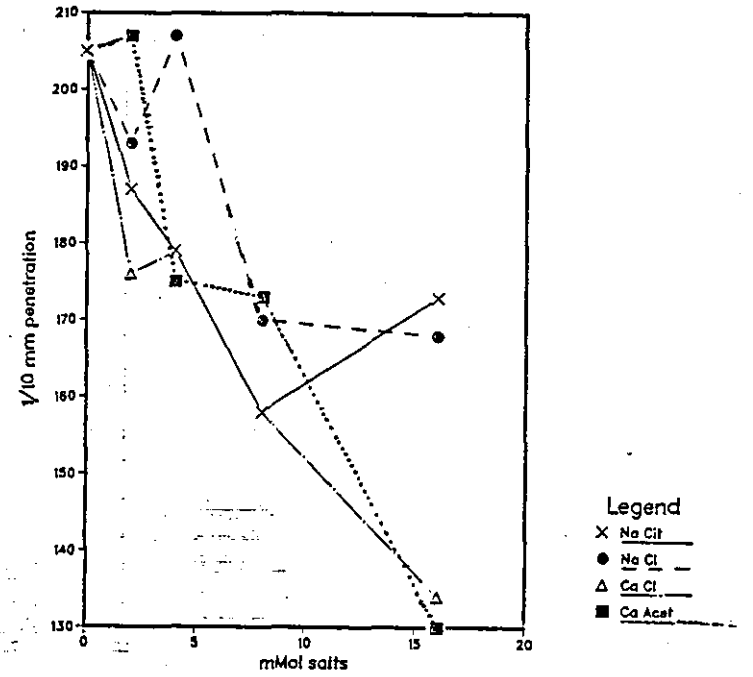
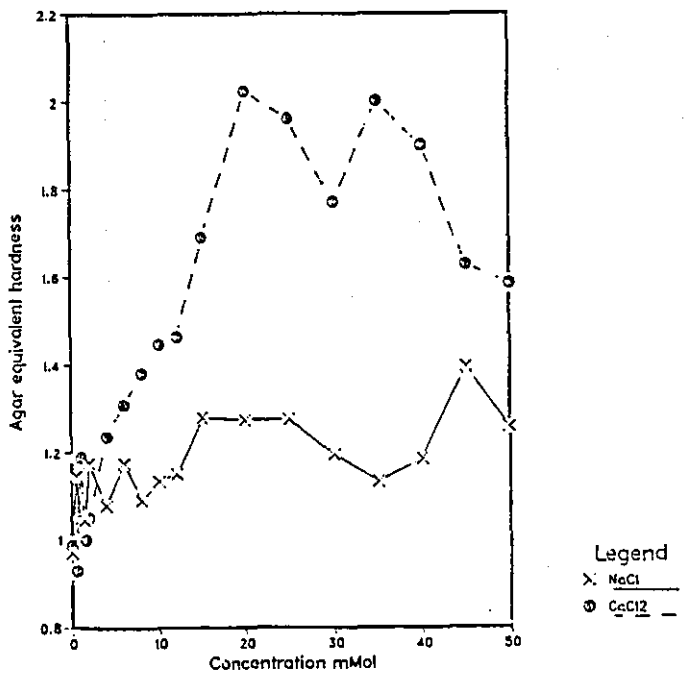


Figure 44 - The effect of a range of concentrations of calcium and sodium chloride on gel hardness of BPP



Kalab and Emmonds (1972) tested various chemicals up to concentrations of 250 mM. They found that low concentrations of sodium and potassium chloride did not effect gel firmness, while divalent cation promoted gelation. Sodium ion concentration seemed to increase viscosity of plasma gels at 0.2M, but to decrease viscosity at concentrations of 0.5 and 1.0 M in the study by Hickson et al (1980). However, using egg albumen no changes were seen on the addition of salt. In the same study 0.2 M calcium gave increased viscosity with plasma, but decreased viscosity with egg. Sodium chloride at 5% was reported to have no effect or to decrease the viscosity and the amount of gelation in the study using fababean, soybean, sunflower and field pea protein by Fleming, Sosulski and Haman (1975).

7.4.G Variation in the amount of Sugar - Appendix 7.4.G

Method

Glucose was added to plasma solution to achieve sugar concentrations of 5 and 10% in solutions of plasma of 8, 9 and 10%. The gels were made and measured by the standard procedure except that if the gels were not strong enough to retard the cone the value of 300 was assigned.

Results and Discussion

At the 8% concentration of BPP the addition of the glucose did not increase the hardness of the gel. However the maximum penetrometer reading obtained was 300 and even if a softer gel was formed on addition of glucose no higher numerical value would be given. At the 9% plasma concentration the 5% glucose did not alter the gel hardness, but the 10% glucose did make the gel softer. At plasma concentrations of 10% the glucose did appear to decrease the hardness of the gel. The softening of the gel being more marked at 5% glucose than with the 10% concentration.

7.4.H Variation in the pH - Appendices 7.4.H

Method

Three concentrations (9, 10, 11%) of plasma solutions were prepared. The pH of each solution was measured and then divided into 3 aliquots. The pH of one aliquot was altered to 5 with 4N hydrochloric acid, another was changed to pH10 with 4N sodium hydroxide. Care was taken to ensure good mixing when adding the acid and alkali to the protein solution. The gels were made and measured according to the standard procedure.

Results and Discussion

Hickson et al (1982) reported that the optimum pH of plasma gels was 7.0. The natural pH for each concentration was 8.3. The gels at 9, 10, 11% concentration and at pH 8.4 and 10 were homogenous clear and light brown in colour. The gels at pH5 were milky white and opaque, see Figure 34. When cut the particulate nature of the gel could be detected. The gels formed at pH5 and 10 were less hard than those formed at the natural pH of 8.4, those being formed at pH5 being softer than the gels at pH10.

Small additions of alkali and acid were made to the samples of altered pH, this would cause slight dilution of the samples. However, in the concentration study it was shown that this slight difference in the powder level could not account for the softening of the gels. The addition of hydrochloric acid and sodium hydroxide will alter the salt balance in the solutions. The increase in ion concentration was only in the order of 0.005 mMolar.

The effect of lowering the pH dramatically alters the type of gel, the gel formed at pH5 being of a cogel nature. At pH5 plasma is not so soluble as at any other pH value. The relationship between solubility and gel hardness was studied by Circle, Meyer

and Whitney (1964). Kojima and Nakamura (1985) studied mixed protein gels and found stable gels at alkaline pH and weak paste like gels at acidic pH. The results reported in this study are similar to those reported by other workers (Hermansson and Lucisano, 1982) and indicate that the final pH of the product must be known for a prediction of the gelation properties of the plasma.

7.4.I. Mechanism of Gelation Appendix 7.4.I

A paper by Howell and Lawrie (1985) discussed the gelation properties of blood plasma proteins when the protein had been modified with various bond breaking agents. To see if similar results could be obtained with the BPP a brief investigation was undertaken using the technique established for gel hardness.

Method

In the Howell and Lawrie (1985) paper gels were made from solutions of 6% protein and 45% sugar w/w. For this study a bulk solution containing 10% BPP was prepared and aliquots of this used to prepare the following solutions.

Code	Material	Bond Effected
A	No addition	Control
B	4M urea	Hydrogen
C	3% w/w SDS (sodium dodecyl sulphate)	Hydrophobic
D	1% w/v mercaptoethanol and 4M urea	Hydrogen and Disulphide
E	1% w/v mercaptoethanol	Disulphide
F	1% w/w Cysteine Hydrochloride	Disulphide
G	24% w/w sugar	Sugar control

Two replicate samples of each example were prepared and the pH measured after 24 hours. Three aliquots of 25 ml were taken from each flask and used for the gelation production and measurement according to the standard procedure.

Results and Discussion

In this study the gels containing the mercaptoethanol showed the highest degree of gel formation. The sample containing urea and mercaptoethanol quickly formed a very clear gel on standing at room temperature. Without the urea the gelling took longer, but would still occur at room temperature, with a cloudy gel being formed on heating equivalent to agar at 2.16%. These results are in marked contrast with those obtained by Howell and Lawrie for the porcine plasma powder where the use of mercaptoethanol and urea reduced gel strength by 82% and the breaking strength by 93%. The use of cysteine hydrochloride, a reagent also said to reduce disulphide bonds, also lowered the gel and breaking strength of porcine plasma in the Howell study. However, the reagent was said to markedly increase the gel strength of porcine serum as found in the present study for BPP.

In this study all gels formed in the presence of urea were pellucid. Gels formed with the urea were not as strong as the controls. In the Howell paper a decrease in gel strength was obtained with urea for the plasma and serum, but an increase occurred for egg white.

No gelation occurred in the presence of SDS in this study, the solution formed was clear. The reported data indicated a lowering of gel strength for the plasma and no gel formation for the serum.

From this brief investigation of the effect of bond breaking agents on BPP notable differences can be seen from the data reported by Howell. The methods of gel measurement vary between the two studies as does the protein and sugar concentration, but the results are so different that this alone cannot explain the variation. Howell used porcine plasma while this study used bovine. The results obtained by Howell for freeze dried porcine serum appear to be similar to those obtained for BPP. In Section 6.8 the protein product Regalbumin (code AlbFib) was assessed and found to gel, but not as well as BPP.

The reason for differences in the data could be variation in the tertiary structure of the proteins. The mechanism discussed by Howell could still be true. Presence of mercaptoethanol could encourage breaking of disulphide bonds causing the structure of the protein to unfold and realign in an ordered form to produce a gel, perhaps reversible in nature and formed from electrostatic and hydrophobic association as well as the reformed disulphide linkages.

The differences between the plasma used by Howell and BPP could be that the BPP had not been so badly damaged in production. A factor in gelling may well be the prehistory of the protein.

CHAPTER 8

CHAPTER 8

SUMMARY OF RESULTS AND CONCLUSIONS

A powdered product was made from bovine plasma and its functional ability was assessed. As no standard methods exist for estimation of functional properties methods had to be developed and other protein products assayed to give some indication of the potential of plasma powder. It was hoped that by doing a range of tests that the important factors relating to functionality could be defined and this would make assessment of products easier. However, the functional tests were dependent on so many parameters that statistical procedures for the modelling of their action would be inappropriate. Although the errors when performing the functional tests had been carefully reduced the normal coefficients of variance were between 10 and 15%. Small variations in the processing of the plasma could not be detected with the functional tests and therefore investigation of small changes in ultrafiltration time and spray drying temperature were not investigated.

A description of the powders produced for this study and their properties are discussed in this Chapter. Comparisons are made between the data obtained for the bovine plasma powder (BPP) and those obtained by authors working on similar products. The quality of the standard tests developed for this project is discussed as are the factors, e.g. concentrations, temperature, pH, that effect the values obtained. Ideas for future work to aid in the assessment of functionality and for monitoring production processes are also given.

8.1 Protein Powders Produced at LUT

A light coloured powder can be made from the plasma fraction of bovine blood, by the process of ultrafiltration and spray drying.

The procedure used produced approximately 3.5 Kg of powder (2.8 Kg protein) from 100 litres of whole blood. The powder formed was stable and exhibited a range of functional properties.

The colour of plasma powders was bland, but the degree of red cell lysis had to be carefully controlled, this was more difficult if porcine blood was used. Some coloration due to the presence of β-carotene was detected. The range of results obtained for the ten bovine plasma powders assayed did not indicate any marked differences in the powders produced. Powders made from frozen plasma, sera, porcine plasma, porcine plasma with Fraction VIII removed and plasma from young barley beef all showed very similar functionality with the tests used. Variations that did occur include lower EC values for a powder not produced at LUT, although made under similar conditions. The amount of insoluble material in the barley beef sample was greater than that expected for BPP. Solutions made from porcine samples were dark in colour. Serum samples had slightly better emulsification and not so good gelation properties as BPP.

High microbial counts in some of the products produced did not seem to radically effect the functionality of the product, but would render it unsuitable for human consumption. To achieve a powder of suitable microbial quality care has to be taken throughout collection and production. Under the conditions used at LUT it was essential to process the plasma within 12 hours of collection and the UF is a critical stage. The apparatus must be constructed so that it can be kept as aseptic as possible. Speed in concentration is also important. The laminar flow in systems such as the DDS UF machine are better for viscous fluids (Thijssen, 1974) and the concentration of the plasma was much quicker with this apparatus. No increase in the microbial load of BPP was found on storing the powder for a year. The need for hygienic production has been noted

by other authors and the off flavours and odours obtained with some of the powders produced by them may have been due to microbial degradation of the plasma during processing.

Ultrafiltration is an economically efficient way to concentrate solutions. The removal of much of the salt added to prevent coagulation may also be beneficial. In spray drying the protein is not subjected to high temperatures and therefore should not be damaged. Electrophoresis of BPP indicated that the major protein fractions still maintained their electrophoretic mobility. The SDS-polyacrylamide gel electrophoresis results of Tornberg and Jonsson (1981) indicated less distinct bands and loss of albumin in spray dried compared with frozen plasma. Although no changes in the agarose electrophoresis pattern could be seen for the BPP, the protein had altered in some way as assays for lactate dehydrogenase indicated a substantial loss of activity after spray drying and slight reduction after UF.

The study of powders collected during spray drying showed that little difference could be detected. However, blockages in the atomiser should be avoided , thus keeping temperatures constant so that particles of the same moisture content and size are produced. It is also necessary that the inside of the drying tower is clean or powder will adhere to the surface thus reducing yield.

Properties exhibited by BPP on first manufacture were retained in the one year stability trial. Powders with β-carotene lost their colour with time. Any initial odour the powders may have had on first production was much reduced after a few hours although heating the powder or solutions of BPP produced a smell.

Solutions of BPP could be made, but it was necessary to stir the powder and water for at least an hour to ensure that full solubility of the powder was achieved. Solutions were cloudy and

darker in colour than the original plasma. The pH of solution formed from dissolving BPP in distilled water was always greater than 8.0 and the colour was pH dependent. At pH 5.0 the solution was more cloudy, but of lighter hue than that at pH 8. The solubility of BPP was very good with over 90% of the powder being soluble at neutral pH values. The lowest solubility, 76%, was shown at pH 5. A decrease in solubility at the iosoelectric point was found with other plasma powder products (Tybor, Dill and Landmann; 1973; Delaney, Donnelly and Bender, 1975). It was established that the degree of solubility varied according to the source and procedure of powder production. The decrease in solubility at pH 5 is most marked when only low concentrations of BPP are used. In this study it was found that the addition of salts or sugar reduced the decrease in solubility when changing the pH.

The ability of the BPP to disperse was not good and there was a risk of further microbial growth if too long a time period was required to achieve full solvation. Many of the other protein powders tested had larger particle sizes and this may have aided in their ability to disperse. If it was necessary to aid dispersion of BPP and alteration of particle size was not sufficient it could be possible to incorporate another substance with the protein. Tybor et al (1973) incorporated lactose when spray drying plasma.

Compared with most of the products tested the functional properties of BPP, as estimated by the given methods, were very good. The amount of oil emulsified by BPP was great and at a ratio of 1:24:75 protein :water:oil the emulsion was very stable. Solutions of the powder also showed good gelling characteristics. Firm gels being formed at 9% solids at 75°C. Only the fresh egg white produced firmer gels when measured with the penetrometer. Egg products also may have formed a slightly more stable foam than BPP, but no differences in the volume of foam obtained could be detected between egg and BPP. The superiority of egg white in cake baking was shown when the utility test of making an angel cake was undertaken.

Foam stabilized by the presence of BPP collapsed to some extent with the introduction of flour and the matrix could not be stabilized after the initial expansion of cooking.

BPP absorbed fat and mixed with oil to form powder/oil mixtures at gravities greater and less than that of the corn oil. This also occurred with some other powder products, but the product produced from plasma by Fibrisol did not form the two layers.

Results of the functional properties of plasma powder, as measured by other workers, have varied. In all cases it must be remembered that assay methods were different as were the powders. In the study of Hickson et al (1980) and Howell and Lawrie (1984 a,b) plasma gels were found to be harder than egg white gels. In the present study no difference or a slightly stronger gel was obtained with the egg products. Work of Howell and Lawrie (1985) on the mechanism of gelation gave very different results from those obtained using BPP, see Section 7.4.I. Kalab and Emmons (1974) investigated various drying techniques for the production of protein powders and found differences in the gelation properties. Whatever the source, the ability of plasma protein to form gels is well established for final food systems. Reports such as plasma increasing the binding strength of frankfurters or beef patties are common (Suter et al, 1976; Terrell et al, 1979).

The foaming ability of plasma in producing confectionery products is less well documented. Only partial replacement of egg white with plasma is possible in cakes, although in model tests the foaming volume for reconstituted plasma powder is as good or better than egg white (Tybor, Dill and Landmann, 1975; Thompson, 1983). The amount of egg that can be replaced with plasma has varied in the opinion of authors, but levels higher than 30% have been considered acceptable. The amount of substitution must also depend on the type of product being made. In this study angel cakes made with 50%

and 100% substitution of egg white did not have the volume, nor the white colour, nor the fine texture of egg albumen cakes.

Reported values for plasma products would indicate that their emulsion characteristics were good. Different studies are difficult to compare as the methods are so varied and no comparative data is given. Tybor et al (1975) found that emulsification properties of plasma were dependent on the manner by which the plasma had been processed. Kinsella (1976) pointed out that the prehistory of the protein was vital for its emulsification properties. The amount of bacterial contamination was found to be relevant in the emulsification determinations by Borton et al (1968).

Results indicate that the plasma powder produced at LUT had similar functional properties to those quoted for other plasma proteins, however there were exceptions. With no standard tests variation in the assay procedure could give the deviant results. Other constituents in the powdered product could also play an important role in some of the functional properties. The method of processing plasma is of vital importance, but this study indicates that the method developed at LUT can be used routinely without much variation in functional properties. BPP does not alter during or after processing. Source of raw material, i.e.; plasma or sera, porcine or bovine, from young or older animals, frozen or fresh plasma, also did not radically alter the results of model tests for functional estimation.

8.2 Factors Effecting Functional Tests

A more detailed study of the behaviour of BPP in some of the functional tests was undertaken in the hope that a clearer perception of the protein's action could be made. The effect of plasma in a food system could only be predicted if factors regulating the functionality had been determined. Knowledge of the results of tests using BPP under different conditions could lead to an understanding of why results of other studies had been so different.

Mixing Procedures

Mixing procedures are of importance in the functional tests. Mixing the dried powder with oil for the fat absorbance did not appear to be critical, except that the powder should not be subjected to rough handling before or during mixing. Passing the powder through the Alpine Classifier obviously had a detrimental effect on its ability to absorb fat.

Care was taken throughout this study when making up the solutions of BPP. If mixed too violently the solution would foam and this indicated unfolding of the polypeptide and its adsorption at the air/solution interface. No emulsions are formed at all if the wrong type of shear force is applied to solutions of BPP and oil. If sheared for too long there is a decrease in emulsion capacity values. Long mixing times produced oil globules of small size and therefore more protein is required as the interface area increases. Foam, another system that requires action of protein at the interface, is very dependent on the method of mixing the two phases. Sparging formed much higher foam volumes than whipping. The size of vessel in which the foam was whipped also plays a role, the larger the vessel the more foam was produced. However, the diameter of the

vessel holding the foam after production did not effect the results. Authors have reported that too long a mixing time decreases the volume of foam produced, but, in this study the longest whip time gave the largest volume although there may have been some loss of stability.

MacDonnell et al (1955) pointed out that the whip time is dependent on the protein. Globular proteins may require more mixing than random coil structures as they have to be denatured before being able to stabilize the foam. An appreciable degree of tertiary structure in the dissolved protein is thought to be needed for maximum stability. However, if a protein is hydrolyzed the protein may give a lower stability, but may foam more readily. Work by Buckingham (1970) indicated that better foaming properties for BSA could be achieved by slightly decreasing the solubility of the protein.

Temperature

Temperature effects most chemical and physical actions. Bovine plasma starts to denature at temperatures in excess of 55°C (Hermansson, 1983) and therefore any tests carried out above this are likely to be different from those at room temperature. Fat absorbance appears to decrease with increasing temperature. Hutton and Campbell (1981) postulated that the change in viscosity of the oil would change the amount of oil entrapped by the powder. It was possible that the high temperature could denature the protein, but studies with BPP that had been held at 85 or 108°C for up to 5 hours showed increased uptake of oil.

An investigation of effect of temperature on the emulsion capacity of BPP was not carried out. Emulsions stabilized by BPP were held at 4, 20 and 80°C and no differences in the amount of oiling off was detected. The volume of foam produced by whipping BPP was temperature dependent with the maximum amount occurring at between 20 and 30°C.

In this study temperature was used to form gels from solutions of BPP. The gels were harder as the temperature was increased from 70 to 90°C. If the temperature was increased to as much as 120°C then the gel would once more become liquid, resetting as the temperature was allowed to fall. Hermansson (1982) found that by increasing temperature from 77 to 91°C a partial disruption of blood plasma gel structure occurred. This was due to a local aggregation phenomenon and as a result there was a decrease in the water binding. The effect of duration of heating was not studied in this project, but after an initial time period further heating is not supposed to effect the gel (Hickson et al, 1980).

Protein Concentration

The amount of protein taking part in any functional test depends on the amount of powder used and the quality of the protein after the processing procedures. In fat absorbance estimations if only small amounts of powder, below 4.8% by weight of the total mixture, were mixed with oil there was an increased amount of oil absorbed per g of powder. At higher proportions of powder the amount of oil absorbed per g of powder remained constant.

Many of the functional tests depend on the protein being in solution. The solubility of the BPP was good with over 90% of the protein soluble at neutral pH with the minimum solubility occurring in the region pH 4 to 5. For the tests involving disperse systems e.g. foaming and emulsification, the protein molecule has to migrate from the bulk phase to the interface. However, when at the interface only small amounts of protein can cover a larger area. Graphs showing the effect of increasing the amount of protein in emulsion and foaming studies are hyperbolic. This indicates that after an initial increase in the property with increasing amounts of protein, further protein does not have much effect. Reports on the amount

of protein required to reach the plateau have varied. Several authors have stated that increasing levels of the protein may increase the strength of the interface (see Section 7.2.D & 7.3.D) but this study did not indicate any increase in stability with high levels of protein. When the amount of water was changed in the foaming and emulsion studies the dominant effect was due to the total amount of protein in the solution and not the volume in which it was suspended.

The units in which the results for functional tests are expressed can be of major significance. For emulsification capacity a common presentation of the results is "g of oil per g of protein". As the actual amount of oil emulsified is not linearly dependent on the weight of powder used the results can be misleading. Results for BPP could be quoted as any value between 160 to 3400 g of oil/ g of powder. Emulsion capacity values are not so affected by protein concentration if they are expressed as oil phase volumes, but the effect of altering the water volume produced non-linear OPV values. Foaming values can be expressed as the "volume of foam formed" or as "foam expansion", neither expression allowing for the protein concentration. If the volume of liquid is altered it affects the values for foam expansion which is the volume of foam divided by the original volume of solution.

It is necessary that a certain concentration of protein is present before there is any gelation, although some increase in aggregation and viscosity can occur at low protein levels. Over the range of concentrations used in this study a hyperbolic curve was obtained for plasma and agar concentrations against the depth to which the penetrometer cone pierced the gel. The curves were linear if log of the penetration value against protein concentration was used.

Salt and Sugar Additions

The solubility of plasma protein is affected by the amount of salt and sugar in solution. Small increases in the amount of salt or sugar, above that already in the powder, enhanced the emulsifying properties, although high levels were not beneficial. Sugar and glucose addition to BPP solutions did not alter the foaming properties to any great degree, but high addition rates may have increased the foam volume a little. For a study of the effect on fat absorbance of additional levels of salt or sugar the compounds would have to be added to the concentrated plasma before spray drying. Little change or a softening in gel hardness was found with the addition of sugar. Adding salts to solutions used for gelation estimations had marked effects. The presence of salt in gelation systems generally promotes aggregated structures on the colloidal level and enhances protein-protein interactions on the molecular level (Hermannson, 1982b). Very low levels of calcium produced hard, white, opaque gels. Low sodium chloride additions also made gels harder, higher concentration not having so much effect. Results for gelation are so dependent on the concentration of various ions, especially highly hydrated cations, that the processing method could be critical. The amount of salt in solution is affected by the UF process. The longer the process continues the less salt there will be in solution as small ions are able to pass through the membrane. The anti coagulant used will also alter the ionic balance of the plasma. Some, but not all, of the variation in the gelation results for the different plasma powders may be explained by the various levels of salts contained in the powders.

pH

Plasma in the body has a strong buffering action and the pH is not readily altered. Spray drying increases the pH of the system

and this could well alter the structural conformation of the protein. The apparent colour of a solution of BPP is altered by reducing the pH. Changes in some functional tests at different pH values have been explained in terms of the alteration in solubility of the protein. Although the total loss of protein solubility at different pH values has been calculated the effect on individual proteins cannot be ascertained. It may be that the loss of a specific protein could alter the functional quality at differing pH values, but such alteration can not be explained just by loss in the total amount of soluble protein. Small amounts of acid added to dry BPP did not effect the FA. It is possible that if the powder had been dried and formed at a different pH the FA values would have been altered. When measuring the EC at different pH values the most noticeable difference was the care with which the mixing had to be undertaken. Emulsions at pH values different from that at which they naturally occur could easily be broken with too much homogenisation. Emulsion capacity of BPP was lowest at pH 5. The pH which produced the greatest amount also produced the most stable foam. Two maxima occurred for foam volume, pH 6.0 and 8.5. The results of other workers were not in agreement for the effect of pH on the foaming properties of solutions made from plasma powders. Alteration in the pH of solutions heated to form gels gave products different in appearance as well as strength.

8.3 Standard Methods

The methods in this study have been adapted from those used by other workers so that they were suitable for use with BPP. As shown a number of factors effect the results and although a range of other protein powders have been assessed alongside BPP it is possible that very different results would have been obtained if the assay methods had been different. As there are no standard assessments it must be advisable to use a well documented powder as a reference for the functional tests, even if the assay methods may not give the same results.

Organoleptic properties are so important for a food product's acceptability that perhaps more attention to the accurate assessment of these should have been carried out. Water absorbance tests are mostly used for products which are not so soluble as the BPP. The uptake of water at certain humidity levels could be a very good indication of storage problems that could occur with powders in damp atmospheres. The method for dispersion was developed due to the difficulty in producing solutions of BPP. For its commercial use some type of instantizing could be investigated.

Fat Absorbance

The method used for fat absorbance produced two layers of powder mixed with oil. On further examination it was found that these layers had approximately the same composition by weight, but the volume occupied by the powder was considerably more in the top layer compared to that at the base. The units used to quote the results could therefore be of great significance. Values in this study give fat absorbance for BPP as ml of fat per gram of powder. The results could look very different if the results are given as ml of fat per ml of powder. Freeze drying bovine plasma

powder produced particles of large size which were not spherical or compact in shape. These particles absorbed more fat than those produced by spray drying but no low density oil/powder mix was formed. Chemically identical protein powders having particles of similar size produced by different methods had different oil absorptions. Globin appeared to absorb more oil than albumin when produced in the same manner.

Emulsification

The method used for the determination of EC was reproducible and small changes in the method could be detected. The units in which the results were quoted were important. Oil phase volumes based on the EC figures were a useful estimation of the emulsifying power of the proteins. The standard procedure for the estimation of stability was not very sensitive and only gross oiling off of the emulsion could be detected. A different method of stability estimation may be required to indicate the property. Estimation of stability rating according to Tornberg (1977) where the fat is determined for the base and top layers of dilute emulsions could be considered. Viscosity measurements cannot be successfully estimated without using a viscometer that cuts a path through the emulsion. A single model system is not able to give a full picture of the emulsifying properties of a protein. The estimations of particle size by any of the methods discussed could prove a useful addition in the estimation of the emulsifying properties.

Foaming

The method of foam formation is critical for the volume of foam produced. Some large standard deviations occurred for the foam stability figures. This was probably due to difficulties in determining the height of an aged column of foam, as the collapse was often ragged with large gaps occurring in the column. Syneresis values

may have been easier to estimate. However, the two values are not the same. Liquid drainage indicates thinning of the lamella and plateau borders, but not necessarily decrease in the foam volume. Lamella rupture can lead to increase in bubble size without collapse. Halling (1981) reported that authors found several different types of profile for the time course of collapse of protein stabilized foams and varied relationships with drainage.

The model test for foaming does not give a true indication of the behaviour of protein in a food product. Testing of BPP in an angel cake preparation indicated that it was not as good as egg white and could only act as a partial substitute for the traditional foaming agent.

Gelation

Gelation properties estimated were based on gel strength and other factors, such as breaking strength, water retention etc., could be important. Better instrumentation could give other values for the gel texture, but the use of an agar standard should act as some control. The amount of salts in the solution to be gelled is critical and therefore should be assayed to give the data on gelation more meaning.

Comments

Although the data from this study do not give any very strong correlation between the different parameters, the protein concentration, pH, salt levels, exact methods of protein production and assessment of functional properties should all be quoted if the results of model tests are to be of value. The inclusion of a well documented protein to act as a standard may help other workers to compare data on functional properties. An assay not carried out for this work,

but which might have been of value is the assessment of hydrophobicity. The theoretical evaluation can be carried out using the amino acid analysis, but the measurement of "effective hydrophobity" may give a better indication of the action of the protein in a functional test (Keshaverz and Nakai, 1979).

8.4 Conclusion

Over 99% of blood from animals slaughtered in the UK is not used for human consumption, yet in other countries the material is successfully marketed and commands a high price. There may be some consumer resistance, but the main reason manufacturers are not eager to incorporate dried plasma into products is that they are unsure of the quality and performance of the protein. Often claims for high functional properties, indicated by model testing, have not been found justified when the product was incorporated into food systems.

Values for functional tests are difficult to compare, but this study would indicate that the powders produced under different regimes may have dissimilar functional properties. Plasma proteins offered to the food industry have therefore varied in two ways, firstly in their manner of manufacture and secondly in the way they were assessed. A third variable could have been due to differences within powders made from the same method. Much of the work reported on blood plasma proteins has been carried out on small trial batches of powder without knowledge of whether production was reproducible.

This thesis outlines the steps taken to estimate if variability within powder samples manufactured in the same way affected the results for BPP. Results showed that bovine plasma could be concentrated by ultrafiltration and spray dried to form the stable product "BPP". This protein was suitable for human consumption and was of a uniform and predictable standard. A predictable standard refers to the values obtained for proximate composition and action in model tests for functionality developed for this study.

To eliminate different assay techniques, which is the second source of variation mentioned, official estimations for functional properties could be developed and used by all those interested in

food products. However, model tests are dependent on a number of factors including those affiliated to the method of manufacture of the protein. It is therefore possible that proteins giving the same results in model tests could still behave in a different manner in a true food system.

Much work has been described in this thesis of how changes in model tests alter values for functionality. The tests need to be carried out according to explicit procedures to monitor the product's functional performance. The model tests could be used to routinely check the quality of the powder, but slight divergences from the standard procedure for production could not be detected. Using the developed range of assays the functional properties of BPP compared favourably with those of other protein products now used in food systems, although it must be recognized that different assay methods may give different results.

BPP could be manufactured commercially to the same quality as that produced for these pilot studies. Procedures for functionality and composition would be used to ensure an acceptable standard of product. Advice could be given to manufacturers as to the best use of BPP and for alterations to food systems to enhance the functionality of the protein. Companies should be encouraged to incorporate BPP, a reproducible powder with functional properties that compare favourably with other proteins, into their foods where appropriate.

Thoughts on Further Work

General Use of Blood Plasma

If blood plasma is to be used by manufacturers the behaviour

of the protein in different food systems should be monitored. Potential markets for the product must be explored and help given to establish the optimum usage of the powder.

Monitoring Functional Properties

Estimation of functionality is important, but difficult to estimate in tests. Model systems were developed as being easier and less complex than a full utility test, but results lack precision and accuracy. Although model tests can be used to give an indication of behaviour they cannot be used to follow small alterations to the protein during manufacture. If the mechanisms of the functional property of a product are to be ascertained then some prior knowledge of the protein or proteins acting in the system must be essential. The primary structure of the protein is probably less likely to be altered by processing than the tertiary and quaternary formation. Methods which indicate the conformation of proteins may correlate well with functional properties. Studying alterations in the protein structure may lead to a further understanding of how processing effects functionality.

Hydrophobicity estimations are meant to correlate well with functional properties, but the estimations have to be done practically on protein in the relevant medium. Theoretical values do not correlate. Spray drying of plasma was found to much decrease the activity of an enzyme requiring a three dimensional structure. This should be more carefully investigated as loss of activity could be used as an assessment of denaturing the protein. It would be a quick assay to perform and the precision would be high. Other enzymes of different molecule weights could be used. Working with plasma would allow for this type of survey during processing. Electrophoresis may be another way of studying the conformation of the proteins and relating the results to functionality.

This work does indicate that it was very hard to monitor processing by assessment of model tests for functionality. Estimations giving arithmetic values may be more helpful. The results may not be directly comparable with functionality in real systems, but neither are the model test results.

If methods to investigate the structure of the protein could be used routinely then a study of all the blood plasma preparations made would be interesting. If plasma powders produced by different methods were assayed by exactly the same functional tests and the range and structure of the proteins were determined, some link between production methods, protein structure and functionality may be ascertained. This knowledge could aid in future planning of production methods for functional compounds and for their functional assessment.

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APPENDICES

APPENDICES FOR CHAPTER 6

Appendix 6.1

Total Viable Counts (TVC) for the different stages of processing the Plasma

Powder No.	TVC in plasma 10^3 per ml	TVC in RBC 10^3 per ml	TVC in conc plasma 10^3 per ml	TVC in powder 10^3 per g
1	690	-	1593	64
2	14	-	5785	540
3	4	-	3443	136
4	41	1285	5250	-
5	136	27100	-	-
6	138	118	-	-
7	59	1158	-	-
8	-	-	-	440
9	47	3740	76000	2770
10	138	320	1647000	5130
11	21	126	55330	7220
12	18	147	64500	3425
13	31	417	187850	490

Microbiology of Plasma During Ultrafiltration (UF)

Time after start of UF (hours)	Run 1 TVCx10^3 per ml	Run 2 TVCx10^3 ml	TVCx10^5 per g protein
0	18	31	4
0.5	27	292	41
1.0	-	176	20
1.5	-	206	20
2.0	3535	37	4
3.0	-	235	21
3.5	-	529	39
4.0	2620	2980	250
22(15 hr. at 4°C)	64500	187850	12042

Appendix 6.1 cont.

The Microbial contamination investigated by the use of Selective Media

Media	Selective for:-
MacConkey (Mac)	Coliforms
Mannitol (Man)	Staphylococci
Desoxycholate Citrate (De)	Salmonella/Shigella
Sabouraud's Glucose (Sab)	Fungi

Sample	Mac 1 in 100	Man 1 in 100	De 1 in 500	Sab 1 in 100
Powder No. 1	ND	45	ND	1
No. 2	1	2	ND	13
No. 3	ND	19	ND	ND
No. 8	ND	430	ND	ND
No. 9	ND	2332	ND	ND
No.10	ND	2160	ND	ND
No.11	10	3928	ND	2
No.12	ND	ND	ND	ND
No.13	14	63	Det	39
Plasma 12	119	ND	33	44
Plasma 13	2	8	5	1
Conc Plasma 13	>2000	>2000	>500	>2000

ND = None detected

Det = colonies present, not countable due to swarmed appearance.

APPENDIX 6.2.1

DATA ON PLASMA FROM INDIVIDUAL CHURNS, PLASMA AFTER SEPARATOR AND CONCENTRATED PLASMA

Haemoglobin g/100 ml	Bilirubin g/100 ml	Total plate count 10^3/ml	pH	Total Protein g/100 ml	Albumin g/100 ml	Total Solids %	Lipids mg/100 ml
Individual Churns							
0.03	0.2	37	7.6	7.6	3.5	8.4	463
0.05	0.1	84	7.6	8.0	3.7	8.5	489
0.07	0.2	54	7.8	8.4	4.5	8.6	472
0.17	0.1	192	7.5	8.7	3.5	8.2	559
0.22	0.2	16	7.5	7.4	3.8	8.8	585
0.23	0.0	53	7.5	8.0	3.9	8.7	559
0.52	0.2	1434	7.6	8.4	3.4	8.8	472
0.64	0.2	9	7.7	8.5	3.5	8.9	550
0.32	0.2	65	7.6	8.2	4.0	8.9	419
0.10	0.2	149	7.7	8.1	4.0	8.4	664
Plasma after Separator							
0.07	0.6	690	7.9	7.0	3.0	8.3	326
0.08	0.2	14	7.7	6.8	3.1	7.5	412
0.18	0.7	4	7.8	8.2	4.1	7.9	410
0.02	0.5	41	7.8	8.3	3.1	8.6	437
0.60	0.3	136	7.5	7.0	3.1	8.3	438
0.26	0.5	138	7.7	7.4	4.0	8.4	463
0.10	0.4	59	7.6	8.2	3.8	8.6	463
-	-	-	-	-	-	-	-
0.48	0.7	47	7.4	7.0	3.6	9.2	172
0.06	0.8	138	7.8	6.5	3.3	8.4	305
0.07	0.4	21	8.7	7.5	3.1	9.1	360
0.02	0.2	18	8.1	7.4	4.1	9.0	241
0.01	0.7	31	8.1	6.9	3.6	8.7	468
Concentrated Plasma							
0.21	0.4	1593	8.4	-	-	9.5	-
0.18	0.3	5785	7.3	13.6	-	13.0	664
0.36	0.4	3443	8.0	15.8	-	15.7	673
0.07	0.8	5250	7.6	12.8	-	10.9	716
0.71	0.4	-	-	19.6	-	16.7	695
1.69	1.0	>6000	6.9	20.3	-	19.4	946
0.14	1.1	1647000	6.9	17.9	-	20.6	1227
0.03	1.5	55330	8.7	18.1	-	17.9	965
0.10	0.8	64500	8.1	17.1	-	18.1	808
-	0.7	187850	7.4	15.6	-	17.9	571

APPENDIX 6.4.1

DATA ON BOVINE PLASMA POWDERS

No.	B. DEN g/ml	L.PS μ	S.PS μ	D50 PS μ	PDR COL
1	0.440	53.5	2.4	14.8	1
2	0.409	87.2	2.4	17.1	1
3	0.414	37.6	2.4	14.1	1
8	0.406	37.6	2.4	14.2	3
9	0.395	53.5	3.0	14.4	8
10	0.395	53.5	2.4	13.4	7
11	0.427	53.5	3.0	15.6	12
12	0.390	87.2	3.0	25.5	7
13	0.420	53.5	2.4	15.7	4
16	0.344	37.6	2.1	13.8	11

No.	Solids %	Protein %	Lipids %	Sod. Cl. %	Calc mg/100 g
1	94	78.6	3.67	2.8	178
2	93	79.6	4.19	4.5	112
3	94	80.0	3.52	4.9	108
8	96	81.5	8.46	2.9	114
9	95	80.7	5.80	3.3	78
10	94	80.2	9.23	2.6	69
11	94	80.0	7.75	2.6	68
12	98	79.8	8.02	2.6	58
13	95	78.9	4.08	3.5	55
16	92	75.7	5.20	3.0	81

No.	Micro 10^3/g	Ash %	In. Sol %	Disp %	Wat Abs ml/100g
1	164	4.29	0.33	49.9	11.33
2	540	4.76	0.05	68.7	15.13
3	136	4.50	0.04	60.7	14.42
8	136	5.36	0.10	55.5	17.93
9	2770	5.61	0.07	50.2	15.17
10	5130	4.46	0.15	67.3	14.77
11	7220	3.00	0.21	54.1	16.03
12	3425	2.77	0.07	52.3	15.97
13	490	5.30	0.03	83.3	16.60
16	2	3.19	0.90	73.8	15.61

No.	Sol.Col	pH	Sp.G g/ml	Visc. cp	Inlet Temp °C	Outlet Temp °C
1	24	8.4	1.027	2.445	185	75
2	19	8.2	1.030	2.112	185	75
3	19	8.5	1.029	2.216	185	75
8	21	8.7	1.018	2.532	185	75
9	23	8.5	1.029	2.480	185	85
10	21	8.4	1.028	2.530	185	85
11	23	8.0	1.027	2.528	185	80
12	20	8.4	1.026	2.262	185	85
13	21	8.1	1.027	2.601	185	80
16	21	9.1	1.027	2.424	173	72

APPENDIX 6.4.1

CONT. DATA ON BOVINE PLASMA POWDER

No.	FAT m/g	FAT B %	FAT T %
1	1.249	12.17	12.38
2	1.303	15.73	9.29
3	1.389	15.64	10.13
8	1.593	13.16	14.37
9	1.634	12.04	15.84
10	1.333	10.81	14.48
11	1.026	7.87	14.77
12	1.627	13.42	14.41
13	1.422	14.47	11.58
16	0.984	13.22	5.20

No.	EM.CAP g oil/g	EM S1 %	EMS14 %	EM VISC.	OPV
1	282	8.1	1.4	52	85.8
2	280	9.8	6.3	54	85.7
3	294	8.9	4.0	54	86.3
8	297	6.8	8.7	39	86.4
9	293	15.2	5.2	57	86.2
10	286	15.9	3.1	54	86.0
11	294	9.4	5.2	53	86.3
12	261	13.7	4.3	46	84.8
13	280	12.0	4.7	52	85.7
16	305	2.2	2.4	35	86.7

No.	FOAM 5 ml	FOAM 25 ml	FOAM 60 ml	F60/5
1	51	41	32	62.3
2	60	41	18	28.5
3	59	39	24	41.0
8	56	39	23	40.0
9	52	35	19	34.7
10	69	52	42	60.6
11	73	55	46	63.0
12	68	53	47	69.2
13	75	59	50	67.1
16	49	35	20	41.2

No.	G 10% 80 mm/10	G 9% 80 mm/10	G 10% 75 mm/10	G 9% 75 mm/10	GEL TOT
1	119	281	229	410	1190
2	210	288	252	328	1078
3	203	279	213	303	998
8	198	353	233	417	1201
9	194	324	238	327	1083
10	186	310	241	302	1039
11	170	208	202	284	864
12	190	354	218	332	1094
13	179	231	186	284	880
16	169	191	218	419	998

APPENDIX 6.4.1.b

Amino Acid Analysis of some Protein Powders

Amino Acid	% of Total protein								
	BPP	PP/O	Alb FV	LSol*	Alo*	Egg P	Cas	Whey	Lact
Asparagine	8.81	10.52	8.72	4.1	6.7	9.26	6.47	9.35	9.87
Threonine	5.17	5.74	4.79	1.6	2.2	4.34	4.19	5.89	6.62
Serine	5.40	4.91	2.70	3.1	4.2	6.20	4.78	3.96	4.14
Glutamic acid	11.70	13.43	14.13	6.2	11.4	12.73	21.21	16.04	16.70
Proline	-	-	8.13	9.8	16.4	2.90	10.88	13.68	9.02
Glycine	4.55	4.57	1.47	25.9	27.5	3.65	1.99	2.13	1.92
Alanine	6.16	5.88	4.37	9.0	11.0	5.93	3.19	3.74	4.50
Cysteine	2.28	1.66	4.87	-	-	2.48	0.15	0.65	2.09
Valine	8.97	6.38	5.04	1.7	2.6	8.13	5.57	4.42	4.69
Methionine	0.46	0.45	1.18	0.4	0.9	1.94	1.66	1.11	1.28
Isoleucine	8.96	8.44	3.54	1.0	1.6	4.42	4.02	4.18	4.54
Leucine	10.18	10.11	9.89	2.3	3.5	8.00	9.89	8.07	9.45
Tyrosine	1.90	2.08	3.78	0.2	0.3	3.38	2.64	1.78	2.62
Phenyl-alanine	5.47	4.36	4.88	1.4	2.2	6.03	4.78	3.90	3.07
Ammonia	4.10	6.37	3.33	3.5	-	6.12	5.51	8.81	4.75
Lysine	8.59	8.10	10.05	4.3	4.5	6.53	7.44	7.54	9.77
Histadine	2.58	2.77	2.00	1.4	0.8	2.34	3.01	2.11	1.94
Arginine	5.38	4.22	5.83	16.1	8.8	5.60	3.70	2.67	3.02
Hydroxy-proline				8.3	14.1				
Hydroxy-lysine				1.1	-				

* Not assayed at LUT

Appendix 6.6 · a

Data on Samples during Spray Drying

Time(min)	B. DEN	L.PS	S.PS	D50 PS
20	0.317	37.6	1.9	12.4
40	0.338	53.5	2.4	15.0
60	0.376	37.6	2.4	14.4
80	0.359	28.1	1.9	10.9
100	0.349	37.6	1.9	12.6
120	0.369	37.6	1.9	11.5
150	0.363	53.5	1.9	14.9
180	0.361	37.6	1.9	11.5
Correlation				
time	0.585	0.051	-0.493	-0.232
Inlet Temp	0.461	0.079	0.381	0.274
Outlet "	0.771	0.121	0.172	0.032

Time	SOLIDS	PROTEIN	LIPIDS	SOD. CL	CALC
20	93.5	75.9	2.63	3.04	249
40	93.2	73.9	3.58	3.07	58
60	91.8	80.9	4.76	3.21	58
80	92.5	73.5	4.12	3.04	42
100	93.7	72.2	4.20	3.17	69
120	92.9	75.8	3.29	3.21	55
150	91.9	79.3	3.65	3.11	49
180	94.4	79.5	3.25	3.32	76
Time	0.165	0.364	-0.025	o.656	-0.490
Inlet Temp	-0.167	0.038	0.787	0.195	0.724
Outlet "	-0.225	-0.002	0.459	0.444	0.934

Time	MICRO 10^2	NON SOL	MEAN INLET TEMP	MEAN OUTLET TEMP
20	2	0.84	146	63
40	1	1.03	181	74
60	1	0.62	184	73
80	6	0.75	183	73
100	2	0.85	183	73
120	1	0.90	154	77
150	2	1.10	180	73
180	1	0.91	180	73
Time	-0.156	0.359		
Inlet Temp	0.267	-0.107		
Outlet "	-0.089	0.142		

Time	pH	Sp. G.	Visc.
20	9.1	1.026	2.413
40	9.1	1.030	2.476
60	9.1	1.027	2.415
80	9.1	1.026	2.440
100	9.1	1.028	2.389
120	9.1	1.026	2.468
150	9.1	1.027	2.580
180	9.1	1.029	2.419
Time	-	0.136	0.320
Inlet Temp	-	0.531	-0.009
Outlet Temp	-	0.256	0.276

Appendix 6.6.a cont

Data on samples during spray drying

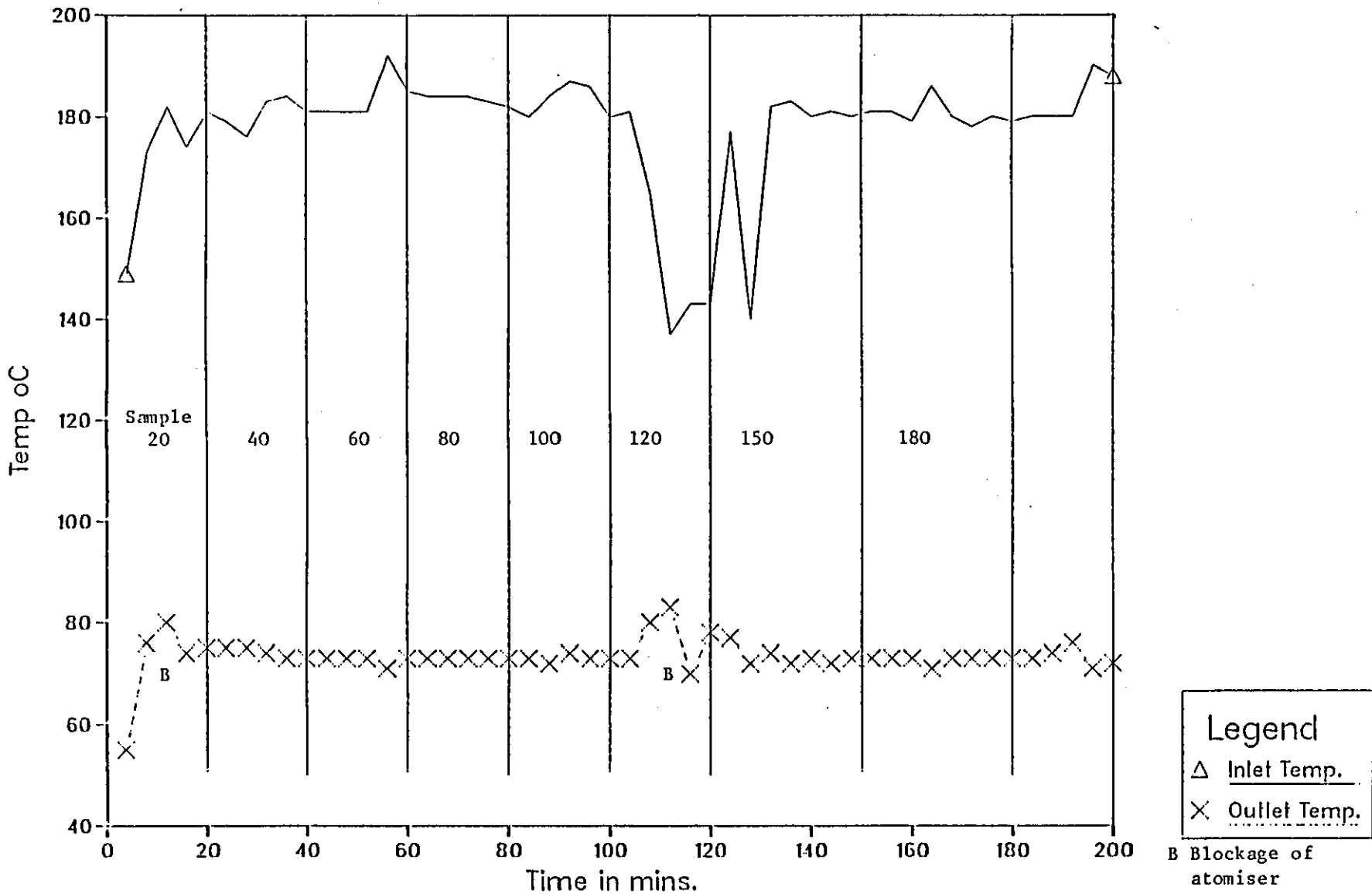
No	FAT	FAT B	FAT T
20	1.477	13.0	11.5
40	1.612	14.5	11.2
60	1.499	13.4	11.3
80	1.398	13.2	10.6
100	1.477	13.5	10.0
120	1.409	13.0	10.9
150	1.545	14.2	10.9
180	1.386	12.9	10.8
Time	-0.237	-0.014	-0.513
Inlet Temp	-0.408	-0.214	-0.170
Outlet "	-0.276		

No	EM CAP	EMS14
20	299	3.0
40	294	1.1
60	273	1.4
80	307	0.8
100	282	2.4
120	282	0.3
150	251	0.4
180	279	3.2
Time	-0.595	-0.039
Inlet Temp	-0.184	-0.098
Outlet "	-0.309	-0.612

No.	FOAM 5M	FOAM 25M	FOAM 60M	F60/5
20	47	26	19	40.4
40	52	35	27	51.9
60	57	39	33	57.9
80	40	31	17	42.5
100	54	34	26	48.2
120	53	32	28	52.8
150	59	37	29	49.2
180	57	35	30	52.6
Time	0.514	0.399	0.438	0.311
Inlet Temp	0.185	0.735	0.323	0.392
Outlet "	0.318	0.630	0.516	0.656

NO.	G 10% 80	G 9% 80	G 10% 75	G 9% 75	GEL TOT.
20	172	195	260	429	1056
40	145	244	365	441	1195
60	153	212	397	389	1151
80	168	196	300	434	1098
100	172	214	237	419	1042
120	145	203	256	348	952
150	132	270	233	360	995
180	142	245	246	340	973
Time	-0.590	0.537	-0.578	-0.831	-0.749
Inlet Temp	-0.143	0.395	0.362	0.098	-0.409
Outlet "	-0.526	0.282	0.127	-0.415	-0.111

APPENDIX 6.6.b A record of inlet and outlet temperatures for spray drying powder 16

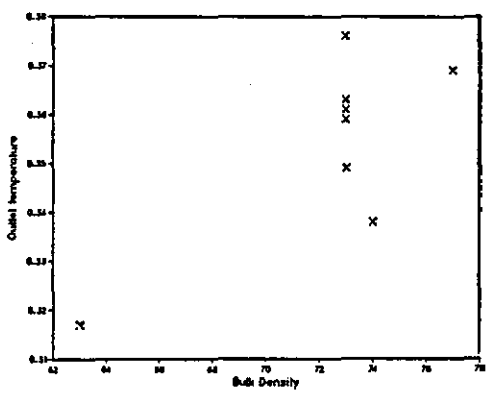


B Blockage of atomiser

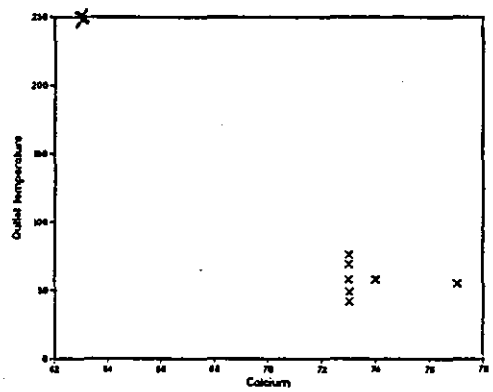
Appendix 6.6c

Data for powders collected during spray drying

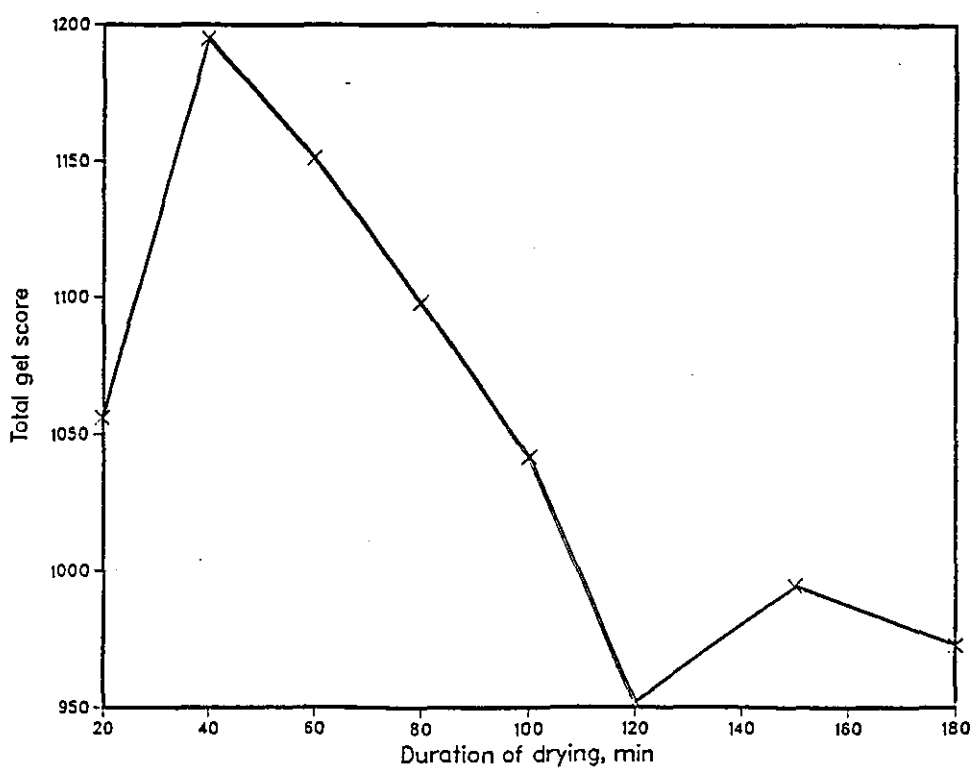
Bulk density against Outlet temperature



Calcium against Outlet temperature



Duration of drying against total gel score



Appendix 6.7 - Stability of Bovine Plasma Powder

Material		B.DEN	LPS	SPS	D50 PS	PDR COL.	SOLIDS	PROTEIN	LIPID	SOD CL	CALC
Samples at start of study		0.352	37.6	1.9	13.0	4	91.9	76.5	3.29	3.5	56
		0.346	37.6	2.4	13.6	4	92.4	76.5	2.68	2.9	86
		0.345	37.6	2.4	14.0	4	92.7	73.9	2.28	2.9	64
		0.336	37.6	1.9	14.3	4	93.3	74.2	2.86	2.8	59
		0.342	37.6	1.9	14.3	4	91.2	77.4	1.95	2.9	61
Samples after one year		0.397	53.5	1.9	11.1	4	92.7	78.5	2.53	3.6	70
		0.404	53.5	1.9	11.1	4	91.4	82.1	2.85	4.2	42
		0.440	53.5	1.9	10.9	4	90.0	77.6	2.72	3.6	63
		0.359	53.5	1.9	10.9	4	93.4	72.5	2.26	4.6	57
		0.369	53.5	1.9	10.8	4	90.5	72.5	2.48	4.0	54
No. of weeks after start of study	1*	0.344	37.6	2.1	13.8	4	92.1	75.7	2.61	3.0	65
	2	0.385	53.5	1.9	12.9	4	91.3	77.1	3.84	3.8	62
	3	0.373	53.5	1.9	13.2	4	91.5	75.6	4.43	3.3	69
	4	0.412	53.5	1.9	12.2	4	90.4	80.1	3.56	3.1	64
	5	0.423	53.5	1.9	12.1	4	90.4	72.4	1.94	3.3	66
	6	0.435	53.5	2.4	12.4	4	90.8	77.2	3.18	3.3	71
	8	0.402	53.5	1.9	12.0	4	90.1	70.0	2.99	3.5	57
	10	0.395	53.5	1.9	11.9	4	91.8	80.2	3.36	3.3	68
	14	0.423	53.5	1.9	11.3	4	92.8	78.1	2.84	4.8	72
	16	0.448	53.5	1.9	12.2	4	92.4	73.5	2.33	3.4	61
	21	0.478	53.5	1.9	12.7	4	90.3	83.3	3.93	4.1	40
	24	0.444	53.5	1.9	13.8	4	90.8	80.0	2.45	3.5	37
	30	0.396	53.5	1.9	11.7	4	90.0	81.8	3.36	2.7	96
	36	0.403	53.5	1.9	10.5	4	92.7	78.9	3.91	3.2	51
	42	0.387	53.5	1.9	12.9	4	90.1	74.9	4.00	3.2	60
	48	0.425	53.5	1.9	12.4	4	91.3	76.4	3.72	3.6[b]	49
	54 +	0.354[a]	53.5	1.9	11.0[c]	4	91.6	78.6	2.57	4.0[b]	57
Correlation with time		0.117					0.007	0.207	-0.117	0.107	-0.277

* = average results for samples at start of study
+ = average results for samples one year

Appendix 6.7 cont.

								FAT ABSORBANCE			EMULSION		
Material	Code	Micro 10^2	NONSOL	SOL.COL.	pH	Sp. G.	VISC.	FAT	FAT B	FAT T	EM CAP	EM S1	EM S14
Samples at Start of Study		1	0.90	21	9.1	1.025	2.411	0.542	11.3	4.9	319	0.0	2.0
		3	0.96	21	9.1	1.026	2.384	0.675	10.2	5.2	294	0.9	1.6
		1	0.89	21	9.1	1.029	2.474	0.969	14.4	5.6	314	0.6	5.0
		4	0.81	21	0.1	1.027	2.415	0.991	14.8	5.4	301	1.2	1.1
		3	0.94	21	9.1	1.026	2.434	1.002	15.4	4.9	299	0.4	1.4
Samples after one year		2	0.77	21	8.6	1.025	2.795	1.217	15.2	7.0	284	0.3	0.6
		3	0.66	21	8.6	1.032	2.769	1.172	15.4	6.4	245	2.8	1.5
		0	0.73	21	8.6	1.025	2.821	1.217	14.9	7.3	266	0.2	0.3
		1	0.80	21	8.6	1.041	3.051	1.206	13.9	8.2	254	0.2	0.9
		2	0.80	21	8.6	1.038	3.026	1.466	15.7	8.7	247	0.2	0.4
No. of weeks after start of study	1	2	0.90	21	9.1	1.027	2.424	0.836	13.2	5.2	305	0.6	2.2
	2	1	0.63	21	9.1	1.026	2.299	0.879	13.3	5.9	287	0.0	1.6
	3	5	0.67	21	9.1	1.029	2.287	0.800	12.5	6.0	296	0.0	0.5
	4	4	0.60	21	9.1	1.027	2.339	0.969	13.0	7.0	306	0.0	9.5
	5	0	0.45	21	9.1	1.027	2.276	0.935	15.8	3.9	317	1.9	1.9
	6	3	0.80	21	9.1	1.036	2.318	0.980	14.5	5.6	287	0.9	5.2
	8	2	0.67	22	9.2	1.043	2.366	1.319	15.4	7.7	290	0.8	1.9
	10	0	0.64	19	9.1	1.031	2.452	1.195	16.0	6.0	293	1.1	0.8
	14	3	0.58	19	9.2	1.030	2.561	1.059	13.9	6.9	235	0.7	2.4
	16	2	0.70	19	9.2	1.025	2.282	0.946	11.9	7.9	305	2.0	0.5
	21	2	0.98	19	8.9	1.030	2.389	1.533	17.0	8.0	288	0.5	0.7
	24	1	0.71	19	9.0	1.037	2.649	1.477	15.5	9.0	310	0.8	1.1
	30	0	0.84	21	9.0	1.029	2.687	1.420	15.5	8.5	303	0.9	1.4
	36	1	1.00	21	8.9	1.030	2.622	1.590	15.5	10.0	321	0.3	0.9
	42	1	0.79	21	8.7	1.022	2.595	1.646	16.0	10.0	288	0.7	2.5
	48	0	0.64	21	8.7	1.019	2.929$_b$	0.788	11.6	6.8	344$_b$	0.0	4.6
	54	2	0.75$_b$	21	8.6	1.032	2.892$_b$	1.256	15.0	7.5	259$_b$	0.7	0.7
Correlation with time		-0.397	0.308		-0.891	-0.257	0.693	0.489	0.160	0.627	0.077	-0.106	0.052

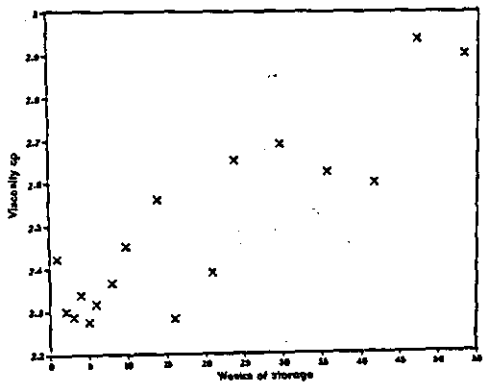
a = significant difference from control $p < 0.05$
b = significant difference from control $p < 0.01$
c = significant difference from control $p < 0.001$

Appendix 6.7 cont.

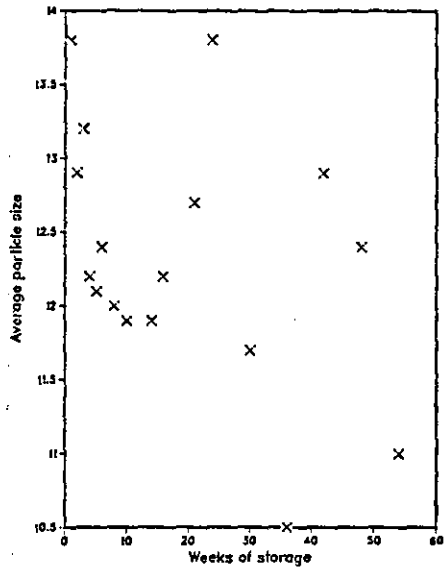
		FOAM FORMATION				GELATION				
Material	Code	Foam 5M	Foam 25M	Foam 60M	FIN % FOAM	G 10% 80	G 9% 80	G 10% 75	G 9% 75	GEL TOT
Samples at start of study		55	42	23	41.8	165	178	217	438	998
		54	40	14	25.9	184	198	215	421	1018
		45	28	25	55.6	177	192	207	402	978
		41	31	20	48.8	147	209	231	434	1021
		52	34	18	34.6	174	180	219	400	973
Samples after one year		52	41	34	65.4	275	349	345	400	1369
		46	32	26	56.5	215	355	280	256	1106
		62	47	43	69.4	185	375	351	245	1156
		61	41	34	55.7	218	270	265	400	1153
		72	51	46	63.9	175	190	210	256	831
No. of weeks after start of study	1	49	35	20	41.3	169	191	218	419	998
	2	55	33	18	32.7	193	268	177	296	934
	3	60	39	32	53.3	215	265	228	404	1112
	4	54	33	22	40.7	173	218	222	268	881
	5	56	31	13	23.2	275	436	285	453	1449
	6	58	36	26	44.8	193	259	414	483	1349
	8	58	38	17	29.3	235	318	277	424	1254
	10	56	40	17	30.4	222	295	340	490	1347
	14	52	32	20	38.5	225	368	275	310	1178
	16	60	34	22	36.7	210	288	397	451	1346
	21	50	40	34	68.0	203	284	362	500	1349
	24	58	41	34	58.1	205	305	248	298	1056
	30	50	34	24	48.0	223	299	325	433	1280
	36	59	33	30	50.8	174	402	221	444	1241
	42	62	42	34	54.8	205	429	208	415	1257
	48	63	46	40	63.5	217	307	193	415	1132
	54	59	42	37[b]	62.2[a]	214	308[a]	290	311[a]	1123
Correlation with time		0.433	0.584	0.750	0.680	-0.053	0.413	-0.127	-0.043	0.071

Appendix 6.7.b Data for stored powders

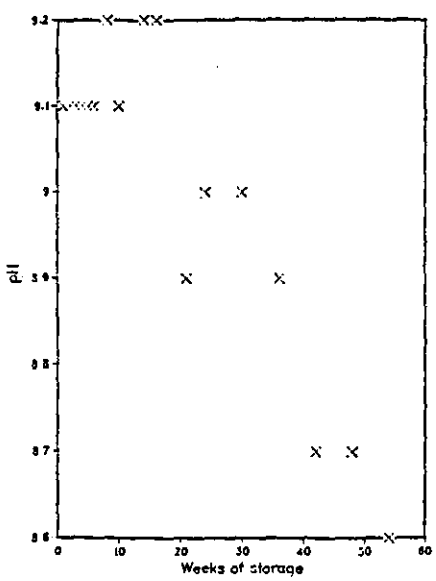
Viscosity of Solution



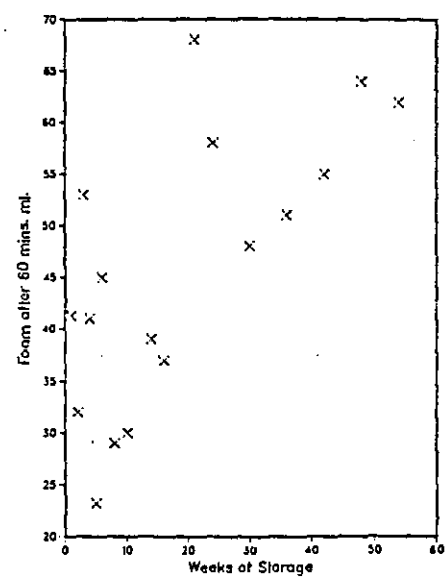
Particle Size



pH of Solution



Foam volume after 60 min



Appendix 6.8 - Data on a Variety of Protein Products

Material	Code	B. DEN	LPS	SPS	D50PS	PDR COL	SOLIDS	PROTEIN	LIPID	SOD. CL	CALC
BPP Mean-2SD	98	0.352	19.1	1.9	8.7	0	91	76.4	1.63	1.6	0
+ 2SD	99	0.456	91.9	3.2	23.0	14	98	82.6	10.35	4.9	179
powders from plasma/sera	BP/U	0.470	53.5	9.0	15.6	11	97	78.6	3.21	4.9	52
	BS	0.354	28.1	1.0	11.0	8	91	86.2	5.03	4.1	1
	BP/M	0.370	53.5	4.8	22.5	5	92	83.0	1.46	6.2	100
	bP/0	0.260	53.5	1.9	11.9	11	94	76.4	2.46	4.2	393
	PP/0	0.432	87.2	7.9	16.5	8	94	73.3	2.82	2.8	80
	PP/F8	0.434	28.1	1.9	10.3	2	93	78.0	3.34	2.4	56
powders associated with blood	RBC	0.578	87.2	4.8	26.3	29	97	93.2	5.98	2.1	26
	BCHy	0.584	87.2	5.2	29.7	6	97	79.5	0.22	17.8	92
	AlbFv	0.298	564.0	5.8	77.3	1	90	85.8	0.22	3.3	24
	AlbFib	0.830	112.8	5.8	39.3	4	94	71.9	3.17	5.1	89
powders of animal origin	Lcou	0.824	87.2	6.9	35.8	8	99	97.9	0.12	3.6	160
	Lsol	0.575	53.5	7.9	22.9	1	96	98.6	0.05	1.0	9
	Alo	0.477	112.8	5.8	40.9	4	95	92.4	0.38	2.6	46
	Alo/P	-	-	-	-	-	94	86.6	1.74	2.5	-
Egg powders	Egg P	0.602	87.2	6.7	24.3	5	94	91.0	3.88	2.8	36
	Egg F	0.598	7000.0	1100.0	5532.0	18	92	78.3	0.89	3.1	32
	Eggli	-	-	-	-	8	13	10.1	0.13	0.4	4
Dairy products	Cas	0.628	87.2	6.9	35.8	1	92	78.2	13.33	0.5	120
	Whey	0.797	87.2	5.9	40.3	5	96	12.7	12.63	2.9	254
	Lact	0.425	87.2	6.2	33.8	1	96	67.4	7.39	1.6	111
Soya products	Soy I	0.528	53.5	7.2	38.6	1	94	77.2	12.75	6.0	18
	Soy F	0.664	53.5	6.3	25.7	6	92	40.6	4.29	5.2	26
Fish waste	Hap	0.626	84.3	5.8	17.0	8	96	37.1	7.60	38.6	132

Appendix 6.8 cont

Material	CODE	MICRO	ASH	IN SOL	DISP	WAT ABS	SOL COL	pH	Sp.G	VISC.
BPP Mean -2SD	98	0	2.14	0.00	39.0	4.6	18	7.8	1.020	2.091
+2SD	99	7093	6.47	0.72	84.2	23.2	24	9.1	1.033	2.735
powders from plasma/sera	BP/U	19	1.94	0.18	65.3	5.5	22	9.0	1.025	2.572
	BS	31	1.86	0.31	78.3	5.7	26	9.0	1.022	2.404
	BP/m	238	2.99	0.24	59.2	4.2	24	9.6	1.038	3.694
	bP/0	50	4.02	1.20	80.1	17.8	22	8.2	1.028	2.256
	PP/0	60	5.31	0.16	58.0	4.6	28	7.9	1.028	2.538
	PP/F8	5	1.67	0.02	76.4	3.9	27	7.8	1.027	2.292
powders associated with blood	RBC	33	0.38	0.36	81.3	2.2	30	8.1	1.018	1.926
	BCHy	15	7.62	0.00	96.0	8.3	16	5.2	1.038	1.376
	AlbFV	0	1.64	0.00	89.6	0.0	13	5.0	1.022	1.548
	AlbFib	1460	9.38	1.30	73.3	24.5	21	9.1	1.038	2.422
powders of animal origin	Lcoll	8	4.41	12.78	10.7	16.3	17	4.4	1.030	-
	Lsol	2	2.02	0.00	87.3	13.8	18	7.9	1.028	6.410
	Al0	0	4.37	0.01	96.2	22.1	7	6.0	1.029	1.769
	Alo/P	321	-	0.11	81.2	-	12	7.0	1.035	1.846
Egg powders	Egg P	7	3.68	0.00	80.7	11.7	6	6.7	1.027	2.053
	Egg F	0	4.82	0.01	88.0	0.0	14	5.2	1.025	1.718
	Egg li	0	0.07	0.00	-	-	10	9.1	1.049	11.836
Dairy products	Cas	3	3.97	5.11	81.6	29.1	6	6.9	1.021	1.947
	Whey	0	7.39	0.30	94.6	9.9	9	5.9	1.038	1.801
	Lact	3	1.92	0.06	84.3	5.4	1	6.5	1.030	2.756
Soya products	Soy I	9	3.26	6.75	23.3	4.6	15	7.2	0.961	-
	Soy F	0	4.99	1.80	28.7	7.4	1	6.3	1.027	-
Fish product	Hap	7	-	4.57	-	15.9	17	6.4	1.049	-

Appendix 6.8 cont.

		FAT ABSORBANCE			EMULSION			
MATERIAL	CODE	FAT	FAT B	FAT T	EM CAP	OPV	EM 51	EMS14
BPP Mean -2SD	98	0.896	12.85	5.69	262	84.9	2.8	0.4
+2SD	99	1.816	17.53	18.81	311	86.9	18.0	8.7
Powders from plasma/sera	BP/u	1.538	10.96	16.10	296	86.4	0.7	11.1
	BS	0.946	6.45	14.96	316	87.1	1.1	13.2
	BP/m	0.946	19.00	7.00	201	81.1	1.1	1.3
	bP/0	0.980	9.80	15.52	262	84.9	4.6	5.2
	PP/0	1.687	11.92	16.41	247	84.1	2.0	1.9
	PP/F8	0.647	15.00	6.00	263	84.9	0.7	3.8
Powders associated with blood	RBC	0.534	8.65	7.69	271	85.3	10.9	12.8
	BCHy	1.229	21.18	0.51	46	49.6	100	100
	Alb Fv	3.352	38.81	0.00	270	85.2	0.4	0.9
	Alb Fib	0.386	16.50	0.00	275	85.5	0.5	13.4
Powders of animal origin	Lcoll	0.885	18.62	0.00	167	78.1	92.2	83.1
	LSol	1.075	16.14	4.17	281	85.7	70.9	100.0
	Alo	0.366	10.50	10.48	216	82.2	4.2	6.0
	Alo/P	0.370	10.19	6.89	262	84.9	4.6	5.2
Egg powders	Egg P	0.921	18.11	0.83	226	82.9	11.8	3.3
	Egg F	0.942	17.70	0.00	240	83.7	3.5	1.1
	Egg Li	-	-	-	215	82.2	0.0	2.5
Dairy Products	Cas	1.767	15.15	11.33	336	87.8	5.2	41.0
	Whey	0.278	13.19	0.00	190	80.3	100	100
	Lact	1.391	15.52	11.02	160	77.4	100	100
Soya Products	Soy I	1.694	18.79	7.05	227	82.9	94.0	88.6
	Soy F	0.833	18.15	0.00	184	79.8	100	100
Fish product	Hap	0.772	21.50	0.00	160	77.4	97.1	99.0

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Appendix 6.8 cont

		FOAM FORMATION				GELATION				
Material	CODE	FOAM 5M	FOAM 25M	FOAM 60M	FIN % FOAM	G 10% 80	G 9% 80	G 10% 75	G 9% 75	GEL TOT
BPP Mean -2 SD	98	43	27	6	21	162	171	182	341	817
+2 SD	99	80	63	58	81	217	399	262	449	1269
Powders from plasma/sera	BP/u	60	30	26	43	153	228	247	289	917
	BS	63	40	30	48	283	457	430	u	1670
	BP/m	45	35	28	62	162	320	274	440	1256
	bP/0	75	45	20	27	159	271	271	340	1094
	PP/0	52	42	37	62	141	170	186	245	742
	PP/F8	70	49	37	53	160	196	227	319	902
Powders associated with blood	RBC	48	33	25	52	195	282	226	282	985
	BCHy	3	0	0	0	x	x	x	x	2400
	AlbFv	93	68	43	46	174	205	217	235	831
	AlbFib	65	44	35	54	276	361	337	433	1407
Powders of animal origin	Lcoll	4	4	1	25	x	x	x	x	2400
	Lsol	55	23	13	24	x	x	x	x	2400
	AlO	36	10	7	19	x	x	x	x	2400
	Alo/P	74	54	36	49	u	u	u	u	2000
Egg powders	Egg P	63	52	47	76	220	312	246	393	1171
	Egg F	66	54	50	76	181	204	275	285	945
	Egg Li	58	46	43	74	134	158	180	198	670
Dairy products	Cas	59	35	9	15	x	x	u	x	2300
	Whey	8	7	5	63	u	u	u	u	2000
	Lact	5	0	0	0	u	u	u	u	2000
Soya products	Soy I	20	18	16	83	u	u	u	x	2100
	Soy F	5	5	2	50	x	x	x	x	2400
Fish product	Hap	14	13	11	79	x	x	x	x	2400

u = gel not strong enough to stop penetrometer cone (value = 500 for total)
x = no gel formation (value = 600 for total)

APPENDIX 6.8.b.

Emulsification properties of proteins when weight used was adjusted to make the protein levels in the sample equivalent to those in bovine plasma powder assays.

Sample	% Protein in product	EC g oil/g protein =protein in BPP	EC g oil/ g powder	ES 14% protein	ES 14% powder wt
PP/O	73.3	258	247	1.3	1.9
EggF	91.0	227	226	3.4	3.3
BCHy	79.5	<160	<160	92.0	100
Soy I	77.2	244	227	81.4	87.6
Soy F	40.6	<160	184	90.9	100
Cas	78.2	284	336	51.1	41.0
Lcoll	97.9	<160	167	100	83.1
Lsol	98.6	310	281	80.7	100
Whey	12.6	210	190	76.1	89.7
AlbFV	85.6	231	270	1.3	0.9

Appendix 6.9

Correlation values between estimations made on ten bovine plasma powders

	B.DEN	D50 PS	SOLIDS	PROTEIN	LIPIDS	SOD CL.	pH	SP. GRAV	FAT	EM CAP	TOT GEL	FOAM 5M
D50 PS	-0.045											
Solids	0.180	-0.691										
Protein	0.503	0.081	0.571									
Lipids	-0.216	0.198	0.442	0.431								
Sod Cl	0.127	-0.168	-0.318	0.047	-0.686							
pH	-0.764	-0.235	-0.204	-0.437	0.024	-0.079						
Sp Grav	0.005	0.001	-0.445	-0.296	-0.486	0.423	-0.303					
Fat	0.118	0.367	0.792	0.676	0.190	0.077	-0.055	-0.286				
Em Cap	-0.293	-0.836	-0.645	-0.222	-0.068	0.087	0.519	-0.160	-0.461			
Tot Gel	0.010	0.087	0.271	0.254	0.096	-0.113	0.406	-0.409	0.465	-0.125		
Foam 5M	0.333	0.331	0.320	0.275	0.334	-0.143	-0.752	0.085	0.008	-0.470	-0.646	
Fin % Foam	0.310	0.386	0.420	-0.062	0.274	-0.596	-0.421	-0.067	-0.065	-0.516	-0.295	0.639

Correlation values between estimations made on a variety of protein powders

D50 PS	0.103											
Solids	0.420	0.014										
Protein	-0.322	0.049	0.558									
Lipids	0.201	-0.172	0.217	-0.277								
Sod. Cl	0.154	-0.064	0.063	-0.310	0.077							
pH	-0.369	-0.301	-0.260	-0.015	0.080	-0.164						
Sp Grav	0.161	-0.031	-0.251	-0.337	-0.369	0.281	0.045					
Fat	-0.439	-0.054	-0.245	0.215	0.071	-0.062	-0.089	-0.313				
Em Cap	-0.230	0.027	-0.004	0.173	0.274	-0.384	0.555	-0.220	0.330			
Tot Gel	0.508	-0.184	0.314	-0.023	0.250	0.330	-0.466	-0.051	-0.244	-0.502		
Foam 5M	-0.421	0.166	-0.137	0.309	-0.208	-0.356	0.384	0.049	0.346	0.617	-0.695	
Fin % Foam	0.177	0.235	-0.218	-0.334	0.217	0.186	0.187	-0.072	0.048	0.192	-0.386	0.299

APPENDIX 6.10. - Solubility of 5% solution of BPP at different pH values and the amount of acid or alkali required to alter the pH of 500 ml.

pH	% Soluble powder		% Soluble protein		ml of 1M NaOH or 1M HCl	
	Mean	SD	Mean	SD	Mean	SD
3	89.5	10.58	71.6	8.46	33.4	2.01
3.5	90.5	3.29	72.4	2.63	26.8	0.35
4	93.9	6.16	75.1	4.93	20.7	1.81
4.5	83.5	3.83	66.8[a]	3.06	15.3	1.78
5	76.0	5.00	60.8[a]	4.00	11.0	0.90
5.5	80.3	10.01	64.2	8.01	7.8	0.79
6	88.3	5.80	70.6	4.64	5.3	0.64
6.5	92.8	3.59	74.2	2.87	3.3	0.60
7	92.9	3.31	74.3	2.65	2.1	0.51
7.5	96.1	4.40	76.1	3.52	1.1	0.52
8	91.9	5.44	73.5	4.35		
8.5	94.1	6.26	75.3	5.01	0.6	0.10
9	94.1	4.93	75.3	3.94	1.0	0.35
9.5	89.4	7.19	71.5	5.75	1.5	0.44
10	94.0	7.76	75.2	6.21	2.8	0.61
10.5	93.8	7.39	75.0	5.91	4.4	0.93
11	93.8	8.51	75.0	6.81	7.1	1.18
11.5	97.1	8.31	77.7	6.65	11.4	2.28
12	91.1	8.28	72.9	6.62	18.2	3.70

a = significantly different from pH 8.0 $p = < 0.05$

Appendix 6.10.b

Solubility at different pH values and concentrations for BPP No 11

Medium	pH	Conc 2.5%		5%		7.5%		10.0%	
		Mean *	SD	Mean*	SD	Mean*	SD	Mean*	SD
distilled water	5.0	52.0	8.98	60.8	4.00	69.5	6.68	69.4	6.55
	8.0	70.9	9.14	73.5	4.35	82.6	6.25	81.9	6.95
	10.0	72.2	5.57	75.2	6.21	82.6	6.43	83.3	3.88
1M NaCl	5.0	74.9	9.35	76.7	3.51	76.9	1.21	74.0	8.60
	8.0	79.1	4.90	80.3	5.26	81.6	2.97	77.0	5.25
	10.0	75.5	8.26	79.9	4.66	78.9	5.05	77.7	4.40
1M Sucrose	5.0	71.2	14.5	74.2	5.08	74.3	8.62	69.2	7.13
	8.0	80.7	7.10	74.6	8.17	73.1	2.97	75.0	6.45
	10.0	69.1	4.68	75.6	2.29	72.4	4.75	74.2	4.64

Significant differences

a = $p < 0.05$ b = $p < 0.01$ c = $p < 0.001$

Medium	Concentration	pH	Probability
Water	2.5% v 10%	5.0	a
	2.5% v 7.5%	5.0	a
	2.5%	5.0 v 8.0	a
	5.0%	5.0 v 8.0	b
	7.5%	5.0 v 8.0	a
	10.0%	5.0 v 8.0	a
	2.5%	5.0 v 10.0	b
	5.0%	5.0 v 10.0	b
	7.5%	5.0 v 10.0	a
	10.0%	5.0 v 10.0	a
1M Sodium Chloride	7.5%	5.0 v 8.0	a
1M Sucrose	2.5% v 5%	10.0	a
	5.0%	8.0 v 10.0	a

* values are (g protein in the solution ÷ total weight of powder used) * 100

Appendix 6.11

Lactate dehydrogenase activity

1st Experiment

	U/litre			U/100 g protein		
	Plasma	Conc Plasma	10% Solution Powder	Plasma	Conc. Plasma	Powder
	3.5	60.0	8.5	50	441	108
	45.0	5.0	8.5	662	32	106
	65.0	8.5	6.5	793	66	81
	10.0	185.0	10.0	143	944	123
	41.5	136.5	8.5	561	672	106
	31.5	225.0	5.0	384	1297	63
			5.0			63
			18.6			234
Mean	33.0	103.0	9.0	432	569	101
SD	23.0	93.0	4.0	294	486	55

T Tests

Samples	Units	T Value	Significant at	Units	T Values	Significa at
Plasma v conc plasma	U/ℓ	-1.807	0.1305	U/100g	-0.589	0.5718
Plasma v powder	U/ℓ	2.518	0.0533	U/100g	2.647	0.0458
Conc. plasma v powder	U/ℓ	2.491	0.0551	U/100g	2.298	0.0700

2nd Experiment

	U/litre			U/100g protein		
	Plasma	Conc. Plasma	10% Solution Powder	Plasma	Conc. Plasma	Powder
	83	107	10	105	82	13
	83	95	12	105	73	15
	68	88	12	86	68	15
	65	77	24	82	59	31
	65	80	25	82	62	32
Mean	73	89	17	92	69	21
SD	9.4	12.1	7.3	12.0	9.2	9.4

T Tests

Samples	Units	T Value	Significant at	Units	T Value	Significa at
Plasma v conc plasma	U/ℓ	-2.424	0.0458	U/100g	3.442	0.0108
Plasma v powder	U/ℓ	10.583	0.0000	U/100g	10.378	0.0000
Conc. plasma v powder	U/ℓ	11.537	0.0000	U/100g	8.095	0.0000

APPENDICES for Chapter 7

Section 7.1

APPENDIX 7.1.B

Fat Absorbance: Mixing with a rod compared to using the Silverson Mixer

	ml oil/g protein	B %	M%	T%
	1.25	8.3	75.4	16.3
ROD	1.52	10.0	73.1	16.9
	1.32	8.7	74.8	16.5
	1.31	9.7	74.9	15.4
Mean	1.35	9.2	74.6	16.3
SD	0.117	0.82	1.01	0.64
	1.63	10.9	72.2	16.9
SILVERSON	1.52	10.3	73.1	16.5
	1.47	9.5	73.6	16.9
	1.50	11.5	73.3	15.2
Mean	1.53	10.6	73.0	16.4
SD	0.069	0.86	0.60	0.81

The values obtained by mixing with the Rod are NOT significantly different for those obtained by mixing with the Silverson

Appendix 7.1.B.b

Fat Absorbance: Oil absorption when oil and powder are associated for varying lengths of time

Time	ml oil/g protein	B %	M %	T %
10 min	1.63	10.9	72.2	16.0
	1.51	10.3	73.1	16.5
	1.47	9.5	73.6	16.9
	1.50	11.5	73.3	15.2
	1.46	10.0	73.6	16.3
2 hour	1.42	10.7	74.0	15.3
	0.92	8.6	78.3	13.1
	1.49	11.2	78.4	15.5
	1.38	11.7	74.4	13.9
24 hour	1.38	12.2	74.4	13.5
	1.16	10.1	76.2	13.7
	1.56	12.4	72.7	14.9

Significant difference between the T% for 10 min and 24 hr. $p < 0.05$

Appendix 7.1.b

Fat Absorbance: Variation in the duration of mixing

Mixing Time: Min	ml oil/g protein	B%	M%	T%
0.5	1.16	8.7	76.2	15.0
0.5	1.36	9.1	74.5	16.3
1.0	1.20	10.1	75.9	14.0
1.0	1.00	9.1	77.6	13.3
1.5	1.32	11.0	74.8	14.2
1.5	1.19	10.0	75.9	14.1
2.0	1.31	10.5	74.9	14.6
2.0	1.25	9.7	75.4	14.9
3.0	1.32	10.5	74.8	14.7
3.0	1.16	8.2	76.2	15.6
4.0	0.98	8.3	77.8	13.9
4.0	1.07	9.5	74.0	13.5

Correlation of mix and B% = -0.175 Mix and B ml = -0.301

M% = 0.372 M ml = 0.225

T% = -0.289 T ml = -0.632

APPENDIX 7.1.C

Fat Absorbance: Variation with temperature

Temp °C	No assayed	ml oil/g protein Mean	SD	B% Mean	SD	M% Mean	SD	T% Mean	SD
4	4	1.45	0.22	11.1	0.64	73.7	1.92	15.2	1.48
20	6	1.49	0.08	12.0	0.69	73.7	0.71	14.7	0.31
40	4	1.38	0.04	11.9	0.50	74.3[a]	0.34	13.8	0.82
60	4	1.38	0.08	11.5	0.85	74.3	0.74	14.2	0.61
80	4	1.31	0.10	10.2	0.64	75.0[b]	0.89	14.9	0.65

Temp °C	No	% oil absorbed Mean	SD	B ml Mean	SD	M ml Mean	SD	T ml Mean	SD
4	4	134.2	20.6	0.86[b]	0.05	5.81[a]	0.34	1.20	0.13
20	6	136.7	7.5	1.07	0.11	6.56	0.25	1.32	0.05
40	4	123.5[b]	3.5	1.08	0.03	6.78	0.09	1.26	0.09
60	4	121.7[a]	7.6	1.05	0.09	6.80	0.06	1.30	0.07
80	4	114.0[b]	9.0	0.90[a]	0.08	6.63	0.25	1.31	0.03

a = significant different from results at 20°C $p < 0.05$
b = significant different from results at 20°C $p < 0.01$
c = significant different from results at 20°C $p < 0.001$

APPENDIX 7. 1.D

Fat Absorbance: Variation in the amount of powder

%	Powder g	No assayed	ml of oil/g protein Mean	SD	B% Mean	SD	M% Mean	SD	T% Mean	SD
2.4	0.5	2	2.33	0.86	3.7	1.91	90.6	2.05	5.8	0.2
4.8	1.0	2	1.42	0.21	4.3	0.50	86.0	0.99	9.8	0.5
7.0	1.5	2	0.98	0.30	3.7	2.05	86.1	2.01	10.4	0.0
9.1	2.0	6	0.77	0.08	6.3	0.14	79.6	0.71	14.1	0.5
11.1	2.5	2	0.70	0.21	7.4	0.99	76.0	2.26	16.6	1.2
13.0	3.0	2	0.73	0.13	9.9	0.00	72.9	0.00	17.2	0.0
14.9	3.5	6	0.89	0.16	11.8	1.24	65.0	2.13	22.2	1.2
16.7	4.0	6	0.79	0.22	13.2	1.98	63.8	3.30	23.0	1.7
18.4	4.5	6	0.75	0.22	14.2	1.55	61.1	3.71	24.8	2.2

No significant differences in the amount of oil absorbed per g of powder when levels of 2, 3.5, 4.0 and 4.5 g powder were used

Correlation of g of powder and B% = 0.935
g of powder and T% =-0.969
g of powder and M% = 0.968

Appendix 7.1.E

Fat Absorbance: Variation of water volume

Sample	ml oil/g powder	Top %	Base %
Water 50 µl	1.586	16.1	11.4
Control	1.789	13.4	15.8

Appendix 7.1.e

Fat Absorbance:- Variation in the oil volume

%	Oil ml	ml oil/g powder	B%	M%	T%
83	10	0.84	12.8	63.0	24.2
88	15	1.10	11.2	70.3	18.5
91	20	1.45	8.0	73.7	18.3
91	20	0.98	7.8	77.8	14.4
93	25	0.75	5.6	83.3	11.1
93	25	0.79	4.0	83.1	12.9
94	30	0.87	3.6	85.1	11.2
94	30	0.74	3.6	85.9	10.5

Correlation of ml oil and B% = -0.979
M% = 0.976
T% = -0.946

Fat Absorbance:- Comparison of oil absorbed using 40 & 60 ml of oil

%	Oil ml	ml oil/g powder	%	Oil ml	ml oil/g powder
91	40	0.600	94	60	0.726
91	40	0.825	94	60	1.146
91	40	1.131	94	60	1.401
91	40	1.076	94	60	1.622
Mean		0.908			1.224
SD		0.245			0.385

No significant difference (T = 1.38)

Appendix 7.1.H.

Fat Absorbance:- Variation in pH

Sample	ml oil/g powder	Top %	Base %
Acid	1.622	15.5	12.3
Control	1.789	13.4	15.8

Appendix 7.1.I

Fat Absorbance: Variation in the oil

Fat	No	ml oil/g protein		B%		M%		T%	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Corn oil	6	1.22	0.04	9.3	0.17	75.7	0.37	15.0	0.45
Lard	3	1.32[a]	0.04	9.3	0.90	74.8[a]	0.35	16.4[a]	0.59
Solid veg oil	4	1.20	0.15	8.7	0.74	75.9	1.33	15.8	0.64

a = Significantly different from the results of corn oil $p < 0.05$

Appendix 7.1.J

Fat Absorbance: Contents of the T and B layers

Layer	Kjeldahl Estimation powder	Ether Extraction Oil	Ether Extraction Residue	S.G. of Layer	S. G. of powder	Volume taken up by 1 g powder
Top	41.9%	45.0%	47.9%	0.775	0.665	1.503
Bottom	38.3%	49.5%	48.7%	0.987	1.097	0.912

Layer	Ratio by weight			Ratio by volume		
	oil		powder	oil		powder
Top	1	:	0.931	1	:	1.282
Bottom	1	:	0.774	1	:	0.646

Appendix 7.1.K

Fat Absorbance: Fine and coarse particles

Sample	Average size μ	ml oil/g powder	Top %	Base %
Original sample	-	1.79	13.4	15.8
fine particles	6	1.31	8.9	16.2
Middle range	15	1.45	6.3	19.9
coarse particles	39	0.96	3.0	19.0

Appendix 7.1.L

FAT ABSORBANCE:- BPP heated for 5 hours at 85°C

Sample	ml of oil/g powder		ml of oil/g dried product		B%		M%		T%	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dried sample	1.23	0.05	1.23	0.05	9.3	0.12	75.6	0.4	15.1	0.3
Control	1.19	0.13	1.27	0.14	9.6	0.90	75.9	1.1	14.5	0.9

BPP dried for various times at 108°C

Sample	ml oil/g powder		% w/w (as is)		% w/w(dried product)		B%		T%	
Sample	1.35	0.12	124	10.7	131	11.4	9.2	0.82	16.3	0.64
2	1.57	0.22	144	20.5	148	21.1	10.6	0.01	16.7	1.92
4	1.54	0.19	141	17.0	141	17.0	10.6	0.11	16.5	1.71
6	1.86	0.12	170	11.2	170	11.2	10.8	0.06	19.0	1.00

		All values	Mean values
Correlation between drying time and	B%	0.749	0.829
	M%	-0.793	-0.923
	T%	0.617	0.823
	ml/g	0.793	0.922
	(as is) %w/w	0.793	0.922
	(dry) %w/w		0.860

Appendix 7.1.M

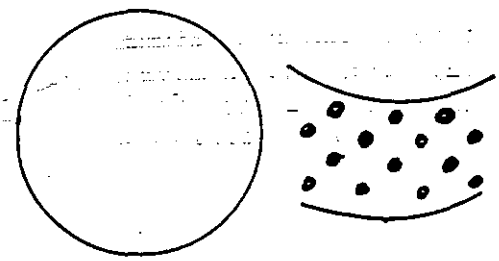
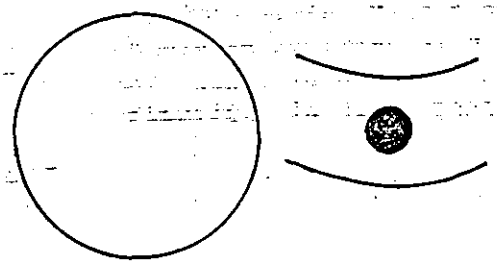
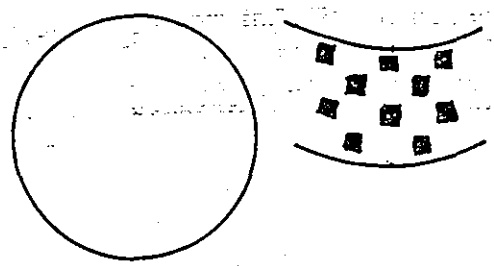
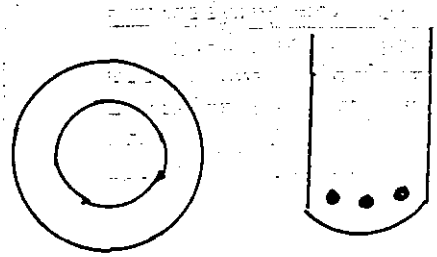
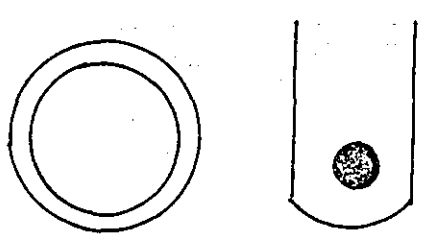
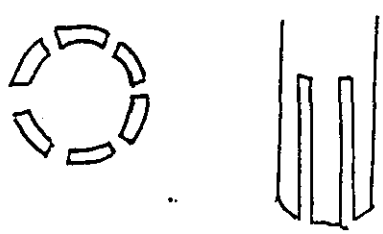
FAT ABOSRBANCE: Protein Fractions of Bovine Plasma

Protein	SG of powder Mixture	PS.L	PS.S	PS.50 ml oil/ g powder		Top%	Bottom %
Pfd	0.933	188	3	46	2.25	0	29
10% fd	0.935	188	2	38	2.85	0	34
20% fd	0.939	188	2	35	2.26	0	29
SDfd	0.890	54	3	15	1.44	16	9
11	0.891	54	3	15	1.21	14	10
2	0.893	87	2	17	1.32	18	13
A	0.932	523	23	188	2.05	0	27
G	0.963	523	18	146	6.35	0	58
FV	0.924	564	6	77	3.92	0	43

Pfd 1 = Freeze dried plasma
10% fd = A solution of 10% BPP No 11 freeze dried
20% fd = A solution of 20% BPP No 11 freeze dried
SD fd = Powder No 11, put through the freeze drying process.
11 = Powder No 11
2 = Powder No 2
A = Bovine Albumin
G = Bovine Globulin
FV = Bovine Albumin Fraction V

SECTION 7.2

APPENDIX 7.2B - Various types of Head used with the Silverson Mixer and the results obtained for emulsions using Bovine Plasma Powder

cross section	type of screen	Description
		Emulsion Screen 1.25 inch Emulsion capacity <147 g oil/g powder Stability 24 hrs. 100% oil released Stability 14 days 100% oil released
		Disintegrating Head 1.25 inch Emulsion capacity <147 g oil/g powder Stability 24 hrs 93% oil released Stability 14 days 100% oil released
		Square Hole High Shear Screen 1.25 inch Emulsion capacity < 147 g oil/g powder Stability 24 hours 100% oil released Stability 14 days 100% oil released
		Emulsifying Head 3/4 inch Designed for the preparation of emulsions with liquid/liquid phases Emulsion capacity 302 g oil/g powder Stability 24 hours 0% oil released Stability 14 days 2% oil released
		Disintegrating Head 3/4 inch Designed for the reduction of agglomerates or semi-solid materials Emulsion capacity 276 g oil/ g powder Stability 24 hours 0% oil released Stability 14 days 1% oil released
		Micro Slot Disintegration Head 5/8 inch Designed for the destruction of fibrous organic animal and vegetable tissues Emulsion capacity 189 g oil/g powder Stability 24 hours 1% oil released Stability 14 days 1% oil released

APPENDIX 7.2b - Emulsions: Duration of Mixing

Mixing Time	EC g oil/ g powder	OPV	ES1 %	ES14 %	Visc cp
0.5	254	0.845	1.9	1.8	26
1	263	0.849	0.2	3.5	32
1.5	286	0.860	0.0	0.8	40
2	299	0.865	0.7	3.3	53
3	244	0.839	0.2	0.9	51
4	235	0.834	0.9	0.8	49

APPENDIX 7.2D - Emulsions: Varying the amount of powder

g Powder in sample	ES1 %	ES14 %	Visc cp
0.125	100	100	11
0.250	100	71	11
0.375	1.68	0.42	49
0.5 *	9.71	9.89	48
0.625	2.88	0.45	48
0.75	1.48	0.58	47
1.0	1.63	3.17	69
1.25	1.59	0.14	50

g Powder in sample	Amount of oil (g) per sample	EC g oil/ g pdr	EC ml oil/ g pdr	Water per g pdr	OPV	1/EC	Abs at 500 nm
0.015	< 80	-	-	-	-	-	-
0.04	137	3423	3737	625	0.857	0.00029	-
0.05	140	2809	3067	500	0.860	0.00036	0.218
0.15	136	907	990	167	0.855	0.00110	0.306
0.2	158	789	861	125	0.873	0.00127	0.317
0.25	155	620	677	100	0.871	0.00161	0.359
0.375	176	468	511	67	0.885	0.00214	0.324
0.5 *	179	357	390	50	0.886	0.00280	0.406
0.625	167	266	290	40	0.879	0.00376	0.391
0.75	184	245	268	33	0.889	0.00408	0.412
1.00	194	194	212	25	0.895	0.00515	0.548
1.25	198	158	172	20	0.896	0.00633	0.578

* amount in standard procedure

APPENDIX 7.2E - Emulsions varying the amount of water

ml of water per sample	ES1 %	ES14 %	Visc cp
0	100	100	7
5	100	100	7
10	97	100	9
15	1	1	45
20	0	3	54
25*	0	1	49
30	1	3	46
35	2	1	41

Emulsion: variation in the amount of water in the Sample

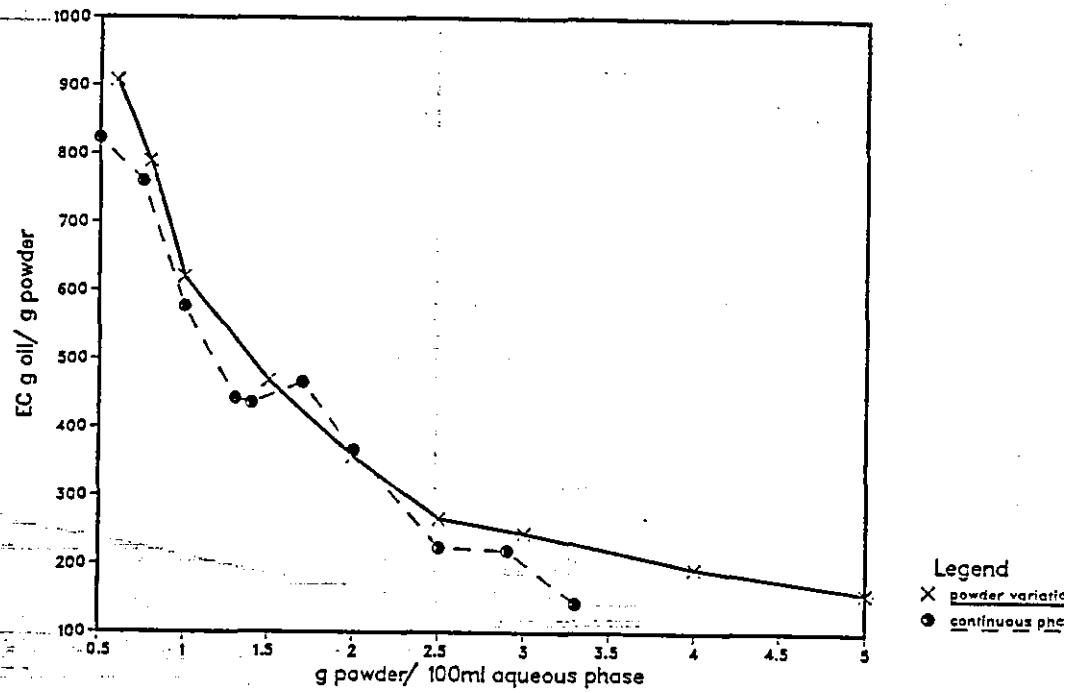
ml of water per sample	conc. of powder g/100 ml Sample	EC g oil/ g protein	OPV	1/EC	Abs at 500 nm
15	3.33	143+	0.839	0.00699	0.542
17.5	2.86	220+	0.873	0.00455	-
20	2.50	224+	0.860	0.00446	0.228
25*	2.00	367	0.889	0.00272	0.236
30	1.67	466	0.896	0.00215	0.204
35	1.43	436	0.872	0.00229	0.257
40	1.25	442	0.860	0.00226	0.225
50	1.00	577	0.863	0.00173	0.296
75	0.67	760	0.847	0.00132	0.197
100	0.50	823	0.818	0.00122	0.156

* value used in standard procedure

\+ values of < 80 ml oil used in initial procedure

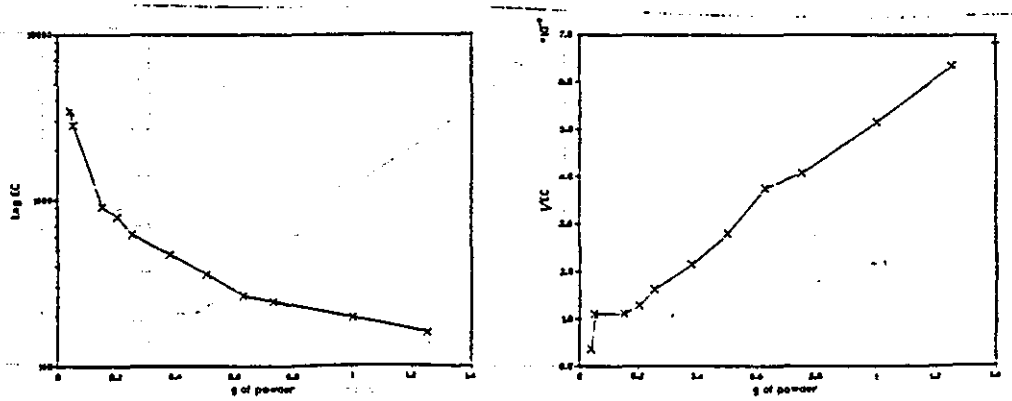
APPENDIX 7.2.E.b

EC Results for varying powder concentrations and volume of aqueous phase when concentrations of powder in solution are converted to g per 100 ml aqueous phase

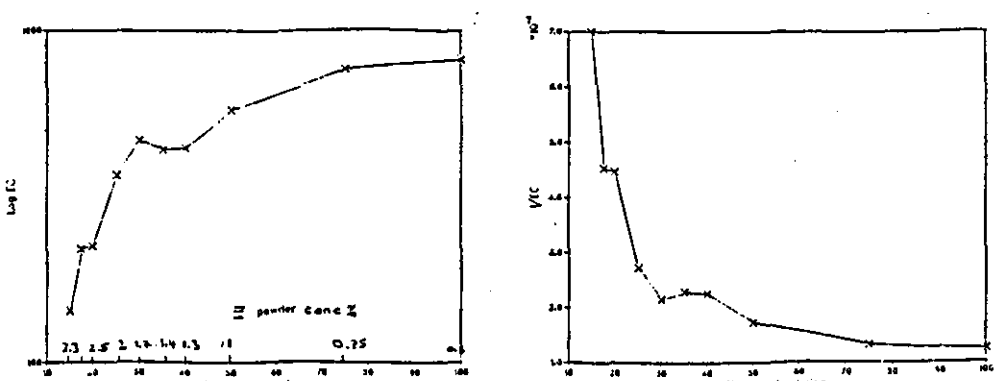


APPENDIX 7.2.E.c

Log EC and 1/EC values for variation in powder amount



Log EC and 1 1/EC values for variation in water volume

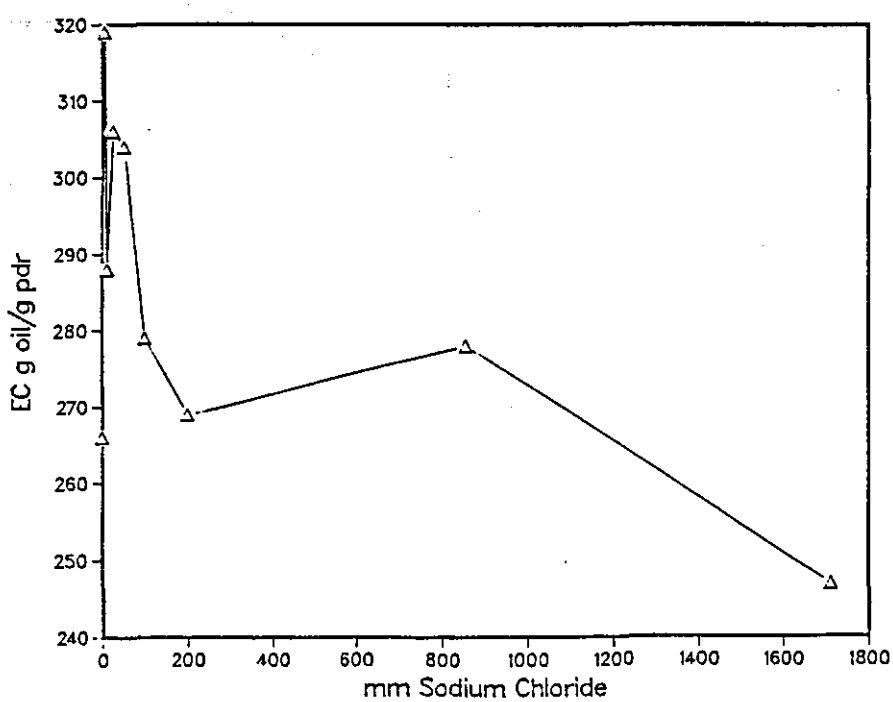


APPENDIX 7.2.F

Emulsions: Levels of Sodium Chloride

Sodium Chloride	EC g oil/g protein	ES1 %	ES14 %	Visc cp
0	266			
6 mM	319	1.96	0.32	40
12 mM	288	0.43	6.60	32
25 mM	306	0.71	0.31	38
50 mM	304	1.58	7.73	34
100 mM	279	1.01	5.21	30
200 mM	269	1.13	0.21	34
856 mM (5%)	278	2.13	3.88	37
1712 mM (10%)	247	0.35	1.57	40

EC levels when additional amounts of sodium chloride was added to BPP solution

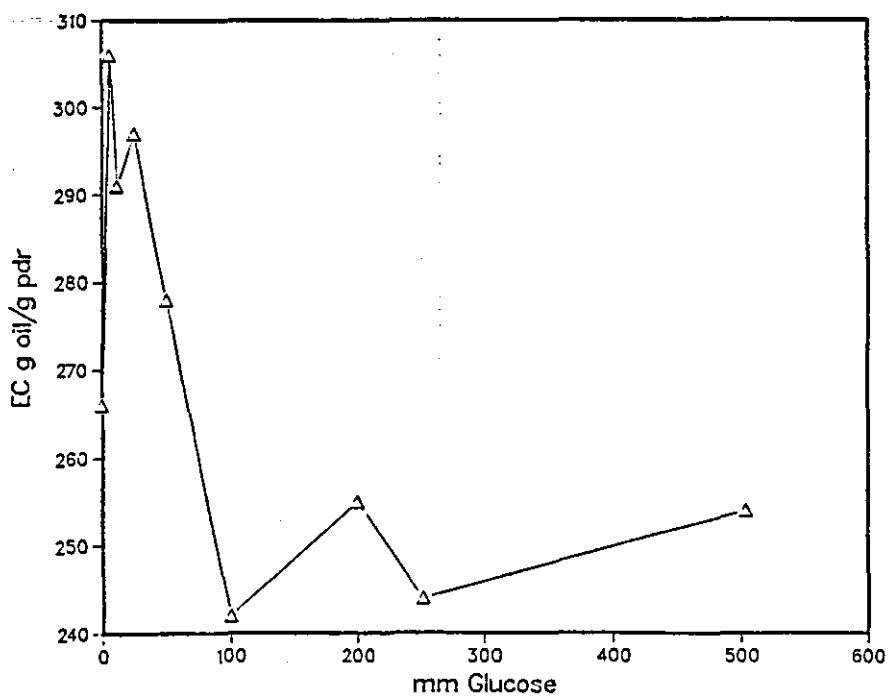


APPENDIX 7.2 G

Emulsions: Levels of Glucose

Glucose	EC g oil/g protein	ES1 %	ES14 %	Visc cp
0	266			
6 mM	306	2.94	1.49	32
12 mM	291	1.72	1.81	33
25 mM	297	4.95	6.85	35
50 mM	278	1.42	6.91	42
100 mM	242	3.01	2.70	46
200 mM	255	4.30	5.30	45
252 mM(5%)	244	0.77	1.38	39
504 mM(10%)	254	1.42	3.69	40

EC levels when additional amounts of glucose was added to BPP solution



APPENDIX 7.2H - Emulsion data with variation in pH

pH of solution	pH of emulsion	ES1 %	ES14 %	EC g oil/ g prot	Visc cp	turbidity Abs 500 nm	EAI	
A								
5	4.9	3.4	11.5	<147	45			oil
6	5.3	3.8	1.2	220	51			code
7	6.0	0.1	4.9	250	52			4226
8	6.4	2.4	1.1	252	53			
9	6.6	1.1	3.5	236	54			use by:
10	6.7	1.6	22.1	261	46			Jul 85
B								
1.5	1.7	2.3	4.8w	233	66	0.184	60	oil
2	2.0	4.2	5.2w	240	74	0.157	51	code
3	3.0	2.6w	8.1w	262	71	0.157	51	4275
4	3.9	11.9	14.1	274	68	0.164	53	
5	4.9	7.7	11.4	212	65	0.116	37	
6	6.0	2.3	10.2	275	48	0.180	58	use by:
7	7.4	0.9	0.1	236	63	0.242	78	Sept 85
8	7.4	1.2	1.5	249	68	0.139	44	
9	7.8	0.5	1.0w	300	63	0.174	55	
10	8.7	0.1	2.3	229	86	0.309	99	
11	10.0	1.8w	7.7w	253	65	0.204	67	
12	11.5	6.7w	12.9	263	98	0.426	138	
12.5	12.5	1.8w	2.6w	285	105	0.551	177	
C								
1.5	1.7			263		0.454	145	oil
2	2.0			268		0.435	140	code
3	2.7			331		0.494	159	5086
4	3.8			259		0.350	113	
4.5	4.2			266		0.568	182	
5	4.8			265		0.290	94	use by:
5.5	5.5			275		0.258	83	Feb 86
6	5.8			302		0.254	83	
6.5	6.2			295		0.308	99	
7	6.5			295		0.396	127	
7.5	6.8			336		0.306	99	
8	7.1			384		0.415	134	
8.5	7.3			333		0.553	177	
9	7.5			307		0.725	233	
9.5	7.8			351		0.502	161	
10	8.0			349		0.552	177	
10.5	8.6			303		0.486	157	
11	9.4			355		0.382	122	
12	11.0			355		0.475	152	
12.5	11.5			<160		0.085	28	

w = water separation

APPENDIX 7.2H.b Emulsion properties of bovine plasma powder at different pH values

pH At Start	pH At Finish	Emulsion capacity (g oil/g protein)		Ave	OPV	R	N	EAI m^2/g
2	2	289	315	302	0.866	2.098	0.024	89.4
3	2.6	288	287	288	0.860	1.801	0.039	104.1
4	3.8	234	265	250	0.843	2.016	0.028	93.0
5	4.6	233	234	234	0.833	1.640	0.051	114.3
6	5.6	237	262	250	0.843	1.414	0.081	132.6
7	6.4	254	253	254	0.845	1.405	0.081	133.5
8	6.8	251	261	256	0.846	1.563	0.058	120.0
9	7.4	252	275	264	0.850	1.626	0.053	115.3
10	8.1	267	277	272	0.853	1.357	0.089	138.2
11	9.5	266	279	273	0.834	1.111	0.165	168.8

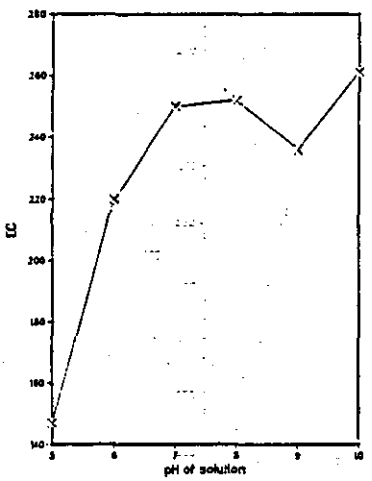
Emulsion properties of BPP when the pH is changed to pH 9.5 in differing manners

	ϕ	R	N	EAI	EC
	0.8225	2.153	0.0200	87.1	260
BULK	0.8281	1.639	0.0450	114.4	252
	0.8326	2.203	0.0190	85.1	303
	0.8214	1.361	0.0780	137.7	272
	0.8348	1.727	0.0390	108.6	261
MEAN	0.82788	1.8166	0.0402	106.58	270[a]
ST DEV.	0.00594	0.357	0.0240	21.6	17.8
SIN	0.8270	1.672	0.042	112.2	303
GLE	0.8348	1.759	0.037	106.6	288
	0.8315	1.359	0.079	138.0	292
	0.8315	2.132	0.020	87.9	314
	0.8315	1.779	0.035	105.4	320
MEAN	0.83126	1.7402	0.4626	110.02	303
ST DEV.	0.00278	0.276	0.0219	18.1	12.3

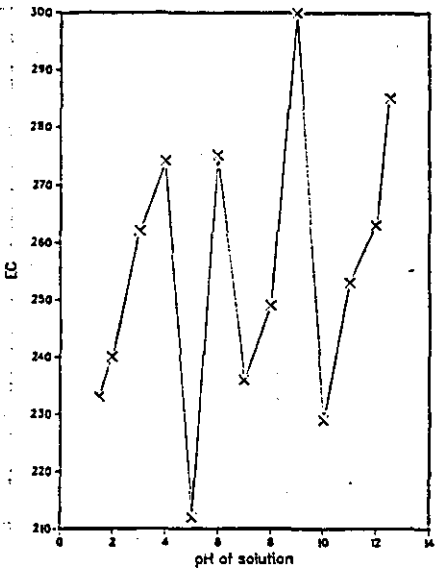
a = significant differences between bulk and single samples $p < 0.05$

APPENDIX 7.2.H.c.EC Values at different pH values of BPP solution

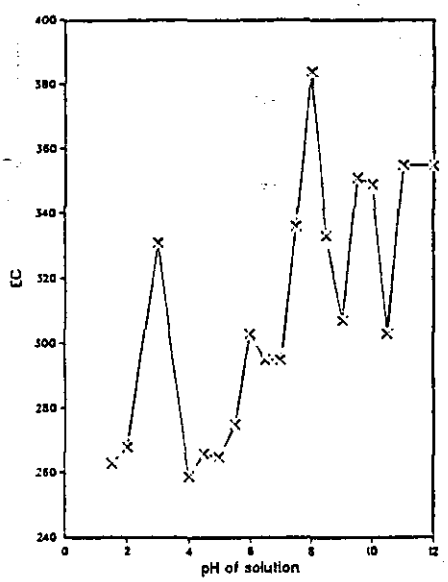
Study A



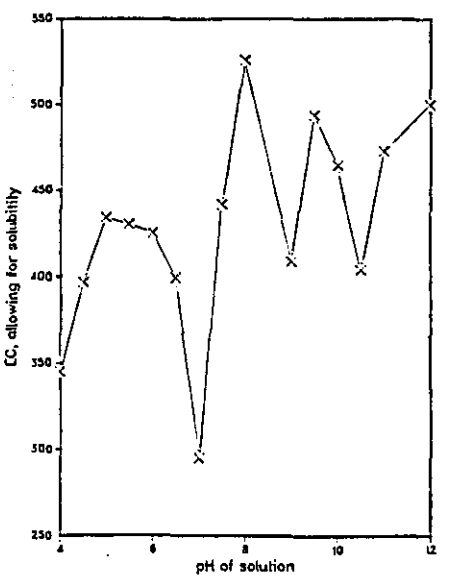
Study B



Study C



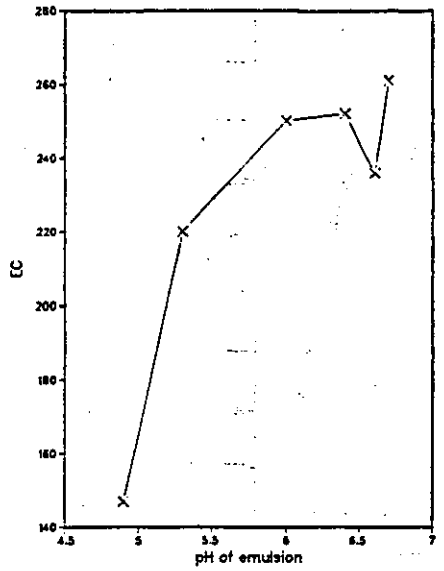
Study C*



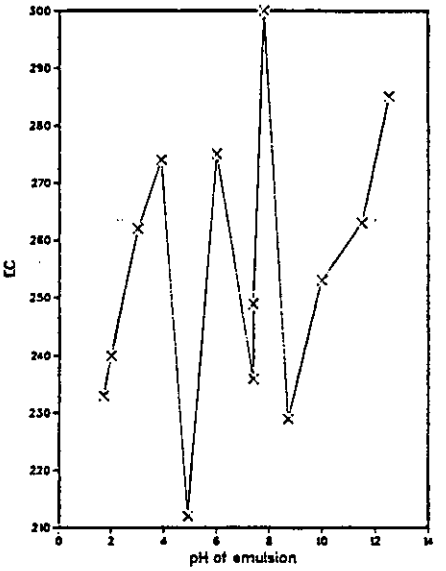
* EC values adjusted for solubility of protein at each pH.

Appendix 7.2.H.d EC values at different pH values of BPP emulsion

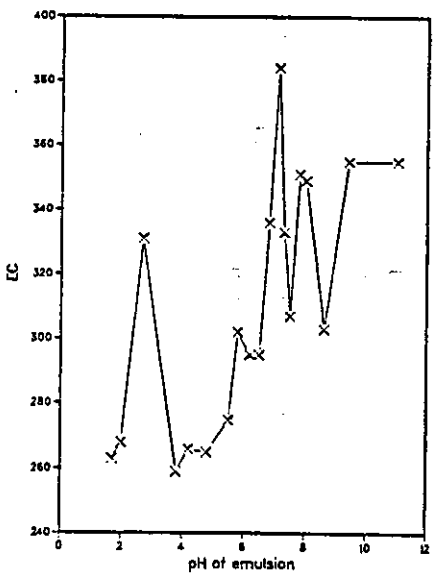
Study A



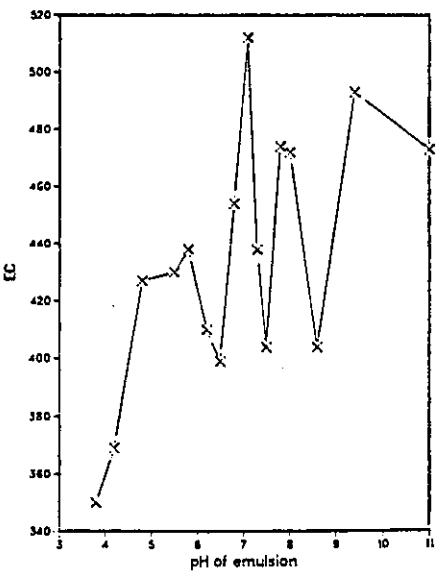
Study B



Study C

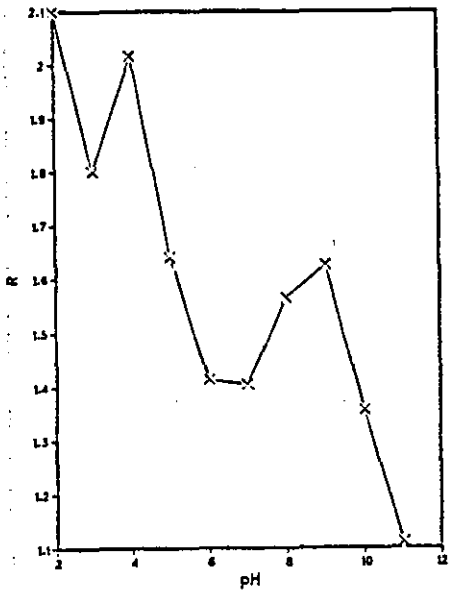


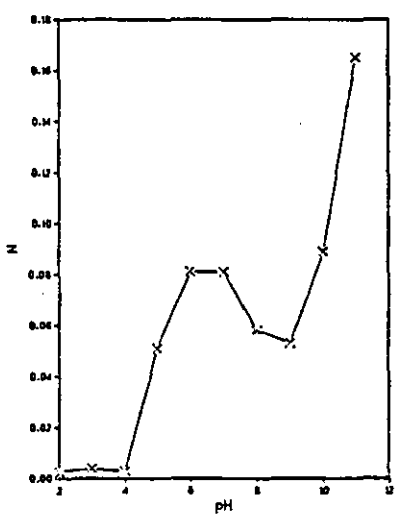
Study C*

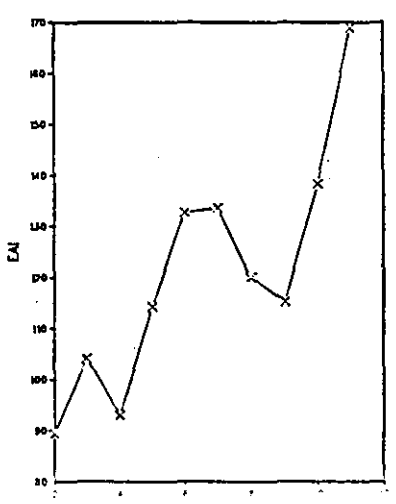


* EC values adjusted for solubility of protein at each pH

APPENDIX 7.2.H.e R, N and EAI values for different pH values of BPP solution





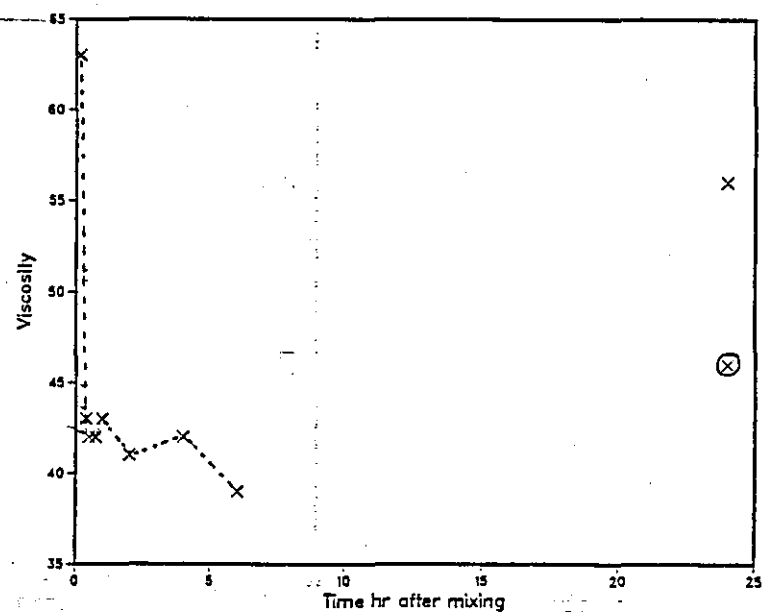


APPENDIX 7.2.I

Emulsions: The Effect of Rate of Oil Addition on Capacity

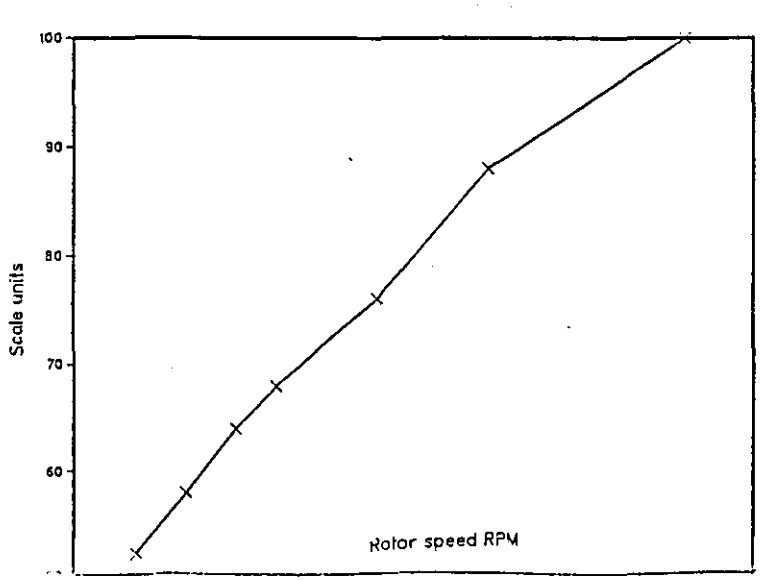
Rate of oil g/min	EC g oil/g protein	OPV	Length of time mixed min
0.55	221	0.80	16.87
1.45	250	0.82	8.97
2.52	243	0.81	4.85
3.45	267	0.83	4.39
6.65	267	0.83	2.29

Appendix 7.2.J - Viscosity of an emulsion at various times after mixing



⊗ viscosity after remixing

Data from Haake Rotovisco for standard emulsion using BPP



LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

Appendix 7.2.K.a

CHEMICAL ENGINEERING DEPARTMENT

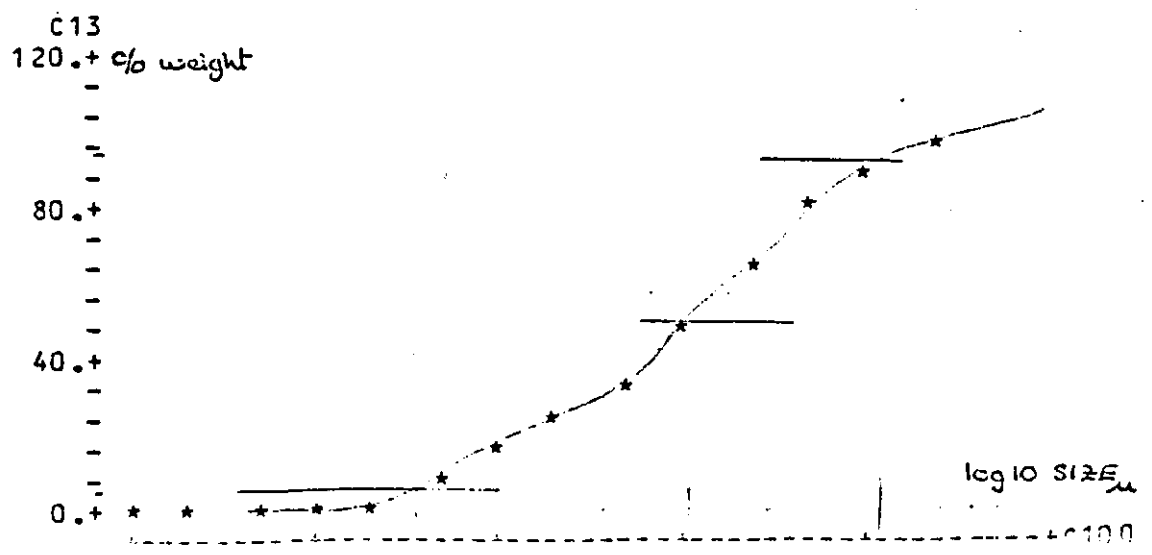
PARTICLE SIZE ANALYSIS BY COULTER COUNTER

SAMPLE REFERENCE:-S HILL. EMULSION. SAMPLE C 5B

JOB NO.:-SH/12 TUBE SIZE:- 200

SIZE MICRONS	SIGMA/N	REL.WT.	CUM%WT<
80.64	.35	4.01	95.98
64	1.24	5.31	90.67
50.8	1.23	11.1	79.56
40.32	.76	14.36	65.2
32	.52	15.32	49.87
25.4	.61	14.55	35.32
20.16	1.16	12.36	22.95
16	1.29	9.08	13.87
12.7	1.11	6.11	7.75
10.08	1.23	3.82	3.93
8	1.11	2.08	1.85
6.35	.75	1.08	.76
5.04	2.1	.48	.27
4	.63	.27	0

BG	DIF.NO.IN EACH RUNS				
0	3	2	3	2	2
0	9	6	15	8	6
0	49	31	39	32	33
0	90	89	107	98	92
0	200	208	205	192	211
0	396	399	370	379	385
0	624	673	663	626	692
1	921	983	1024	942	950
0	1329	1256	1252	1330	1322
2	1647	1594	1554	1651	1678
1	1797	1833	1745	1744	1717
2	1833	1825	1893	1660	1812
4	1587	1685	1644	1573	1785
19	1904	1967	1919	1900	1905
0	0	0	0	0	0
0	0	0	0	0	0



Appendix 7.2.K.b.

Print out of particle size for the Malvern

MALVERN 2200/3300 PARTICLE SIZER V3.1

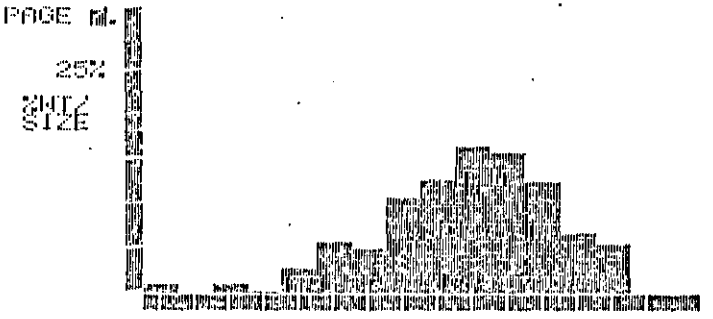
MALVERN INSTRUMENTS LTD,SPRING LANE,MALVERN,ENGLAND.

PRINTING RESULTS FROM DATA BLOCK 2

TIME 00-53-30 RUN NO. 7 LOG ERROR = 3.54

SAMPLE CONCENTRATION = 0.0070 % BY VOLUME
OBSCURATION = 0.23

SIZE BAND UPPER	SIZE BAND LOWER	CUMULATIVE WT BELOW	WEIGHT IN BAND	CUMULATIVE WT ABOVE	LIGHT ENERGY COMPUTED	LIGHT ENERGY MEASURED
188.0	87.2	100.0	0.0	0.0	393	302
87.2	53.5	94.6	5.4	0.0	492	493
53.5	37.6	87.6	7.0	5.4	704	700
37.6	28.1	75.1	12.5	12.4	947	963
28.1	21.5	59.3	15.8	24.9	1262	1239
21.5	16.7	42.6	16.7	40.7	1592	1622
16.7	13.0	29.7	12.9	57.4	1897	1885
13.0	10.1	18.8	10.9	70.3	2047	2044
10.1	7.9	13.6	5.2	81.2	2040	2047
7.9	6.2	8.0	5.6	86.4	1923	1924
6.2	4.8	5.1	2.9	92.0	1740	1705
4.8	3.8	4.8	0.3	94.9	1555	1516
3.8	3.0	3.7	1.1	95.2	1390	1359
3.0	2.4	3.7	0.0	96.3	1257	1175
2.4	1.9	2.5	1.2	96.3	1265	1196



WEIGHT ON DIODE SIZES
BEST LOG ERROR = 3.54

APPENDIX - Emulsion values calculated by globule size
7.2.K.c.

		COULTER COUNTER						MALVERN						TURBIDITY DATA							
Experiment	Samples	CC.D50		CC.S		CC.L		M.D50		M.S		M.L.		ϕ		R		N		EAI	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Controls	1	27.6		11.2		56.0		21.9		1.9		64.1		0.8337		1.259		0.100		149	
	2	31.7		12.7		59.9		25.2		1.9		69.1		0.7765		1.328		0.079		141	
Mixing Time 2 min	3	33.5		12.0		68.1		26.0		1.9		65.0		0.8315		1.622		0.047		116	
Protein Conc 2%	4	34.2		12.6		68.3		26.5		1.9		68.3		0.8304		1.573		0.050		119	
pH 8.1	5	37.3		13.4		93.8		28.9		1.9		87.2		0.8370		1.742		0.037		108	
Oil volume 80	6	37.3		13.4		100.0		28.7		1.9		87.2		0.8203		1.812		0.033		104	
	Mean	33.6		12.6		74.4		26.2		<1.9		73.5		0.8216		1.556		0.058		123	
	SD		3.67		0.85		18.2		2.58		0.00		10.8		0.0228		0.221		0.0263		18.4
Mixing Time	0.5 min	42.4	4.81	16.4[a]	0.71	92.1	5.9	33.5[b]	0.99	<1.9	0.00	87.2	0.00	0.8338	0.0080	2.093	0.524	0.0260	0.0184	92	23.1
	4.0 min	30.5	1.70	11.2[a]	0.07	62.8	10.7	21.7[b]	0.14	<1.9	0.00	76.4	2.12	0.8353	0.0069	1.2545[a]	0.0007	0.1005[a]	0.0007	150[a]	0.1
Protein Conc	5%	26.3	8.84	11.2	3.04	58.0	11.7	19.0	5.02	<1.9	0.00	51.9	21.6	0.8307	0.0009	1.164	0.298	0.1525	0.108	6.7	17.0
	0.5%	46.4	4.03	17.2	1.84	79.3	9.9	35.0	3.39	2.3	0.14	87.1	0.2	0.8293	0.0007	1.594	0.035	0.049	0.003	471[c]	10.3
pH	pH5.0	29.0	2.62	11.9	2.55	63.4	13.7	21.2[a]	1.06	<1.9	0.0	53.7[b]	1.6	0.8287	0.0024	1.221[b]	0.139	0.1125[b]	0.038	155[b]	17.6
	pH9.0	29.1[a]	0.64	12.0	1.27	51.6[a]	6.7	21.8[a]	0.35	<1.9	0.0	51.3[b]	2.9	0.8298	0.0025	1.134[b]	0.051	0.1365[b]	0.018	166[b]	7.4
Oil volume	40 ml	39.8[b]	0.07	13.9	2.90	62.6	10.1	28.1	1.7	<1.9	0.0	83.7	5.0	0.6720[c]	0.0113	1.303	0.109	0.0740	0.017	144	12.2
	100ml	29.4	1.13	12.3	0.05	71.1	0.1	22.0	2.1	2.0	0.1	87.2	0.0	0.8710[b]	0.0014	1.303	0.116	0.096	0.086	145	12.9

a = significantly different from controls p <0.05
b p <0.01
c p <0.001

No significant difference between pH 5.0 and pH 9.0
M.D50 significantly different from CC.D50 p <0.01

SECTION 7.3

NOTE

Stability data normally Appendix . . . a
Volume data normally Appendix . . . b

APPENDICES 7.3.B. Foam Variation in the Mixing Procedure

	Mixing time	5 min Total vol	5 min Foam	25 min Total vol	25 min Foam	60 min Total vol	60 min Foam	
Silverson	1	92	57	90	46	87	41	Bovine
	2	97	68	97	56	92	49	Bovine
	2	94	63	93	52	93	47	Egg
Waring blender	1	104	68	103	65	100	50	Bovine
	1	60	20	58	110	57	13	Egg
	2	96	59	80	35	70	32	Bovine
	2	75	35	73	32	73	28	Egg

APPENDIX 7.3.B.b - Foam: Stability of foams for variation in diameter of Mixing Vessel

Vessel	ID of Mixing Vessel	F25/5	F60/5	F25/60
Plastic cylinder 200 ml	35.6	78.84	69.23	113.8
Pyrex 100 ml beaker	47.8	74.66	64.00	116.6
Storage Jar 120 ml	51.8	74.35	65.38	113.7
Pyrex high form 200 ml beaker	58.7	75.00	66.66	112.5
CSN Simax 200 ml beaker	65.4	80.72	68.67	117.5
Pyrex flat sample dish	76.7	74.28	59.04	125.8
Large Pyrex flat sample dish	90.4	68.69	48.69	141.0
Correlation against ID of mixing vessel		-0.611	-0.828	0.861

APPENDIX 7.3.B.d - Foam: Stability of foams for variation in diameter of Holding Vessel

Holding Vessel	ID of holding vessel	F25/5	F60/5	F25/60
Glass 100 ml measuring cylinder	27.9	71.21	60.60	117.5
Plastic cylinder 200 ml	35.6	76.19	68.25	111.6
Pyrex 100 ml beaker	47.8	80.64	69.35	116.2
Storage jar 120 ml	51.8	85.50	50.86	140.4
Pyrex high form 200 ml beaker	58.7	87.30	79.36	110.0
CSN Simax 200 ml beaker	65.4	81.42	67.14	121.2
Pyrex flat sample dish	76.7	87.32	77.46	112.7
Large Pyrex flat sample dish	90.4	82.85	74.28	111.5
Correlation against ID of holding vessel		0.701	0.636	0.178

Appendix 7. 3B.c- Foaming volumes for variation in diameter of mixing vessel

I.D. of Mixing Vessel	L.VOL.A	F.VOL.E	T.VOL.C	GAS. B	L.FOAM. D	OVER RUN	FOAM. P.V.	GAS. P.V.	FOAM. VOL
AFTER 5 MIN									
35.60	50	52	82	32	20	64	0.615	0.390	104
47.80	50	75	98	48	27	96	0.640	0.489	150
51.80	50	78	102	52	26	104	0.666	0.509	156
58.70	50	84	102	52	32	104	0.619	0.509	168
65.40	50	83	109	59	24	118	0.710	0.541	166
76.70	50	105	122	72	33	144	0.685	0.590	210
90.40	50	115	130	80	35	160	0.695	0.615	230
Correlation against ID		0.977		0.987	0.825	0.986	0.748	0.961	0.977
AFTER 25 MIN									
35.60	50	41	82	32	9	64	0.780	0.390	82
47.80	50	56	98	48	8	96	0.857	0.489	112
51.80	50	58	101	51	7	102	0.879	0.504	116
58.70	50	63	102	52	11	104	0.825	0.509	126
65.40	50	67	109	59	8	118	0.880	0.541	134
76.70	50	78	122	72	6	144	0.923	0.590	156
90.40	50	79	120	70	9	140	0.886	0.583	158
Correlation against ID									0.969
AFTER 60 MIN									
35.60	50	36	80	30	6	60	0.833	0.375	72
47.80	50	48	93	43	5	86	0.895	0.462	96
51.80	50	51	97	47	4	94	0.921	0.484	102
58.70	50	56	98	48	8	96	0.857	0.489	112
65.40	50	57	103	53	4	106	0.929	0.514	114
76.70	50	62	109	59	3	118	0.951	0.541	124
90.40	50	56	100	50	6	100	0.892	0.500	112
Correlation against ID									0.808

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Appendix 7.3.B.e Foaming volumes for variation in diameter of holding vessel

ID of holding vessel	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B.	L.FOAM.D	OVER RUN	FOAM.P.V.	GAS.P.V.	FOAM.VOL
AFTER 5 MIN									
27.90	50	66	95	45	21	90	0.681	0.473	132
35.60	50	63	95	45	18	90	0.714	0.473	126
47.80	50	62	99	49	13	98	0.790	0.494	124
51.80	50	69	108	58	11	116	0.840	0.537	138
58.70	50	63	101	51	12	102	0.809	0.504	126
65.40	50	70	108	58	12	116	0.828	0.537	140
76.70	50	71	109	59	12	118	0.830	0.541	142
90.40	50	70	111	61	9	122	0.871	0.549	140
Correlation against ID				0.889	-0.857	0.889	0.888	0.888	0.650
AFTER 25 MIN									
27.90	50	47							94
35.60	50	48							96
47.80	50	50							100
51.80	50	59							118
58.70	50	55							110
65.40	50	57							114
76.70	50	62							124
90.40	50	58							116
Correlation against ID									0.827
AFTER 60 MIN									
27.90	50	40							80
35.60	50	43							86
47.80	50	43							86
51.80	50	42							84
58.70	50	50							100
65.40	50	47							94
76.70	50	55							110
90.40	50	52							104
Correlation against ID									0.876

Appendices 7.3.b.a

- Foam: Stability of foams for variation in duration of Mixing

Duration of Mixing	F25/5	F60/5	F25/60
0.50	86.95	73.91	117.64
1.00	80.70	71.92	112.19
1.50	78.78	66.66	118.18
2.00	82.35	72.05	114.28
2.50	77.94	79.41	98.14
3.00	79.45	68.49	116.00
4.00	75.34	65.75	114.58
5.00	75.90	68.67	110.52
Correlation against Mixing Time	-0.817	-0.388	-0.239

Appendix 7.3.b.b

Foaming Volmes for variation in Duration of Mixing

Duration of Mixing	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B	L.FOAM.D	OVER RUN	FOAM.P.V.	GAS.P.V.	FOAM.VOL
AFTER 5 MIN									
0.50	50	46	85	35	11	70	0.760	0.411	92
1.00	50	57	92	42	15	84	0.736	0.456	114
1.50	50	66	80	30	36	60	0.454	0.375	132
2.00	50	68	97	47	21	94	0.691	0.484	136
2.50	50	68	98	48	20	96	0.705	0.487	136
3.00	50	73	100	50	23	100	0.684	0.500	146
4.00	50	73	97	47	26	94	0.643	0.484	146
5.00	50	83	103	53	30	106	0.638	0.514	166
Correlation against Mixing Time		0.920	0.759	0.759	0.538	0.759	-0.189	0.725	0.920
AFTER 25 MIN									
0.50	50	40	84	34	6	68	0.850	0.404	80
1.00	50	46	90	40	6	80	0.869	0.444	92
1.50	50	52	79	29	23	58	0.557	0.367	104
2.00	50	56	97	47	9	94	0.839	0.484	112
2.50	50	53	97	47	6	94	0.886	0.484	106
3.00	50	58	100	50	8	100	0.862	0.500	116
4.00	50	55	96	46	9	92	0.836	0.479	110
5.00	50	63	103	53	10	106	0.841	0.514	126
Correlation against Mixing Time									0.873
AFTER 60 MIN									
0.50	50	34	80	30	4	60	0.882	0.375	68
1.00	50	41	87	37	4	74	0.902	0.425	82
1.50	50	44	78	28	16	56	0.636	0.358	88
2.00	50	49	92	42	7	84	0.857	0.456	98
2.50	50	54	91	41	13	82	0.759	0.450	108
3.00	50	50	95	45	5	90	0.900	0.473	100
4.00	50	48	92	42	6	84	0.875	0.456	96
5.00	50	57	102	52	5	104	0.912	0.509	114
Correlation against Mixing Time									0.832

Appendix 7.3.C.a Stability of foams for variation in temperature

Temperature °C	F25/5 8	F60/5 8	F25/60 8
4	71.42	76.19	93.75
20	82.35	72.05	114.28
30	73.91	65.21	113.33
40	74.19	64.51	115.00
50	75.38	64.61	116.66
60	71.18	57.62	123.52
70	64.40	44.06	146.15
80	73.58	52.83	139.28
Correlation against Temp	-0.440	-0.918	0.919

Appendix 7.3.D.a Stability of foams for variation in powder concentration

Conc. of BPP	F25/5	F60/5	F25/60
12.0	78.77	63.26	124.19
10.0	80.19	64.35	124.61
8.0	76.53	65.30	117.18
6.0	73.73	62.62	117.74
4.0	72.22	61.11	118.18
2.0	76.11	65.67	115.90
1.0	74.00	60.00	123.33
0.5	83.87	74.19	113.04
0.25	82.60	56.52	146.15
0.1	81.81	63.63	128.57
Correlation against conc.	-0.209	0.014	-0.200

Appendix 7.3.E.a Stability of foams for variation in the volume of the protein solution

Powder Conc	Vol ml	F25/5 6	F 60/5 6	F25/60 6
3	150	71.79	64.10	112.00
2.5	125	75.35	67.60	111.45
2	100	76.87	68.70	111.88
1.5	75	79.10	75.37	104.95
1	50	69.56	65.21	106.66
0.5	25	83.05	71.18	116.66
Correlation against volume		-0.450	-0.452	-0.026

Appendix 7.3.C.b Foaming volumes for variation in temperature

Temp °C	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B	L.FOAM D	OVER RUN	FOAM. P.V.	GAS. P.V.	FOAM. VOL
AFTER 5 MIN									
4	50	42	82	32	10	64	0.761	0.121	84
20	50	68	97	47	21	94	0.691	0.216	136
30	50	69	98	48	21	96	0.695	0.214	138
40	50	62	92	42	20	84	0.677	0.217	124
50	50	65	96	46	19	92	0.707	0.197	130
60	50	59	92	42	17	84	0.711	0.184	118
70	50	59	91	41	18	82	0.694	0.197	118
80	50	53	90	40	13	80	0.754	0.144	106
Correlation against Temp.		0.082	0.132	0.132	0.013	0.132	-0.012	0.026	0.082
AFTER 25 MIN									
4	50	30	80	32	0	64	1.066	0.390	60
20	50	56	97	47	9	94	0.839	0.484	112
30	50	51	97	47	4	94	0.921	0.484	102
40	50	46	92	42	4	84	0.913	0.456	92
50	50	49	94	44	5	88	0.897	0.468	98
60	50	42	88	38	4	76	0.904	0.431	84
70	50	38	80	30	8	60	0.789	0.375	76
80	50	39	84	34	5	68	0.871	0.404	78
Correlation against Temp		-0.015	-0.359	-0.359	0.446	-0.359	-0.648	-0.346	-0.115
AFTER 60 MIN									
4	50	32	80	30	2	60	0.937	0.375	64
20	50	49	95	45	4	90	0.918	0.473	98
30	50	45	92	42	3	84	0.933	0.456	90
40	50	40	87	37	3	74	0.925	0.425	80
50	50	42	88	38	4	76	0.904	0.431	84
60	50	34	80	30	4	60	0.882	0.375	68
70	50	26	70	20	6	40	0.769	0.287	52
80	50	28	74	24	4	48	0.857	0.324	56
Correlation against Temp		-0.563	-0.629	-0.629	0.786	-0.629	-0.759	-0.649	-0.569

Appendix 7.3.D.b Foaming volumes for variation in concentration

Conc. %	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B	L.FOAM.D	OVER RUN	FOAM.P.V.	GAS.P.V.	FOAM. VOL
AFTER 5 MIN									
12.00	50	98	103	53	45	106	0.540	0.514	196
10.00	50	101	109	59	42	118	0.584	0.541	202
8.00	50	98	108	58	40	116	0.591	0.537	196
6.00	50	99	108	58	41	116	0.585	0.537	198
4.00	50	90	105	55	35	110	0.611	0.523	180
2.00	50	67	95	45	22	90	0.671	0.473	134
1.00	50	50	87	37	13	74	0.740	0.425	100
0.5[illegible]	50	31	76	26	5	52	0.838	0.342	62
0.25	50	23	70	20	3	40	0.869	0.285	46
0.10	50	11	60	10	1	20	0.909	0.166	22
Correlation against Conc		0.859	0.789	0.789	0.911	0.789	-0.862	0.733	0.859
AFTER 25 MIN									
12.00	50	77	103	53	24	106	0.688	0.514	154
10.00	50	81	109	59	22	118	0.728	0.541	162
8.00	50	75	108	58	17	116	0.773	0.537	150
6.00	50	73	108	58	15	116	0.794	0.537	146
4.00	50	65	105	55	10	110	0.846	0.523	130
2.00	50	51	94	44	7	88	0.862	0.468	102
1.00	50	37	83	33	4	66	0.891	0.397	74
0.5[illegible]	50	26	73	23	3	46	0.884	0.315	52
0.25	50	19	66	16	3	32	0.842	0.242	38
0.10	50	9	58	8	1	16	0.888	0.137	18
Correlation against Conc		0.891	0.802	0.802	0.995	0.802	-0.968	0.754	0.891

Appendix 7.3.D.b cont. Foaming volumes for variation in concentration

AFTER 60 MIN									
12.00	50	62	102	52	10	104	0.838	0.509	124
10.00	50	65	107	57	8	114	0.876	0.532	130
8.00	50	64	107	57	7	114	0.890	0.532	128
6.00	50	62	105	55	7	110	0.887	0.523	124
4.00	50	55	100	50	5	100	0.909	0.500	110
2.00	50	44	89	39	5	78	0.886	0.438	88
1.00	50	30	77	27	3	54	0.900	0.350	60
0.50	50	23	70	20	3	40	0.869	0.285	46
0.25	50	13	60	10	3	20	0.769	0.166	26
0.10	50	7	56	6	1	12	0.857	0.107	14
Correlation against Conc		0.865	0.839	0.839	0.960	0.839	0.138	0.787	0.865

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Appendix 7.3.E.b- Foaming volumes for variation of continuous phase

Vol. of continuous phase	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B	L.FOAM.D	OVER RUN	FOAM P.V.	GAS.P.V.	FOAM.VOL
AFTER 5 MIN									
150	150	156	250	100	56	66.667	0.641	0.400	104
125	125	142	222	97	45	77.600	0.683	0.436	113
100	100	147	200	100	47	100.000	0.680	0.500	147
75	75	134	171	96	38	128.000	0.716	0.561	178
50	50	92	116	66	26	132.000	0.717	0.568	184
25	25	59	69	44	15	176.000	0.745	0.637	236
Correlation against vol		0.913	0.913	0.859	0.965	-0.979	-0.962	-0.988	-0.979
AFTER 25 MIN									
150	150	112	246	96	16	64.000	0.857	0.390	74
125	125	107	220	95	12	76.000	0.887	0.431	85
100	100	113	201	101	12	101.000	0.893	0.502	113
75	75	106	170	95	11	126.667	0.896	0.558	141
50	50	64	106	56	8	112.000	0.875	0.528	128
25	25	49	68	43	6	172.000	0.877	0.632	196
Correlation against vol		0.864	0.980	0.835	0.965	-0.932	-0.242	-0.952	-0.933
AFTER 60 MIN									
150	150	100	240	90	10	60.000	0.900	0.375	66
125	125	96	214	89	7	71.200	0.927	0.415	76
100	100	101	194	94	7	94.000	0.930	0.484	101
75	75	101	168	93	8	124.000	0.920	0.553	134
50	50	60	104	54	6	108.000	0.900	0.519	120
25	25	42	57	32	10	128.000	0.761	0.561	168
Correlation against vol		0.826	0.976	0.812	0.064	-0.925	0.651	-0.927	-0.949

APPENDIX 7.3.F.a Foam - Stability of foams for variation in Sodium Chloride Concentration

mm NaCl	F25/5	F60/5	F25/60
0.00	53.01	68.67	129.54
6.00	48.83	67.44	138.09
12.00	55.00	72.50	131.81
25.00	57.14	71.42	125.00
50.00	57.47	71.26	124.00
100.00	56.81	70.45	124.00
200.00	56.81	71.59	126.00
856.00	56.12	71.42	127.27
1716.00	61.38	70.29	114.51
Correlation Against NaCl	0.625	0.072	-0.691

APPENDIX 7.3.G.a Foam - Stability of foams for variation in Glucose Concentration

mm Glucose	F25/5	F60/5	F25/60
0	71.01	56.52	125.64
6	67.60	57.74	117.07
12	68.05	59.72	113.95
25	75.00	63.38	117.39
50	71.76	57.64	124.49
100	71.08	60.24	118.00
200	71.42	57.14	125.00
252	71.42	61.90	115.38
504	68.88	63.33	108.77
Correlation Against Glucose	-0.143	0.492	-0.543

APPENDIX 7.3.H.a Foam- Stability of foams for variation in pH

pH	F 25/5	F 60/5	F25/60
4.5	76.31	71.05	107.40
5.0	76.38	69.44	110.00
5.5	79.45	69.86	113.72
6.0	73.68	67.10	109.80
6.5	72.97	66.21	110.20
7.0	72.85	68.57	106.25
7.5	75.36	66.66	113.04
8.0	72.22	65.27	110.63
8.5	73.97	68.49	108.30
9.0	74.64	69.01	108.16
9.5	84.12	63.49	132.50
Correlation Against pH	0.131	-0.636	0.440

Appendix 7.3.F.b- Foaming values for variation in the amount of sodium chloride

mM NaCl	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B.	L.FOAM.D	OVERRUN	FOAM.P.V.	GAS.P.V.	FOAM.VOL
AFTER 5 MIN									
0	50	83	100	50	33	100	0.602	0.500	166
6	50	86	102	52	34	104	0.604	0.509	172
12	50	80	100	50	30	100	0.625	0.500	160
25	50	84	99	49	35	98	0.583	0.494	168
50	50	87	104	54	33	108	0.620	0.519	174
100	50	88	103	53	35	106	0.602	0.514	176
200	50	88	103	53	35	106	0.602	0.514	176
856	50	98	108	58	40	116	0.591	0.537	196
1716	50	101	108	58	43	116	0.574	0.537	202
Correlation against NaCl		0.918	0.830	0.830	0.919	0.830	-0.682	0.819	0.918
AFTER 25 MIN									
0	50	57	100	50	7	100	0.877	0.500	114
6	50	58	100	50	8	100	0.862	0.500	116
12	50	58	99	49	9	98	0.844	0.494	116
25	50	60	98	48	12	96	0.800	0.487	120
50	50	62	103	53	9	106	0.854	0.514	124
100	50	62	103	53	9	106	0.854	0.514	124
200	50	63	103	53	10	106	0.841	0.514	126
856	50	70	108	58	12	116	0.828	0.537	140
1716	50	71	108	58	13	116	0.816	0.537	142
Correlation against NaCl		0.898	0.837	0.837	0.742	0.837	-0.487	0.825	0.898
AFTER 60 MIN									
0	50	44	90	40	4	80	0.909	0.444	88
6	50	42	87	37	5	74	0.880	0.425	84
12	50	44	87	37	7	74	0.840	0.425	88
25	50	48	90	40	8	80	0.833	0.444	96
50	50	50	95	45	5	90	0.900	0.473	100
100	50	50	95	45	5	90	0.900	0.473	100
200	50	50	95	45	5	90	0.900	0.473	100
856	50	55	100	50	5	100	0.909	0.500	110
1716	50	62	108	58	4	116	0.935	0.537	124

Appendix 7.3.G.b- Foaming values for variation in the amount of glucose

mM Glucose	L.Vol.A.	F.Vol.E.	T.Vol.C.	Gas.B	L.Foam.D.	Overrun	Foam.P.V.	Gas.P.V.	Foam.Vol.
AFTER 5 MIN									
0	50	69	93	43	26	86	0.623	0.462	138
6	50	71	95	45	26	90	0.633	0.473	142
12	50	72	95	45	27	90	0.625	0.473	144
25	50	72	97	47	25	94	0.652	0.484	144
50	50	85	100	50	35	100	0.588	0.500	170
100	50	83	102	52	31	104	0.626	0.509	166
200	50	84	102	52	32	104	0.619	0.509	168
252	50	84	104	54	30	108	0.642	0.519	168
504	50	90	106	56	34	112	0.622	0.528	180
Correlation against Glucose		0.799	0.863	0.863	0.622	0.863	-0.006	0.847	0.799
AFTER 25 MIN									
0	50	49	92	42	7	84	0.857	0.456	98
6	50	48	92	42	6	84	0.875	0.456	96
12	50	49	92	42	7	84	0.857	0.456	98
25	50	54	97	47	7	94	0.870	0.484	108
50	50	61	100	50	11	100	0.819	0.500	122
100	50	59	102	52	7	104	0.881	0.509	118
200	50	60	101	51	9	102	0.850	0.504	120
252	50	60	104	54	6	108	0.900	0.519	120
504	50	62	106	56	6	112	0.903	0.528	124
Correlation against Glucose		0.704	0.825	0.825	0.245	0.825	0.600	0.806	0.704
AFTER 60 MIN									
0	50	39	85	35	4	70	0.897	0.411	78
6	50	41	87	37	4	74	0.902	0.425	82
12	50	43	87	37	6	74	0.860	0.425	86
25	50	46	92	42	4	84	0.913	0.456	92
50	50	49	97	47	2	94	0.959	0.484	98
100	50	50	97	47	3	94	0.940	0.484	98
200	50	48	94	44	4	88	0.916	0.468	96
252	50	52	100	50	2	100	0.961	0.500	104
504	50	57	106	56	1	112	0.982	0.528	114
Correlation against Glucose		0.864	0.862	0.862	-0.710	0.862	0.732	0.835	0.864

Appendix 7.3.II.b Foaming values for variation in pH

pH	L.VOL.A	F.VOL.E	T.VOL.C	GAS.B	L.FOAM D	OVER RUN	FOAM.P.V.	GAS.P.V.	FOAM.VOL
4.5	50	76	96	46	30	92	0.605	0.479	152
5.0	50	72	94	44	28	88	0.611	0.468	144
5.5	50	73	96	46	27	92	0.630	0.479	146
6.0	50	76	99	49	27	98	0.644	0.494	152
6.5	50	74	98	48	26	96	0.648	0.489	148
7.0	50	70	96	46	24	92	0.657	0.479	140
7.5	50	69	95	45	24	90	0.652	0.473	138
8.0	50	72	96	46	26	92	0.638	0.479	144
8.5	50	73	97	47	26	94	0.643	0.484	146
9.0	50	71	97	47	24	94	0.661	0.484	142
9.5	50	63	92	42	21	84	0.666	0.456	126
Correlation against pH		-0.680	-0.222	-0.222	-0.850	-0.222	0.835	-0.229	-0.680
AFTER 25 MIN									
4.5	50	58	96	46	12	92	0.793	0.479	116
5.0	50	55	94	44	11	88	0.800	0.468	110
5.5	50	58	94	44	14	88	0.758	0.468	116
6.0	50	56	98	48	8	96	0.857	0.489	112
6.5	50	54	97	47	7	94	0.870	0.484	108
7.0	50	51	95	45	6	90	0.882	0.473	102
7.5	50	52	95	45	7	90	0.865	0.473	104
8.0	50	52	99	49	3	98	0.942	0.494	104
8.5	50	54	97	47	7	94	0.870	0.484	108
9.0	50	53	94	44	9	88	0.830	0.468	106
9.5	50	53	92	42	11	84	0.792	0.456	106
Correlation against pH		-0.703	-0.160	-0.160	-0.423	-0.160	0.354	-0.172	-0.703
AFTER 60 MIN									
4.5	50	54	96	46	8	92	0.851	0.479	108
5.0	50	50	94	44	6	88	0.880	0.468	100
5.5	50	51	94	44	7	88	0.862	0.468	102
6.0	50	51	96	46	5	92	0.901	0.479	102
6.5	50	49	94	44	5	88	0.897	0.468	98
7.0	50	48	93	43	5	86	0.895	0.462	96
7.5	50	46	92	42	4	84	0.913	0.456	92
8.0	50	47	96	46	1	92	0.978	0.479	90
8.5	50	50	96	46	4	92	0.920	0.479	100
9.0	50	49	93	43	6	86	0.877	0.462	98
9.5	50	40	86	36	4	72	0.900	0.418	80
Correlation against pH		-0.740	-0.518	-0.518	-0.621	-0.518	0.511	-0.523	-0.740

Appendix 7.3.H.c

Foam at natural pH and at pH 6.6

pH	Volume	5	25	60	F60/5
	ml	ml	ml	ml	
8.4	102	73	57	46	63
	96	66	52	44	67
	93	61	49	44	72
	100	70	56	49	70
	99	66	53	47	71
	98	67	53	45	67
Mean	98	67	53	46	68
SD	3.16	4.07	2.88	1.94	3.33
6.6	107	77	65	58	75
	100	69	55	47	68
	101	73	67	53	73
	101	73	58	54	74
	103	75	60	57	76
	102	74	60	53	72
Mean	102^{a}	74^{a}	60^{b}	54^{b}	73^{a}
SD	2.50	2.66	4.45	3.88	2.83

a = significant difference from pH 8.4 p = <0.05

b = pH 8.4 p = <0.01

Appendix 7.3.I

Foaming Properties with Olive Oil and Milk

Conc	Volume ml	5 min	25 min	60 min	F60/5
0	95	56	49	31	55
	93	58	47	34	59
	96	67	51	38	57
	108	73	47	34	47
	94	62	47	36	58
	94	58	48	20	34
Mean	97	62	48	32	52
SD	5.65	6.53	1.60	6.40	9.67
2000 ppm	100	66	50	27	41
Olive Oil	93	56	39	24	43
	100	70	48	32	46
Mean	98	64	46	28	43
SD	4.04	7.21	5.86	4.04	2.52
2000 ppm	66	20	14	7	35
Milk	65	19	14	7	37
	60	28	16	5	18
Mean	67 c	22 c	15 c	6 c	30
SD	2.65	4.93	1.15	1.15	10.4
100 ppm			44		
Milk			42		
			46		
Mean			44		
SD			2.00		
20 ppm			48		
Milk			49		
			52		
Mean			50		
SD			2.08		

c = significant difference from controls p = <0.001

SECTION 7.4

APPENDIX 7.4.A

Gelation: Penetration (1/10mm) of a cone into gels made from various concentrations of agar

Conc %	No.	1/10 mm Mean	SD
4	8	48.5	8.2
3.75	4	51.0	3.6
3.5	8	65.3	18.6
3.25	4	56.0	5.48
3	8	82.1	23.7
2.75	4	68.5	8.5
2.5	12	93.1	18.1
2.25	8	101.9	8.8
2	14	125.3	17.2
1.75	8	130.1	19.0
1.5	14	155.6	23.0
1.25	8	176.9	19.2
1	18	205.1	29.5
0.9	7	207.9	23.4
0.8	7	215.0	13.4
0.7	8	251.9	33.3
0.6	8	353.3	60.6
0.5	6	450	0.0

Regression equations

Concentration = 3.90 - 0.0127 (mm/10 Penetration)

Correlation = -0.893

log of penetration values

Concentration = 11.6 - 4.48 (log mm/10 penetration)

Correlation = -0.952

1/Penetration values

Concentration = 4.08 - 0.00133 (1: mm/10 penetration)

Correlation = 0.910

APPENDIX 7.4.C

Gelation: A table comparing gels of plasma concentrations 9, 10, 11% at varying temperature by measuring amount, in 1/10 mm, a cone penetrates the gels

Temperatures	Concentrations of Plasma											
	9%				10%				11%			
	No.	Mean pen	SD	Agar ≡	No.	Mean pen	SD	Agar ≡	No.	Mean pen	SD	Agar ≡
70°C	4	400.0	0.0	<0.5	4	400.0	0.0	0.5	4	324.8	51.2	0.55
75°C	4	302.3	21.7	0.58	4	241.3	18.1	0.73	4	193.5^{a}	6.4	1.02
80°C	4	310.0	49.2	0.55	4	186.3^{e}	5.1	1.09	4	151.3^{be}	12.7	1.54
85°C	4	240.5^{d}	22.7	0.73	4	193.0^{e}	8.0	1.02	4	141.3^{bd}	19.6	1.69
90°C	4	195.5^{egj}	3.7	1.00	4	161.3^{e}	20.1	1.40	4	117.5^{bfi}	9.5	2.06

Significant different from value at the same concentration at

70°C	a	=	p	<0.05	b	=	p	<0.01	c = p <0.001
75°C	d		"		e		"		f "
80°C	g		"		h		"		i "
85°C	j		"		k		"		l "

APPENDIX 7.4.D

Gelation: Penetration (1/10 mm) of a cone into gels made from various concentrations of bovine plasma powder

Conc %	No	Penetration Mean	SD	Agar Equivalents
8	22	288	31.3	0.6
9	37	222	35.4	0.8
10	12	180	31.1	1.2
11	12	156	23.2	1.5
12	8	116	26.8	2.1
13	8	91	26.1	2.6
14	5	94	15.0	2.5
15	6	68	32.9	3.2

Regression equations

Concentration = 14.7 - 0.0237 (mm/10 penetration)

Correlation = -0.883

log of penetration values

Concentration = 30.3 - 8.97 (log mm/10 penetration)

Correlation = - 0.964

1 ÷ penetration values

Concentration = 7.61 + 390 (1 ÷mm/10 penetration)

Correlation = 0.987

APPENDIX 7. 4.F

Gels of plasma at concentrations 8, 9, 10% at sodium chloride concentrations of 5 and 10% by measuring the amount, in 1/10 mm, a cone penetrates the gel

	Concentrations of Plasma											
	8%				9%				10%			
	No.	Mean pen	SD	T-value	No.	Mean pen	SD	T-value	No.	Mean pen	SD	T-value
Control	22	288.0	31.3	Control	37	221.5	35.4	Control	12	179.8	31.1	Control
5%	10	181.4	18.7	11.95[c]	10	163.8	9.5	8.83[c]	12	162.2	13.6	1.80
10%	20	207.6	16.9	10.48[c]	20	165.5	22.0	7.36[c]	4	244.5	12.7	3.21[b]

T-test between 5% and 10% Salt conc at 8% plasma = 3.733[b]

9% = 0.287

10% = -2.365

a = significant difference from control p <0.05

b = p <0.01

c = p <0.01

APPENDIX 7.4 F.b

Gelation: Hardness of gel with the addition of various salts to a 10% plasma solution

		Mol Wt.
1	Sodium citrate $Na_3C_6H_5O_7.2H_2O$	294.1
2	Sodium chloride NaCl	58.4
3	Calcium chloride $CaCl_2.2H_2O$	147.0
4	Calcium acetate $CaC_4H_6O_4$	158.2

	1/10 mm penetration			
Solution mM conc	1	2	3	4
0	205	205	205	205
2	187	193	176	207
4	179	207	179	175
8	158	170	158*	173*
16	173	168	134*	130*

* increase in the opacity of the gel

Appendix 7.4F.c. Gelation: Various concentrations of Sodium Chloride and Calcium Chloride on the effect of gelation.

	Sodium Chloride				Calcium Chloride			
m Molar Conc	1/10 mm Penetration No tested	Mean	SD	Agar Equivalents	1/10 mm Penetration No	Mean	SD	Agar Equiv
50	4	173.5	22.1	1.258	4	149.50	28.9	1.585
45	4	162.5[a]	20.0	1.395	4	149.50[a]	21.5	1.628[a]
40	4	178.5	15.8	1.185	4	129.75[a]	35.0	1.900
35	4	182.5	11.3	1.133	4	124.50[a]	34.9	2.003[a]
30	4	178.3	18.2	1.193	4	135.75[b]	16.5	1.770[b]
25	4	171.5	20.4	1.275	4	123.50[c]	14.6	1.960[b]
20	4	170.5[a]	12.4	1.273[a]	4	120.25[b]	19.3	2.023[b]
15	4	170.3	10.5	1.278[b]	4	140.75[c]	6.3	1.690[c]
12	4	181.0[a]	9.6	1.150[a]	4	156.75[a]	20.5	1.463[a]
10	4	182.8	10.0	1.133	4	159.00	24.2	1.445
8	4	186.8	14.1	1.090	4	163.00	23.6	1.380
6	4	179.5	17.7	1.175	4	169.25	22.0	1.308
4	4	188.5	15.5	1.078	4	175.50	22.2	1.235
2	4	179.0	13.6	1.175	4	194.00	27.6	1.050
1.5	4	190.5	6.7	1.045	4	197.00	17.9	1.000
1.0	4	191.0	9.3	1.048	4	181.75[a]	32.0	1.188
0.5	4	181.0[a]	5.6	1.146[a]	4	205.50	19.8	0.930
0.0	4	199.3	10.0	0.970	4	196.75	9.4	0.988

a = significant difference from '0' value $p < 0.05$

b = significant difference from '0' value $p < 0.01$

c = significant difference from '0' value $p < 0.001$

* gels were milky white in appearance

APPENDIX 7.4.G

Gels of plasma at concentrations 8, 9, 10% at Glucose concentrations of 5 and 10% by measuring the amount, in 1/10 mm, a cone penetrates the gels

	Concentrations of plasma											
	8%				9%				10%			
	No.	Mean per	SD	T-value	No	Mean per	SD	T-value	No	Mean per	SD	T-value
Control	22	288.0	31.3	Control	37	221.5	35.4	Control	12	179.8	31.1	Control
5%	8	300	0	-	8	218.5	34.7	0.222	8	282.1	33.7	-6.87[c]
10%	8	266.5	62.1	0.935	8	296.3	10.6	-10.803[c]	8	214.3	33.0	-2.34[a]

T-test between 5% and 10% Glucose concentration 8% plasma = -

9% plasma = 4.071[d]

10% plasma = 6.056[e]

a = significant difference from control p <0.05

b = p <0.01

c = p <0.001

d = between 5 & 10% p <0.01

e = between 5 & 10% p <0.001

APPENDIX 7.4H

Gels of plasma at concentrations 9, 10 & 11% at varying pH by measuring the amount in 1/10mm, a cone penetrates the gels.

	Concentrations of plasma											
	9%				10%				11%			
	No.	Mean per	SD	T-value	No.	Mean per	SD	T-Value	No.	Mean per	SD	T-value
Control	37	221	35.4	Control	12	180	31.1	Control	12	156	23.2	Control
pH 5	8	284	24.0	-6.05[c]	8	249	46.0	-3.74[b]	8	221	32.2	-4.85[c]
pH 10	8	271	40.5	-3.211	8	203	24.7	-1.82	8	185	18.3	-3.04[b]

a = significant difference from control of same plasma concentration p <0.05

b = p <0.01

c = p <0.001

Appendix 7.4.I

Gels formed when various substances had been added to the 10% solutions of BPP

Sample	pH	Penetrometer Reading	Comments
A Control	7.5	164, 162, 132 128, 178, 147 Ave = 152	A cloudy firm gel, which could be inverted and retard the conc. Agar equivalent = 1.53%
B Urea	8.1	254, 352, 275 370, 364, 399 Ave = 336	A clear gel, stable on inversion and was equivalent to agar of 0.54%
C SDS	8.3	>600	Very clear solution, no gel
D mercaptoethanol + Urea	7.8	These samples gelled on standing for only 1 hour at room temperature. The gel was clear and firm and could not be transferred to the universals for measurement.	
E mercaptoethanol	7.7	120, 124, 116 92, 116, 100 Ave = 111	Some gelling was present at 24 hours, before heating took place. On On heating the gel was cloudy and very firm. The agar equivalent was 2.16%
F Cysteine Hydrochlorine	5.9	139, 132, 154 162, 130, 137 Ave = 144	A milky white gel which, after heating was stable on inversion and retarded the penetrometer cone. The agar equivalent was 1.95%
G Control Sugar	7.2	187, 187, 222 181, 212, 176 Ave = 161	The cloudy gel that was formed was darker in colour than F. The gel was stable on inversion and retarded the penetrometer cone. The agar equivalent was 1.40%