Aspects of structure and functionality in legumin and vicilin from vicia faba seeds

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ASPECTS OF STRUCTURE AND FUNCTIONALITY IN LEGUMIN AND VICILIN FROM VICIA FABA SEEDS.

BY

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DECLARATION

I declare that no part of this thesis has previously been submitted for a degree at this or any other University. Also, that the work presented in this thesis is entirely my own.

ABSTRACT.

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Soy beans command a large proportion of the vegetable protein market of the food processing industry; however, their cultivation is limited by climatic factors. A crop able to compete in these markets, but suited to growth in cooler, temperate conditions would have political and economic advantages for Europe. One such crop under consideration is Vicia faba minor. A new faba bean line (IVS-G), developed by a breeding programme at Durham University, was compared to an established commercial variety (Maris Bead) for chemical, physical and functional characteristics. A selection of processing methods was used to produce proteinaceous materials equivalent to soy products already on the commercial market. The methods used were defatting, air-classification, and the production of isolates, proteinates and micellar protein preparations. The materials were assessed and compared to soy products for functionality in tests designed to assess solubility, gelling, foaming and emulsifying properties. The storage proteins, legumin and vicilin, are the major protein constituents of the bean seed, and their structural and functional properties were also investigated.

The bean products had similar functional properties to equivalent soy products, when protein contents were also similar. However, the higher initial in vivo protein content of the soy bean does give the latter a competitive advantage. Also, the marketing of by-products of faba bean, eg. starch and fibre, may prove difficult.

Abbreviations

BSA - bovine serum albumin

CWM - cell wall material

DSC - differential scanning calorimetry

DTT - dithiothreitol

EDTA - ethylenediamine tetraacetate

PAGE - polyacrylamide gel electrophoresis

PSE - protein separation efficiency

PY - protein yield

SAXS - small angle X-ray scattering

SDS - sodium dodecyl sulphate

SEM - scanning electron microscopy

SSE - starch separation efficiency

SY - starch yield

Tris - tris(hydroxymethyl)aminomethane

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CHAPTER 1

INTRODUCTION

The expansion of the food processing industry and the development of markets for convenience foods have led to greatly increased demands for low cost protein containing materials. Animal proteins are expensive in terms of land required for their production, and market price. Attempts have been made, therefore, to replace them with alternative sources. Potential candidates have been identified as oil seed, grain legume, fish, leaf and single cell proteins (Mattil, 1971).

Proteins in foods contribute to both nutritional and functional properties. In many poorer countries legume proteins are a major source of dietary protein; in South America 30% of total protein intake consists of bean proteins. The use of legumes as protein supplements in low protein cereal based foods (eg. spaghetti and bread) has been investigated (Pomeranz and Finney, 1973; Fleming and Sosulski, 1977; Bahnassey et al., 1986).

However, whilst proteins are nutritionally essential, common protein foods in developed countries owe their commercial success to gastronomic appeal. Proteins may contribute specific functional attributes to foods which give them their characteristic properties,

eg. organoleptic, kinesthetic, hydration, surface, structural, textural and rheological properties. In the food processing industry the latter properties are more important than nutritional considerations, since the overall diet is normally balanced for protein and amino acids anyway. Most attempts to incorporate vegetable proteins into foods have tried to imitate as closely as possible more familiar animal protein products. This results in a direct competition between animal and plant proteins, with the latter required to perform at least as well as animal proteins before consumer acceptance can be gained.

Soy proteins are currently the most widely utilized vegetable proteins in the fabrication of foods. Their physical and chemical properties have been reviewed by Wolf (1977). However, climatic and agronomic conditions in temperate regions do not suit the growth requirements of the soy crop. The E.E.C. must currently import large quantities of soy beans to meet the requirements of the food and animal feed industries. Economic and political reasons make it desirable to find a substitute crop more suited to the endemic growth conditions (Hebblethwaite, 1984). One crop under consideration is Vicia faba minor. The beans have a relatively high protein content compared to other grain legumes. Currently V-faba minor seeds are utilized predominantly in livestock feed compounds, although interest in their use in food applications has been expressed, eg. Vaisey et al. (1975).

However, the crop is not very popular in Europe. This has been attributed to a number of factors (Hebblethwaite, 1984):

1) low yields in comparison with cereals,

2) low profitability compared to cereals,

3) unstable yields compared to other crops,

4) uneven maturity in cool temperate countries,

5) disease, eg. Botrytis,

6) problems in utilization and marketing,

7) rapid expansion of more profitable break crops for cereals, eg. oilseed rape.

In contrast to other leguminous crops (eg. soy and Phaseolus beans) faba bean seeds do not contain harmful concentrations of toxic constituents. However, they do contain a number of substances which may impair nutritional quality, eg. tannins, phytate, protease inhibitors, vicine and convicine (Nitsan, 1971; Griffiths and Jones, 1977; Martin-Tanguy et al., 1977; Griffiths, 1979; 1981; 1982; 1984; Moseley and Griffiths, 1979; Fowler, 1980; Griffiths and Thomas, 1981; Olaboro et al., 1981; Pitz et al., 1981). However, significant variation in the content of these anti-nutritive factors in Vicia faba lines has been demonstrated, suggesting the possibility of breeding for varieties with improved nutritional quality (Frauen et al., 1984).

In Durham a breeding programme has been undertaken to construct a faba bean ideotype based on the independent vascular supply reproductive architecture, in an attempt to counter the unstable yields frequently observed for this crop (White, 1985). The most promising seeds produced by this programme were termed 'IVS-G', and their properties have been compared with a commercial variety (Maris Bead) in this study.

To facilitate the fabrication of protein foods from cheap raw materials it is necessary to have an understanding of the structural and functional properties of the new material. A similar objective has already been achieved in the margarine industry, where a fundamental knowledge of the physical and chemical properties of the relatively small and less complex glyceride molecules, and the relationship of these properties to functional and performance characteristics, has been used to create an acceptable consumer product.

Structural Properties.

The major storage proteins of Vicia faba are globulins, and can be separated into two major types termed legumin and vicilin. The widespread occurrence of homologous proteins has been demonstrated in many dicotyledonous plants, and also in some monocotyledenous ones (Derbyshire et al., 1976). They are characterised by their complex quaternary structure, a tendency to association - dissociation reactions, low contents of α-helix (approx. 10%), and a large β-sheet complement (approx. 50%) (Fukushima, 1968; Blagrove et al., 1984; Zirwer et al., 1985).

Legumin from V.faba has been studied by Bailey and Boulter (1970), Wright and Boulter (1974), Mori and Utsumi (1979), Utsumi et al. (1980) and Matta et al. (1981a), and has been compared to pea legumin by Croy et al. (1979). The accepted model of legumin structure is a protein with an approximate Mr. of 340 000, composed of six acidic + basic subunit pairs. This has been supported by small-angle x-ray scattering (SAXS) studies of V.faba legumin in solution (Plietz et al., 1984). Similar structures have been observed for pea legumin (Miles et al., 1985), soy glycinin (Badley et al., 1975; Miles et al., 1984), and the 11S proteins from sunflower and rape seeds (Plietz et

al., 1978; 1983b).

The legumin subunits from V.faba exhibit a considerable degree of heterogeneity (Wright and Boulter, 1974; Utsumi and Mori, 1980; Matta et al., 1981a; Maplestone et al., 1985). Similar heterogeneity has been observed for 11S proteins from pea (Thomson et al., 1978; Thomson and Schroeder, 1978; Krishna et al., 1979; Matta et al., 1981b), soy bean (Kitamura et al., 1980; Mori et al., 1981b), ground nut (Tombs, 1965) and lupin (Blagrove and Gillespie, 1978).

Each acidic + basic subunit pair is joined by a disulphide linkage, and pairing between subunits is specific (Staswick et al., 1981; Matta et al., 1981b; Utsumi and Mori, 1983; Horstmann, 1983). This is due to the synthesis of the subunit pairs as single polypeptides and subsequent processing by proteolytic cleavage of the precursors. Precursors have been identified in pea (Evans et al., 1979; Croy et al., 1980; 1982; Lycett et al., 1984; Boulter, 1984), V.faba (Croy et al., 1980), soy (Tumer et al., 1981; 1982; Barton et al., 1982; Nielsen, 1984), rice (Yamagata et al., 1982) and oats (Brinegar and Peterson, 1982; Matlashewski et al., 1982; Walburg and Larkins, 1983).

Vicilin from Vicia faba has been studied by Bailey and Boulter (1972) and Mori and Utsumi (1979). Native vicilin exists as a trimer with an approximate Mr. value of 120 000. The shapes of 7S proteins in solution have been observed using SAXS for phaseolus beans (Plietz et al., 1983a; c), and pea (Miles et al., 1985). Three 'Y' shaped subunits with deep solvent clefts have been observed. Association is controlled via hydrophobic interactions. Considerable heterogeneity of vicilin proteins has also been demonstrated.

The major differences between the legumin and vicilin proteins are:

a) the larger size of the legumin protein,

b) the involvement of disulphide linkages in stabilizing the subunit structure of legumin,

c) the higher sulphur containing amino acid content of legumin,

d) the small quantities of carbohydrate associated with vicilin (Gatehouse et al., 1980; 1981).

However, structural similarities between legumin and vicilin storage proteins have been demonstrated (Argos et al., 1985).

Legumins from different legumes also show significant homology. Wright (1983) compared the amino acid content of pea, field bean and soy bean legumins. Only proline, arginine and threonine contents differed by more than 10%, and these differences were less than 20%. Pea and field bean legumins were more closely related than either of these two with soy beans. Generally non-polar and dicarboxylic acid residues were conserved.

Amino acid sequence homology between subunits, and between legume proteins from different species, has been demonstrated by Gilroy et al. (1979), Moreira et al. (1979), Casey et al. (1981a; b) and Walburg and Larkins (1983).

Soy protein structure has been reviewed by Badley et al. (1975) and Peng et al. (1984).

Considering the extent of the similarities between storage proteins from a variety of plant sources it would seem reasonable to expect similar functional properties. However, large quantities of purified proteins are not easily obtainable without considerable effort and expense, and this renders them impractical for use by the food industry. However, a variety of processing methods have been developed to produce useful materials for food fabrication. These materials range widely in their physical, chemical and functional properties, and can be tailored to meet the requirements of specific products. The processing methods used in this thesis were defatting, air classification, and isolate, proteinate and micellar protein preparation.

Air classification is a technique in which particles differing in size, density and mass are separated in a current of air. By careful selection of the operating conditions it is possible to determine the composition of the coarse and fines fractions produced. The technique has proved useful for producing protein rich and starch rich fractions initially from cereals, and subsequently from many starchy grain legumes, eg. California small white beans (Kon et al., 1977), navy beans (Patel et al., 1980) field peas and horse beans (Vose et al., 1976), mung beans and lentils (Sosulski and Youngs, 1979; Tyler et al., 1981). Its application to wheat, barley, malted barley, oats, triticale, rice, sorghum, potatoes and grain legumes has been reviewed by Vose (1978).

The procedure for isolate production was first developed for soy beans (Anson and Pader, 1957), but has been adapted for rape seed (Gillberg and Tornell, 1976), faba beans (Bramsnaes and Olsen, 1979), mung beans (Thompson, 1977), phaseolus beans (Satterlee et al., 1975), lupins (King et al., 1985), peas and horse beans (Vose, 1980) and

cotton seed (Lawhon and Cater, 1971). Commercial production of isolates usually involves alkaline extraction of proteins from the flour and removal of insoluble material, followed by precipitation of the protein at the isoelectric point. The isolate is then washed and spray dried, or neutralized with sodium hydroxide and spray dried as a sodium proteinate. The ultimate aim of the method is to obtain an efficient protein extraction, and to recover the protein in a soluble form so that its functional properties can be fully exploited. The method of protein isolation may influence the functional properties of a protein to a greater extent than the actual nature of the protein. Therefore, it is important to avoid harsh extraction or precipitation conditions. Large yields of isolates can be obtained from food sources possessing proteins with good solubility characteristics at the extraction pH, and low solubility at the isoelectric point.

An even gentler processing method is used in the production of micellar proteins. The procedure was developed using field beans and exploits hydrophobic interactions between protein molecules (Murray et al., 1978). Many food proteins have high hydrophobicity values and a tendency to form quaternary structures (Bigelow, 1967). Some of these hydrophobic residues may be exposed on the surface if insufficient polar residues are available to bury them in the interior of the molecule (Klotz, 1970; Lee and Richards, 1971) and these would tend to come together in an aqueous environment if they were present in sufficient concentrations. To enhance this reaction pH should be close to neutrality as at more extreme pH values repulsion between charged amino acid residues will result in increased solubility. When these hydrophobic reactions occur extensive protein precipitation takes place, and the micellar protein can be collected and dried. Once formed the protein micelles are relatively stable, possibly due to the

formation of disulphide linkages. The effects of pH and various anions on micellar protein formation by vicilin from Vicia faba have been investigated by Ismond et al. (1986a; b).

Generally protein is extracted in a weak salt solution. The supernatant is then diluted with distilled water to promote hydrophobic interactions (Murray et al., 1981). Micellar proteins can be produced by this method from field peas, chickpeas, peanuts, rape seed, soy beans and oats (Murray et al., 1981). Other proteins which have the capacity to aggregate into a micellar arrangement include β-casein (Evans and Phillips, 1979) and membrane proteins (Simons et al., 1978). Electrolyte mediation is essential for the formation of casein micelles (Slattery, 1976).

The merits and problems associated with these methods are discussed at greater length in the discussion.

Functional Properties.

To assess the functional properties of new proteins a systematic evaluation of their behaviour in a variety of environments has to be undertaken (Kinsella, 1976; 1979). Traditionally new ingredients were tested in food products under practical conditions. This approach is expensive and time consuming, although ultimately it is necessary before a new food protein is accepted in a marketable product. Many simple model functional tests have been developed with the intention of measuring the behaviour of proteins objectively and of extrapolating the results to predict behaviour in food systems. Ideally tests should be performed using a wide range of pH values, salt concentrations, temperatures and in the presence of various ions, to represent the range of conditions found in food systems. The

development of micro-methods which are quick, accurate and use small quantities of material facilitates this process. Micro-methods have been developed to assess foaming (Waniska and Kinsella, 1979; Kato et al., 1983b), gelling (Utsumi et al., 1982) and emulsifying (Yamauchi et al., 1982) properties. However, these methods are not in widespread use.

a) Solubility.

Many important functional properties of food proteins relate to water-protein interactions, eg. solubility and dispersibility, viscosity, gelation, foaming and emulsification (Kinsella, 1976; Hermansson, 1979). A solubility profile of a new food protein will provide information important for determining a) the optimum extraction procedure for that protein, and b) its potential application in fabricated food systems, eg. maximum solubility at acidic pH values for carbonated beverages, or good solubility and an absence of heat precipitation if the protein is to be used in milk type beverages or coffee whiteners (Wolf and Cowan, 1971). The balance between protein-protein and protein-solvent interactions can be affected by pH, concentration, temperature, nature of the solvent, processing method and presence of other components, eg. lipid and phytate (Hermansson, 1979; de Rham and Jost, 1979).

For maximum functionality, solubility should be high over a broad pH range; low solubility over a broad pH range is generally a sign of severe denaturation. Wolf and Cowan (1971) suggested that the nitrogen solubility index (NSI) provides useful information concerning the extent of heat damage sustained during the processing of soy beans. The effects of processing methods on subsequent solubility have been studied for isolates from soy bean and rape seed (Hermansson,

1979), coconut (Samson et al., 1971), peanut (Rhee et al., 1973), alfalfa leaves (Lu and Kinsella, 1972) and cottonseed (Lawhon and Cater, 1971). However, a better method of determining the extent of protein denaturation is differential scanning calorimetry (DSC). This is a useful thermoanalytical technique for monitoring changes in physical or chemical properties of materials as a function of temperature. Its use in food research has been reviewed by Biliaderis (1983). An inert material and a sample are heated at a constant rate, and the differential heat flow to the sample is recorded as a peak. The area under the peak is directly proportional to the enthalpic change in the sample. A relatively undenatured protein would be expected to produce a larger enthalpic change than a denatured one. In this study the effects of the processing method used on the dispersibility profiles and DSC thermograms of protein products were investigated.

b) Gelling.

Heating an aqueous protein dispersion often causes an increase in viscosity, and at sufficiently high concentrations gelling may occur. This property is important in foods where thickening or gelling properties are required, eg. custard type puddings, sauces, soups, beverages, batters, sausage type meats, cheese, yoghurt and baked products. Egg and muscle proteins, wheat gluten and carbohydrates have been traditionally employed as thickening or gelling agents. Heating an 8% solution of soy protein may cause gelation (Catsimpoolas and Meyer, 1970). However, gels from globular proteins generally require 10 x the protein concentration compared to gels formed from gelatin or carbohydrate sources (Tombs, 1974).

Gels can be described as a 3-dimensional network structure which may act as a matrix to hold water, lipids, dissolved proteins, carbohydrates and other food components. Muscle proteins are largely responsible for the physical and chemical stabilization of fat and water in comminuted meat products (Ziegler and Acton, 1984; Samejima et al., 1985; Hermansson et al., 1986). The mechanisms involved in gel formation are difficult to study as most current rheological instruments are designed to evaluate the final product. However, the gelation mechanisms of egg proteins have been studied by Hegg et al. (1979), Nakamura et al. (1982), Gossett et al. (1984), Grinberg et al. (1985) and Watanabe et al. (1985). Many studies have also been made on the gelling mechanisms of soy proteins and these are more extensively reviewed in the discussion.

c) Foaming.

The behaviour of proteins at interfaces is of fundamental significance in food systems such as emulsions and foams. Foams are found in whipped toppings and creams, souffles, mousses, meringues, ice cream, leavened bread and baked products. They are enjoyed for their lightness and mouth feel. Foam formation involves the entrapment of air by a protein film, and these air cells act to impart body and smoothness, ensure uniform rheological properties and facilitate dispersion of flavours in the product. Egg white is currently the most widely used foaming agent, but gelatin, casein, milk, whey proteins, gluten and soy proteins are also used (Kinsella, 1981).

There are three widely used methods for determining foaming properties: a) whipping (Eldridge et al., 1963; Lawhon and Cater, 1971), b) shaking (Yasumatsu et al., 1972; Wang and Kinsella, 1976)

and c) sparging (Buckingham, 1970; Mita et al., 1977). The amount of protein usually employed in each test varies, ie. 3-4% for whipping, 1% for shaking and 0.1-2% for sparging. The volumes required are also much larger for whipping. Proteins are subjected to different forces during foam formation by the three methods. Foam bubbles are continuously formed and broken by shear forces during whipping. In contrast, once bubbles are formed by sparging the rate of rupture is a function of lamella thickness and interfacial viscoelasticity (Mita et al., 1977). The methods of producing foams have been compared by Halling (1981). In this study results obtained by the sparging and whipping methods were compared.

d) Emulsifying properties.

Emulsifying agents are used extensively in foods such as coffee whiteners, milk type beverages, mayonnaise, gravies, comminuted meats and ice cream. The most widely used emulsifying ingredients are meat and milk proteins.

Three indices of emulsifying properties are in general use, (1) emulsifying capacity (EC), (2) emulsion activity (EA) and (3) emulsion stability (ES). The EC index was introduced by Swift et al. (1961). A protein solution is vigorously stirred while oil is steadily added. When a critical volume of added oil is reached the solution undergoes a sudden change in viscosity, and this is taken as the end point of the titration. Using EA as an index was introduced by Yasumatsu et al. (1972). An emulsion is formed by mixing set volumes of oil and protein solution. This is then centrifuged and the height of the unseparated layer is measured. Determining ES involves monitoring the rate of depletion of oil from the bottom of an emulsion

over a time period.

The effects of different blending methods, and speed and duration of blending on emulsion formation have been investigated by Tornberg and Hermansson (1977) and Pearce and Kinsella (1978). Generally increasing blending speed results in smaller oil droplets and a decreased % oil phase volume (Swift et al., 1961; Carpenter and Saffle, 1964; Ivey et al., 1970; Crenwelge et al., 1974). The type of oil, protein concentration and oil volume fraction also affect emulsion formation (Carpenter and Saffle, 1964; Pearce and Kinsella, 1978). This makes it difficult to compare results obtained by different groups. Comparative studies of emulsifying properties of vegetable proteins have been made for chick pea, faba bean, field pea, great northern bean, lentil, lima bean, lupin, mung bean, pea bean, peanut, pecan, rape seed, soy bean and sunflower proteins (Inklaar and Fortuin, 1969; Lin et al., 1974; Sosulski et al., 1976a; Hutton and Campbell, 1977; McWatters and Cherry, 1977; Ramanatham et al., 1978; McWatters and Holmes, 1979a; b; Volkert and Klein, 1979). A review on the use of vegetable proteins as emulsifying agents was conducted by McWatters and Cherry (1981).

Aims.

The aims of this study were to compare the structural and functional properties of protein-based products prepared from a commercial bean variety (Maris Bead), with those from the new IVS-G line. Also, a variety of processing methods were used to produce materials with a range of physical and chemical properties, and these were assessed for functionality in a number of simple tests. The potential of a dry processing method, namely air classification, for

producing protein concentrates with equivalent protein contents and functional properties to commercial soy products was assessed. The functional properties considered were solubility, dispersibility, gelling, thermal, foaming and emulsifying. Any observed functional differences should reflect the variations generated by the processing methods in chemical and physical properties, and lead to a better understanding of the relationships between these properties and functional properties.

The ultimate aim was to assess, in terms of functionality, the potential of the field bean preparations as competitors to those of the soy bean as a source of protein for the food industry.

CHAPTER 2

MATERIALS

2.1 BIOLOGICAL MATERIALS.

Seeds of Vicia faba minor cv. 'Maris Bead' were obtained from Tyneside Seed Stores, Gateshead, Tyne and Wear. Seeds of Vicia faba (IVS-G) were donated by Dr. P. Gates, Department of Botany, University of Durham.

Sodium caseinate was obtained from Unilever Research, Colworth Laboratory, Sharnbrook, Bedfordshire, MK44 1LQ.

Full-fat and defatted soy meal were obtained from British Drug Houses (BDH) Limited, Poole, Dorset, BH12 4NN.

Soy protein concentrate was supplied by Lucas Ingredients Limited, Moravian Road, Kingswood, Bristol, BS15 2NG.

Pea (Birte, Filby or Progreta) and soy concentrates, isolates, proteinates and micellar proteins were prepared at the AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich, Norfolk, NR4 7UA.

Pea (Birte) globulins, and 7S and 11S fractions were also produced at the AFRC Institute of Food Research. The globulins were

obtained by salt extraction, and the 7S and 11S fractions by ammonium sulphate precipitation.

Spray dried egg white was supplied by Allmey and Layfield Limited, Windsor Mills, Holden Street, Liverpool.

Purified proteins were obtained from the Sigma Chemical Company, Fancy Road, Poole, Dorset, BH17 7NH. They were: BSA type A4503, γ-globulin type G5009, hemoglobin type H2500, lysozyme type L-6876, myoglobin type M1882 and ovalbumin type 5503.

2.2 CHEMICALS AND REAGENTS.

Trizma base and coomassie brilliant blue R were obtained from the Sigma Chemicals Company, Fancy Road, Poole, Dorset, BH17 7NH.

All other chemicals were supplied by British Drug Houses (B.D.H.) Ltd., Poole, Dorset, BH12 4NN, and were of AnalaR grade or the best available.

2.3 OTHER MATERIALS.

HA-Ultrogel was obtained from LKB Instruments Ltd., South Croydon, Surrey, CR2 84D. Superose 6 was obtained from Pharmacia (G.B.) Ltd., London, W5 5SS.

Visking dialysis tubing was supplied by Medicell International Limited, 239, Liverpool Road, London, N1.

CHAPTER 3

METHODS

3.1 PREPARATION OF EXPERIMENTAL MATERIAL.

3.1.1 Preparation Of Flour For Air-classification.

Seeds were split on a Pascal End Runner Mill and the splits passed through an Alpine Kolloplex 160Z pin mill, as described by Wright et al. (1984).

3.1.2 Preparation Of Flour For Protein Purification.

Seeds were dehulled manually and freeze-dried. The cotyledons were ground for 30 s in a water-cooled Janke and Kunkel mill. The flour was defatted twice with hexane (1:10 w/v) at 4°C for 30 min. Excess hexane was removed under vaccuum.

3.2 FRACTIONATION OF FLOURS.

3.2.1 Air-Classification.

Flour was fractionated using an Alpine Zig-Zag Classifier model A100 MZR. Classification of flours was investigated using classifier speeds ranging from 3000 to 11000 rev./min, and corresponding air flows from 52 to 44 N m^3h^{-1} . Percentage weight of

coarse and fines fractions was determined for each experiment. Due to unavoidable losses from the fines fraction, percentage weight of fines was calculated as 100 - percentage weight of coarse fraction. All weights were corrected for moisture content to negate the effects of water loss during the air-classification process.

3.2.2 Re-classification Of Coarse Fraction.

1 kg Maris Bead flour was air-classified at 6500 rev./min. This speed was chosen as it produced the coarse fraction with the highest protein content when both protein percentage and fraction yield were considered. The coarse fraction was re-milled as described previously. 200g twice-milled flour was re-classified at 5500, 6500 and 7000 rev./min. The percentage weight of the coarse and fines fractions was determined.

3.2.3 Sequential Air-classification.

1kg Maris Bead and IVS-G flours were milled as described previously. 600g flour was air-classified at 11000 rev./min. The coarse fraction was re-classified at 9000 rev./min, and the process repeated at 8000, 7000, 6500, 6000, 5500, 5000, 4000, and 3000 rev./min. Percentage weight of coarse and fines fractions was determined after each air-classification.

3.2.4 Protein Yield, Protein Separation Efficiency, Starch Yield And Starch Separation Efficiency.

Protein Yield (P.Y.) and Protein Separation Efficiency (P.S.E.) were calculated as defined by Tyler et al. (1981).

$$P.Y. = \frac{\left[\begin{matrix}\% \text{ weight fines} \\ \% \text{ protein in fines}\end{matrix}\right] + \left[\begin{matrix}\% \text{ weight coarse} \\ \% \text{ protein in coarse}\end{matrix}\right]}{\% \text{ protein in starting material}}$$

$$P.S.E. = 100 - \frac{(\% \text{ weight coarse} \times \% \text{ protein in coarse})}{\% \text{ protein in starting material}}$$

Starch Yield (S.Y.) was determined by the same method as (P.Y.). Starch Separation Efficiency (S.S.E.) was obtained from the following equation:

$$S.S.E. = 100 - \frac{(\% \text{ weight fines} \times \% \text{ starch in fines})}{\% \text{ starch in starting material}}$$

3.3 ANALYSIS OF FLOURS AND AIR-CLASSIFIED FRACTIONS.

3.3.1 Moisture Content Determination.

Moisture content was measured by dry weighing samples at 105°C overnight. Tests were performed in duplicate.

3.3.2 Protein Determination.

Nitrogen content of oven-dried samples was measured using a Carlo Erba ANA 1400 Automatic Nitrogen Analyser (N x 6.25). All results were the average of duplicate tests. A nitrogen to protein conversion factor of 6.25 was used throughout this thesis for ease of comparison with other functionality studies.

3.3.3 Starch Determination.

Starch determination was conducted on oven-dried samples according to the glucoamylase method of Blake and Coveney (1978). All results were the average of duplicate tests.

3.4 ISOLATE, PROTEINATE AND MICELLAR PROTEIN PRODUCTION.

Isolates and proteinates were produced by a modification of the method of Sumner et al. (1981).

3.4.1 Isolate Preparation.

Flour was extracted in water (1:10 w/v) at pH 7 and room temperature for 1 h, centrifuged and the supernatant fraction collected. The pH of the supernatant fraction was adjusted to 4.6 - 4.7 and the precipitate centrifuged and collected. It was resuspended in a small quantity of distilled water and freeze dried.

3.4.2 Proteinate Preparation.

The isolate procedure was followed as far as the second centrifugation. The precipitate was resuspended in a small quantity of distilled water and the pH adjusted to > 7. It was stirred until stable and freeze dried.

3.4.3 Micellar Protein Preparation.

(a)

Flour was extracted in 0.4M NaCl (1:10 w/v) at pH 6 and room temperature for 30 min. The mixture was filtered through two layers of muslin and the filtrate centrifuged.

The supernatant was reduced in volume by 80% using an Amicon Model DC2 laboratory dialyzer/concentrator and diluted (1:5) with chilled, distilled water. The precipitate was allowed to settle. The supernatant was decanted and the residue centrifuged and freeze dried.

(b)

Micellar protein was prepared by a modification of the method of Murray et al. (1978). Flour was extracted in 0.4M NaCl (1:10 w/v) at pH 7 and room temperature for 3 h. The mixture was centrifuged. The supernatant fraction was diluted (1:3) with chilled, distilled water and left at 4^{0}C overnight. The precipitated protein was collected (after an extra centrifugation if necessary) and freeze dried.

3.5 PURIFICATION OF STORAGE PROTEINS.

Extraction and purification of the 7S and 11S storage proteins was conducted by a modification of the method of Gatehouse et al. (1980).

3.5.1 Crude Extraction Of Storage Proteins.

Defatted flour was extracted (1:10 w/v) in 50mM potassium phosphate buffer with 0.4M NaCl, pH 7.8 at 4^{0}C for at least 3 h. The mixture was centrifuged at 20000 g for 30 min and the pellet discarded. The solution was fractionated by $(NH_4)_2SO_4$ precipitation, and the material precipitating in the range 60 - 100% saturation was collected by centrifuging at 20000 g for 30 min. The crude globulin pellet was resuspended in 50mM potassium phosphate (pH 7.8) and

dialyzed extensively into the same buffer.

3.5.2 Separation Of Crude Globulins On HA-Ultrogel.

Buffers contained 0.05% (w/v) sodium azide and were filtered through 0.22 μM pore size cellulose acetate filters. The dialyzed globulins were filtered as above and loaded on to a column of HA-Ultrogel. Excess extract was eluted by washing with 50mM potassium phosphate buffer, pH 7.8. The column was eluted with a linear concentration gradient of potassium phosphate buffer, pH 7.8 (50mM - 0.75M, 500 ml + 500 ml), at a flow rate of 40 ml/h. 10 ml fractions were collected and freeze dried. They were subsequently analyzed by SDS-PAGE.

3.6 ANALYSIS OF PROTEINS.

3.6.1 Polyacrylamide Gel Electrophoresis (PAGE).

(a) Preparation of samples for SDS-PAGE.

The following procedure was used for samples already in solution. 200 μl sample containing approximately 100 μg protein was taken and 1.2 ml 0.05% 880 NH_3 in acetone added. The solution was chilled at -20°C for 20 min. The sample was centrifuged at room temperature and the supernatant decanted. Excess acetone was allowed to evaporate. 95 μl 10mM Tris, 5mM EDTA, 20% (w/v) SDS (pH 7.85) and 5 μl 2-mercaptoethanol were added. The sample was mixed thoroughly.

(b) SDS-PAGE.

Samples were run on 15% (w/v) polyacrylamide slab gels according to the system of Laemmli (1970). Molecular

weights were determined using the following standards: phosphorylase b (Mr. 92000), BSA (67000), ovalbumin (45000), lactic dehydrogenase (35000), chymotrypsinogen (25000), β-lactoglobulin (18500) and ribonuclease (12500).

3.6.2 Analysis Of Purified Proteins On Superose 6.

To test homogeneity the purified proteins were passed through a Superose 6 gel filtration column according to the method of Lambert (1985). All buffers contained 0.02% (w/v) sodium azide and were filtered through 0.22 μm cellulose acetate membrane filters. The samples were dialyzed in 50mM Tris, 200mM NaCl, 1mM DTT (pH 8.0), at 4°C overnight. They were filtered and loaded on a 10 ml Superose 6 column and eluted at a flow rate of 0.4 ml/min. Retention times were compared to the following standards: thyroglobin (Mr. 669000), ferritin (440000), glucose oxidase (154000), BSA (67000), ovalbumin (45000) and myoglobin (18000).

3.6.3 Analytical Ultracentrifugation.

Samples were tested for homogeneity on an MSE Centriscan 7S. 0.5-5% (w/v) protein solutions were dialyzed into 0.035M KH_2PO_4, 0.4M NaCl, 1mM DTT, 0.05% (w/v) sodium azide (pH 7.6), at 4°C for 16 h. Samples were centrifuged at 50000 rev./min and 20°C. Scans were made at 10 min intervals and sedimentation coefficients were measured and corrected to $S_{20,w}$ values using density and viscosity values given by Svedberg and Pederson (1940). Values of $S_{20,w}$ were obtained at four protein concentrations and $S^0_{20,w}$ values calculated by linear regression analysis.

3.7 MEASUREMENT OF SOLUBILITY AND GELLING ABILITY.

3.7.1 Measurement Of Solubility And Gelling Ability As A Function Of pH.

Insoluble aggregates, soluble aggregates, nitrogen solubility and gelling ability were measured for solutions of proteinates, isolates and micellar proteins over the pH range 2 - 11.

Samples were homogenized in distilled water (10 or 20% w/v) until a homogeneous dispersion was obtained. 18 ml aliquots were adjusted to the desired pH level with dilute acid or alkali. 2 x 8 ml were transferred to a graduated centrifuge tube (10 ml) and centrifuged in a MSE Minor (Setting 5) for 5 min.

(a) Measurement of insoluble aggregates.

Insoluble aggregates were measured as the volume of precipitate (cm^3) after centrifugation.

(b) Measurement of soluble aggregates.

Soluble aggregates were measured by reading the optical density at 420 nm after centrifugation, using distilled water as the zero value.

(c) Measurement of soluble protein.

Soluble protein was determined by the method of Lowry et al. (1951), with BSA as the standard. 500 μl aliquots were taken from each sample before gelling. Duplicates were taken at two concentrations for each sample.

(d) Measurement of gelling ability.

Samples were gelled in the centrifuge tube at 95°C for 30 min. They were allowed to cool and left at 4°C overnight.

Samples were centrifuged in a MSE Minor (Setting 5) for 5 min and the volumes of gel and supernatant recorded. A qualitative assessment of the gel was conducted (Voutsinas et al., 1983b). Gels were assigned a number on the following scale:

-4: a smooth liquid

-3: a slightly granular liquid

-2: a moderately granular liquid

-1: granular but collapses with inversion

0: a viscous gel-like semi liquid

+1: a soft gel

+2: a medium gel

+3: a firm gel

3.7.2 Assessment Of Gelling Properties By A Compression Test And Stress Relaxation Measurements.

Compression and stress relaxation measurements were performed using an Instron Universal Testing Machine, Model 1122. Samples were homogenized in distilled water (1:10 w/v) until a homogeneous dispersion was obtained. The pH was adjusted and the sample centrifuged in a MSE Minor (Setting 5) for 10 min. The solution was poured into the templates (15mm diameter teflon cylinders embedded in a steel block) and gelled at 95°C for 30 min. The gels were allowed to cool and left at 4°C overnight. Gels were removed from the templates and coated with silicon fluid (Dow Corning 200/100 cs) to prevent evaporation. Prior to testing the crosshead platen and steel plate were also coated.

(a) Compression test.

The sample was compressed at 50mm/min until material was expelled from between the platens. The stress induced in the sample was measured against time and data recorded using a Hewlett Packard HP85. Data was fitted to the following expression (Blatz et al., 1974).

$$F_{(\lambda)} = \frac{2G}{n}(\lambda^{n} - \lambda^{-2n})$$

Where $F_{(\lambda)}$ = stress

λ = $\frac{\text{new height}}{\text{initial height}}$

G = shear modulus or 'rigidity'

n = a material constant

Rupture strength (Nm^{-2}) was expressed as the force applied to the sample when the surface yield point was reached.

(b) Stress relaxation measurement.

The sample was compressed at a constant rate (50 mm/min) to a pre-determined strain. The stress detected by the load cell was recorded using a Hewlett Packard HP85. Data collection was stopped when no significant change in signal was observed. Relaxation times were obtained by curve fitting the data to an exponential series (Peleg and Normand, 1983) of the form:

$$F(t) = F_{\infty} + A_1 \exp\frac{(-t)}{\tau_1} + A_2 \exp\frac{(-t)}{\tau_2} + A_3 \exp\frac{(-t)}{\tau_3}$$

Where F_{∞} is the residual force at 'infinite' time

A = the amplitude of the individual stress components

τ = time required for a stress component to reduce by 1/e

F(t) = the decaying parameter (ie. force)

Force is used here as the area is assumed to be constant throughout relaxation.

Co-operative flow analysis as described by Bohlin (1980) was carried out on the data using the calculated relaxation times. The theory considers viscous flow to be the major component in stress relaxation, and that a flow unit can be identified. The relationship between a flow unit and the bulk system is defined by the cooperative coordination number, z . The rate equation describing the relaxation process is given by:

$$\frac{ds}{dt} = \frac{-(1)s(s+\alpha)^{z}}{\tau}$$

where s = relative stress (or force)

τ = the relaxation time

α = a measure of the strength of the cooperativity

The co-ordination number, z, was obtained directly from the inverse slope of the linear section of a graph of

$$\frac{-d\ (F/F_0)}{d\ \ln t} \quad \text{versus} \quad \frac{F}{F_0}$$

where F = force

F_0 = the initial force

t = time

The intercept of this line with the F/F_0 axis was also recorded.

3.8 ASSESSMENT OF FOAMING ABILITY.

3.8.1 Determination Of Foaming Ability By A Large-scale Whipping Method.

Foaming ability was determined by a modification of the method

of Patel (1985). 250 ml 0.5% (w/v) solution in 0.1M potassium phosphate (pH 7), was stirred for 1 h at room temperature. The solution was transferred to a Kenwood Chef Model A901 with a balloon whisk attachment and whipped at maximum speed for 5 min. The foam and residual liquid were transferred to a 2 l measuring cylinder with a plastic spatula. Air pockets in the foam were removed by two sharp downward shakes. The total volume (cm^3) and volume of drained liquid (cm^3) were measured and recorded 5 min after whipping had finished (To), and at 10 min intervals for 1 h. Duplicates were performed for each sample. The results were used to calculate the following parameters:

(a) Foam Expansion (F.E.)

$$\text{F.E.} = \frac{(\text{initial foam volume } (cm^3) - 250)}{250} \times 100$$

where initial foam volume includes the residual liquid volume.

(b) Foam Volume Stability (F.V.S.)

$$\text{F.V.S.} = \frac{\text{foam volume } (cm^3) \text{ after 30 min}}{\text{initial volume of foam } (cm^3)} \times 100$$

where initial foam volume includes the residual liquid volume.

(c) Foam Liquid Stability (F.L.S.)

$$\%\ \text{Liquid Drainage (L.D.)} = \frac{\text{Volume of liquid drained } (cm^3) \text{ after 30 min}}{250}$$

$$\text{F.L.S.} = 100 - \text{L.D.}$$

3.8.2 Determination Of Foaming Ability By A Small-scale Sparging Method.

Foaming ability was measured by a modification of the method of Kato et al. (1983b). The apparatus is shown in Figure 1. The flow rate was set at 50 $cm^3 min^{-1}$ using $5cm^3$ distilled water. The sinter and burette were cleaned and dried. 25 ml of 0.5% (w/v) solution in 0.1M potassium phosphate buffer (pH 7) was stirred for 1 h. 5 ml was pipetted into the burette. Gas was directed through the vent until the flow rate was stable and then redirected through the sinter for 1 min. Data was recorded on a chart recorder for 10 min after sparging. Readings were taken manually from the chart recorder at 0.25 min intervals and a linear regression obtained for the time interval 1 - 4 min. Each sample was tested in duplicate. It was essential to use the same sinter throughout the experiment and to clean it with chromic acid between samples to obtain reproducible results.

Foam expansion and foam stability were determined by two methods, as described by Kato et al. (1983b).

Firstly, foam expansion was determined by measuring the conductivity of the foam immediately after sparging was complete, i.e. initial conductivity (Ci). Foam stability was obtained from the following equation:

$$Co.\Delta t/\Delta c$$

Where Δt is represented by the time interval 1 - 4 min.

Δc is the change in conductivity, c, occurring during the time interval Δt.

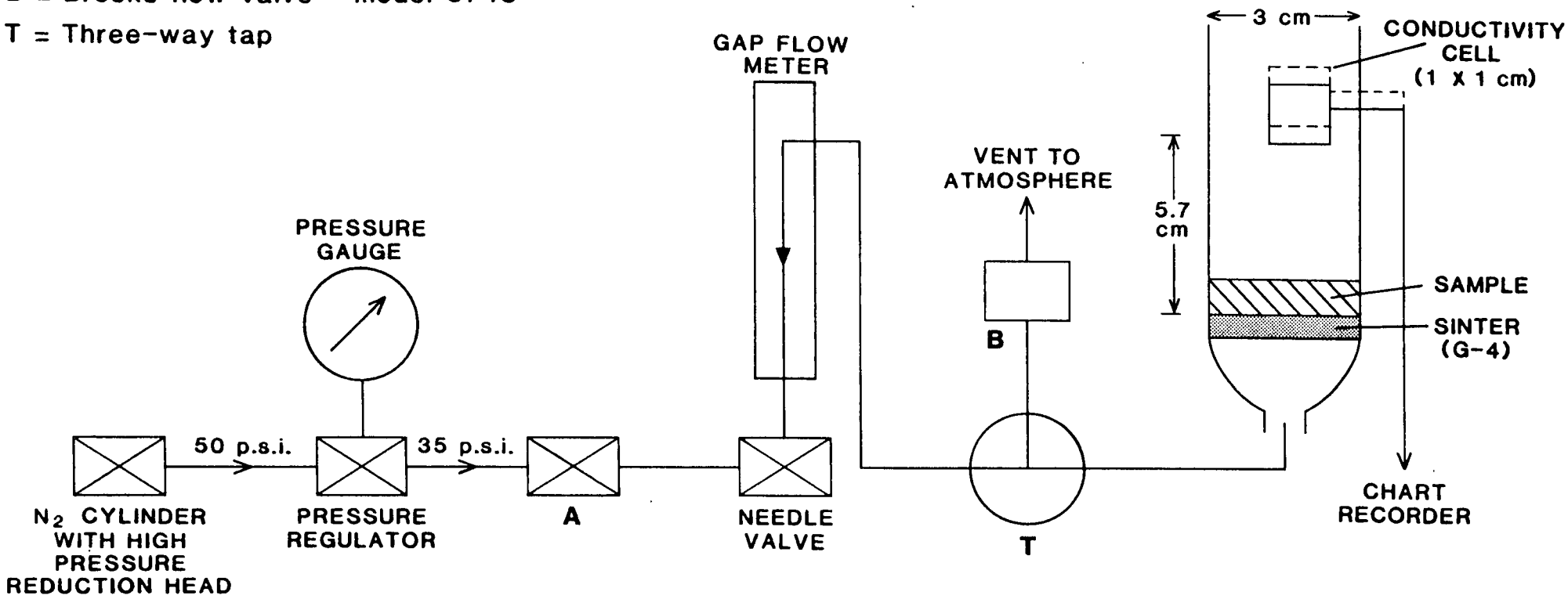
and Co is the conductivity at 0 time obtained from the extrapolation of a linear conductivity versus time plot for the time interval 1 - 4 min.

Figure 1. Sparging apparatus used for determining foaming properties.

A = Brooks flow valve - model 8744

B = Brooks flow valve - model 8743

T = Three-way tap



Secondly, foam expansion (F.E.) and foam volume stability (F.V.S.) were determined using modifications of the equations described in the previous section.

$$F.E. = \frac{(\text{initial foam volume } (cm^3) - 5) \times 100}{5}$$

where initial foam volume is the volume of foam and liquid in the burette immediately after sparging.

$$F.V.S. = \frac{\text{foam volume } (cm^3) \text{ after 10 min} \times 100}{\text{initial volume of foam } (cm^3)}$$

Where initial foam volume is defined as above.

3.8.3 Comparison Of Whipping And Sparging Methods For Determining Foaming Properties.

The whipping and sparging methods were compared by the method of least squares. Comparisons were made between a) F.E. from whipping experiments and Ci from sparging experiments, b) F.V.S. from whipping experiments and $Co.\Delta t/\Delta c$ from sparging experiments, c) F.E. from whipping and sparging experiments and d) F.V.S. from whipping and sparging experiments for both commercially obtained protein standards ($n = 6$) and all samples tested ($n = 42$).

F.E. and F.V.S. from the whipping method were also compared with

a) $\log \left[\frac{\Delta c}{t}\right]$

where t is 0 to 0.9 min

and b) $\frac{\text{conductivity at 3 min}}{\text{conductivity at 0 min}}$

respectively (Hemmant, 1986). Comparisons were made for a) protein

standards (n = 6) and b) all samples tested (n = 43).

3.8.4 Investigation Of The Effect Of Sucrose On Foaming Properties.

The effect of sucrose on F.E., F.V.S. and F.L.S. of 0.5% (w/v) defatted Maris Bead proteinate solution was determined using the large-scale whipping method described above. Samples were either stirred in 0.1M potassium phosphate buffer, pH 7, containing 0, 15, 30 or 60% (w/v) sucrose for 1 h, and foamed as previously, or sucrose (6, 12, or 24% (w/v)) was added 2 or 4 min after the start of whipping. All tests were conducted in duplicate.

3.9 DETERMINATION OF EMULSION ACTIVITY.

500 mg sample in 10 ml distilled water was stirred for 1 min and the pH adjusted to 7.5 with dilute acid or alkali. The mixture was stirred for 1 h at room temperature whilst maintaining the pH. 5 ml dispersion was added to 3 ml pure sunflower oil in a 10 ml vortex beaker and homogenized using a MSE homogenizer (Setting 2) for 20 s. The emulsion was transferred to a 10 ml graduated centrifuge tube and centrifuged in a MSE Super centrifuge at 3000 rev./min for 15 min. The volumes (cm^3) of free oil and emulsion were recorded. Results were expressed as a) ml oil absorbed/g sample and b) ml oil absorbed/g protein. Tests were performed at least in duplicate.

3.10 DIFFERENTIAL SCANNING CALORIMETRY.

Differential scanning calorimetry was performed using a Perkin-Elmer DSC 2B as described by Wright et al. (1977). Samples were dialyzed in 50mM sodium phosphate, 10% (w/v) NaCl, 1mM DTT, 0.02% (w/v) sodium azide, pH 7, and centrifuged before analysis. Thermal

transitions were defined in terms of Tmax (peak temperature) and Tm (extrapolated onset temperature). Transition enthalpies were calculated from peak areas.

3.11 SCANNING ELECTRON MICROSCOPY.

Gel samples were prepared as for testing with the Instron Universal Testing Machine. Preparation of the gels was conducted by a modification of the method of Yasui et al. (1979). Gels were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7) for 48 h, post-fixed in OsO_4 for 5 h and dehydrated in an ethanol series. After dehydration they were transferred to 50 and 100% amylacetate and critical point dried. Gels were observed with a Philips 501B scanning electron microscope.

CHAPTER 4

RESULTS

4.1 AIR CLASSIFICATION AND ANALYSIS OF FLOURS.

Maris Bead and IVS-G flours were analysed for moisture, protein and starch contents, and the results are shown in Table 1. Moisture and protein contents were shown to be similar, but the starch content of IVS-G flour was lower than that of Maris Bead flour.

TABLE 1. Moisture, protein and starch contents of Maris Bead and IVS-G flours.

Sample	Moisture content %	Protein content %	Starch content %
Maris Bead			
Full-fat flour	11.5	31.9	42.6
Defatted flour	5.0	32.4	
IVS-G			
Full-fat flour	11.2	32.0	35.2
Defatted flour	5.2	33.2	

Protein and starch percentages were calculated on a dry weight basis.

Air classification of Maris Bead and IVS-G flours yielded the results shown in Table 2. At lower speeds IVS-G gave higher yields of coarse fraction than Maris Bead. Selected speeds were used to classify IVS-G flour subjected to a second pass through a Kolloplex pin mill. These flours gave higher yields of fines fraction compared to flour subjected to a single pass through the mill (Table 2a).

TABLE 2. Air classification of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Coarse Fraction Yield (%)		Fines Fraction Yield (%)	
	Maris Bead	IVS-G	Maris Bead	IVS-G
3	17.1	27.1	82.9	72.9
4	23.2	36.4	76.8	63.6
5	38.3	58.9	61.7	41.1
5.5	46.8	62.0	53.2	38.0
6		68.7		31.3
6.5	62.5	77.1	37.5	22.9
7	74.8	78.7	25.2	21.3
8	79.1	82.6	20.9	17.4
9	82.9	84.9	17.1	15.1
10	86.8		13.2	
11	88.8	88.1	11.2	11.9

TABLE 2a. Air classification of IVS-G flours subjected to a second pass through a Kolloplex mill.

Classifier Speed rev./min x 1000	Coarse Fraction Yield (%)	Fines Fraction Yield (%)
3	25.1	74.9
5.5	40.7	59.3
6.5	61.8	38.2
7	65.5	34.5

Each result was produced by a single air classification.

Percentage yield fines of air classified Maris Bead and IVS-G flours was plotted against cut point (in μm) and the results are shown in Figure 2. IVS-G flour gave lower yields of fines fraction than Maris Bead at all cut point values. However, IVS-G flour subjected to a second pass through the pin mill gave higher yields of fines fraction at low cut point values than Maris Bead subjected to a single pass through the pin mill. Cut point was obtained from a calibration

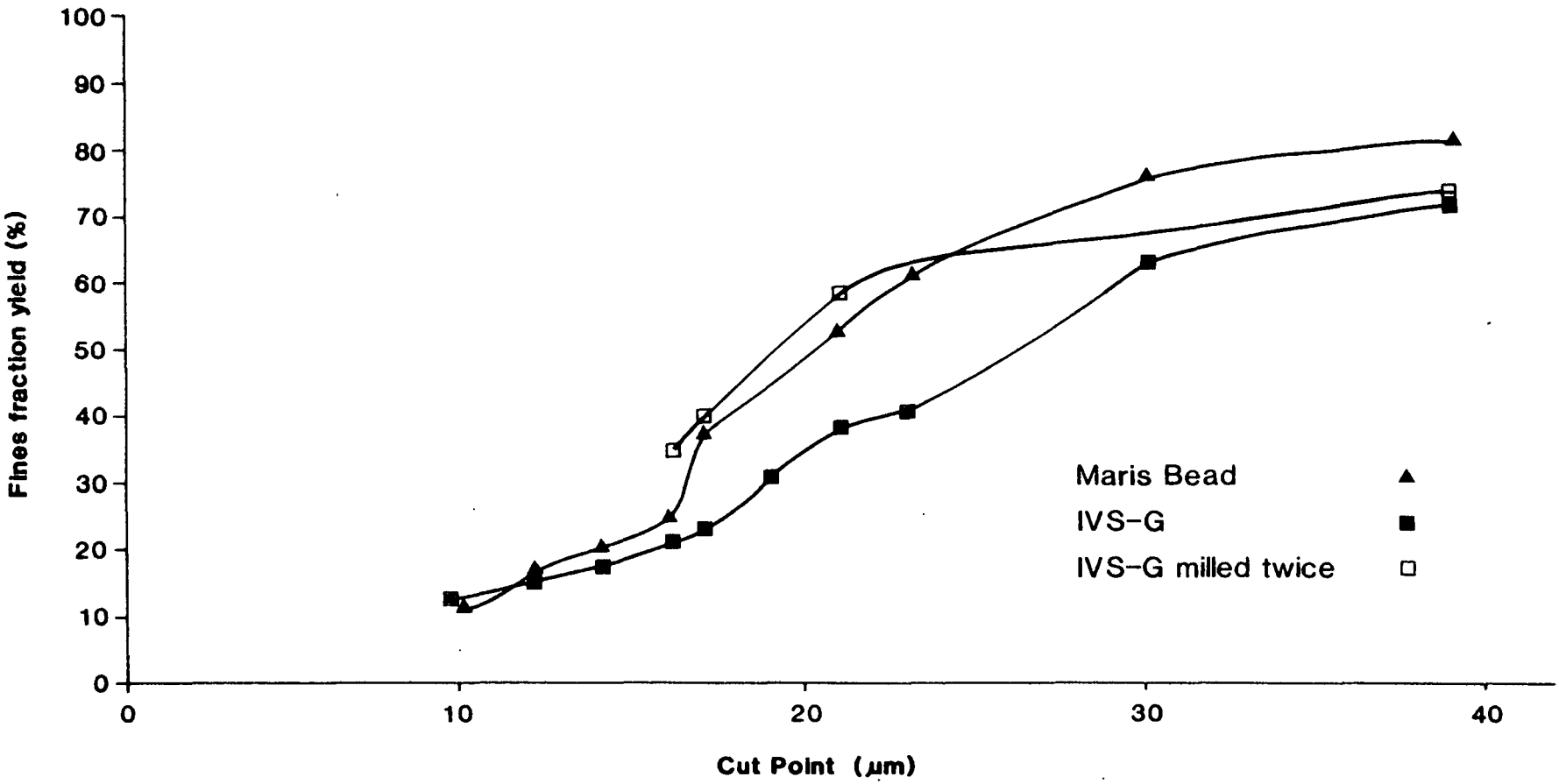
TABLE 3. Percentage protein of air classified fractions of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Protein (%)			
	Coarse		Fines	
	Maris Bead	IVS-G	Maris Bead	IVS-G
3	18.7	15.9	29.9	37.0
4	20.6	18.0	33.2	37.9
5	22.1	24.0	38.0	41.3
5.5	19.5	19.3	39.5	53.9
6		21.0		53.8
6.5	17.0	22.1	51.2	61.5
7	19.7	22.9	58.3	65.7
8	21.4	24.1	64.2	68.0
9	21.8	24.3	67.8	70.8
10	22.9		70.9	
11	23.1	25.9	75.1	74.5

TABLE 3a. Percentage protein of air classified fractions of IVS-G flours subjected to a second pass through a Kolloplex mill.

Classifier Speed rev./min x 1000	Protein (%)	
	Coarse	Fines
3	15.6	33.5
5.5	25.2	35.4
6.5	22.1	45.6
7	21.0	48.2

Figure 2. Relationship between cut point and percentage fines of air-classified fractions of Maris Bead and IVS-G flours.



graph of classifier speed versus cut point for the Alpine A100 MZR Zigzag air classifier (Wright et al., 1984).

Protein and starch percentages of the air classified fractions are shown in Tables 3 and 4 respectively. These results show that percentage protein in the fines fractions of IVS-G flour was generally greater at equivalent speeds than that of Maris Bead flour. Starch percentages of both coarse and fines fractions of IVS-G were lower than those of Maris Bead with the exception of the fines fractions obtained at 6500 and 7000 rev./min.

TABLE 4. Percentage starch of air classified fractions of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Starch (%)			
	Coarse		Fines	
	Maris Bead	IVS-G	Maris Bead	IVS-G
3	13.5	12.9	44.6	38.9
4	19.0		49.5	
5	34.8		45.4	
5.5	46.3	32.4	38.6	34.1
6.5	55.3	37.3	22.0	28.0
7	51.7	36.5	13.7	27.2
8	49.7		9.8	
9	47.8		6.3	
10	46.4		3.4	
11	46.7		2.5	

IVS-G flour was subjected to a second pass through a Kolloplex mill before air classification.

Protein yields (P.Y.) and protein separation efficiencies (P.S.E.) are shown in Tables 5 and 5a. P.Y. varied from 90 - 100%, but was generally higher for IVS-G. P.S.E. was generally higher for Maris Bead.

TABLE 5. Protein yields and protein separation efficiencies of air classified fractions of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Protein Yield		Protein Separation Efficiency	
	Maris Bead	IVS-G	Maris Bead	IVS-G
3	100	98	90	87
4	97	96	85	80
5	100	97	73	56
5.5	94	101	71	63
6		98		55
6.5	93.5	97	67	47
7	92	100	54	44
8	95	99	47	38
9	93	98	43	36
10	92		38	
11	91	99	36	29

TABLE 5a. Protein yields and protein separation efficiencies of air classified fractions of IVS-G flours subjected to a second pass through a Kolloplex mill.

Classifier Speed rev./min x 1000	Protein Yield	Protein Separation Efficiency
3	91	88
5.5	98	68
6.5	97	57
7	95	57

Starch yields (S.Y.) and starch separation efficiencies (S.S.E.) are shown in Table 6. S.Y. varied from 92 - 100%, but was generally higher than P.Y.. S.S.E. was greater than 90% at speeds above 6500 rev./min for Maris Bead.

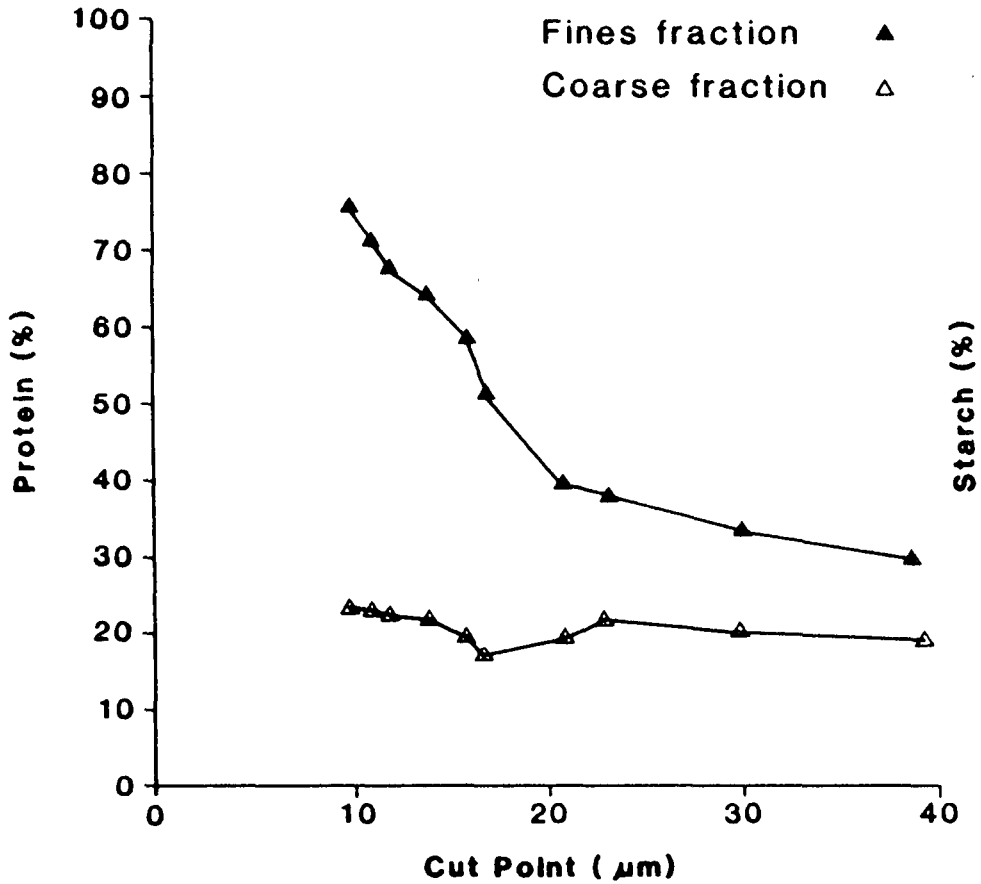
Protein and starch percentages of air classified fractions from Maris Bead and IVS-G flours were plotted against cut point in Figures 3 and 4 respectively. Protein percentage of the fines fraction decreased as cut point increased, but only small differences in protein percentage of the coarse fraction at different cut points were observed. Maris Bead and IVS-G gave similar results. Starch percentage

TABLE 6. Starch yields and starch separation efficiencies of air classified fractions of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Starch Yield		Starch Separation Efficiency	
	Maris Bead	IVS-G	Maris Bead	IVS-G
3	92	92	5	9
4	100			
5	97		31	
5.5	99	95	51	37
6.5	100	96	79	65
7	99	95	91	68
8	97		92	
9	96		93	
10	96		95	
11	98		98	

IVS-G flour was subjected to a second pass through a Kolloplex mill before air classification.

Figure 3. Relationship between cut point and a) percentage protein and b) percentage starch of air-classified fractions of Maris Bead flour.



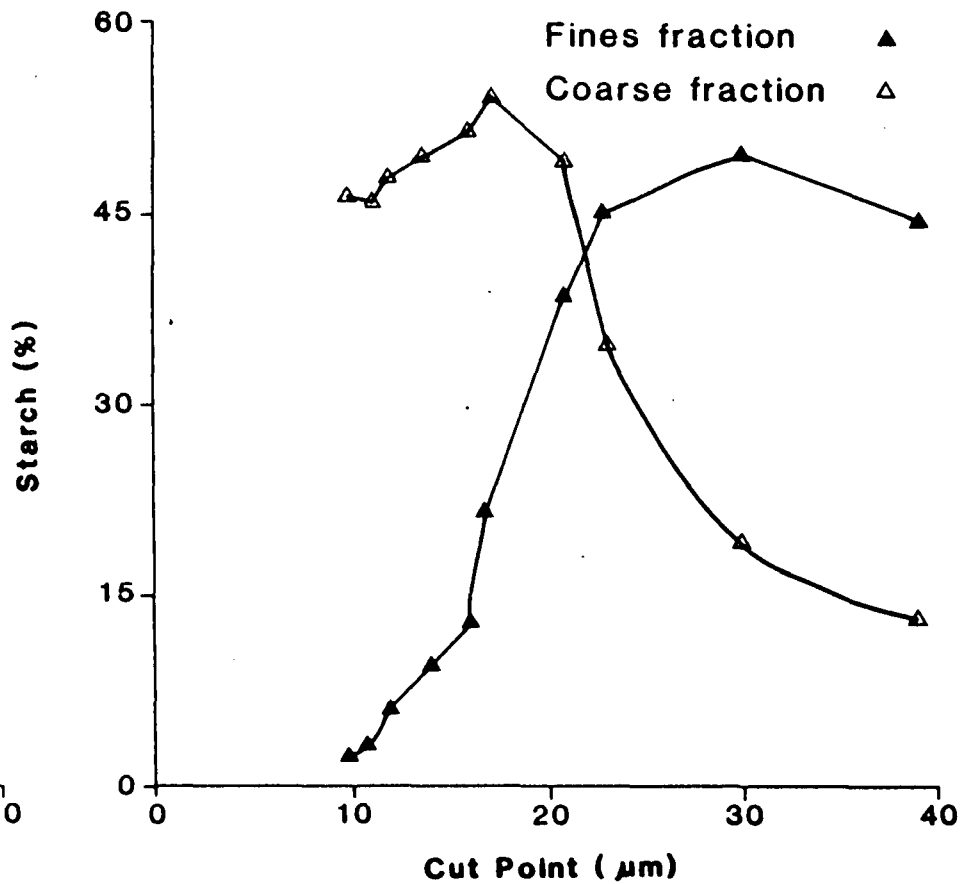
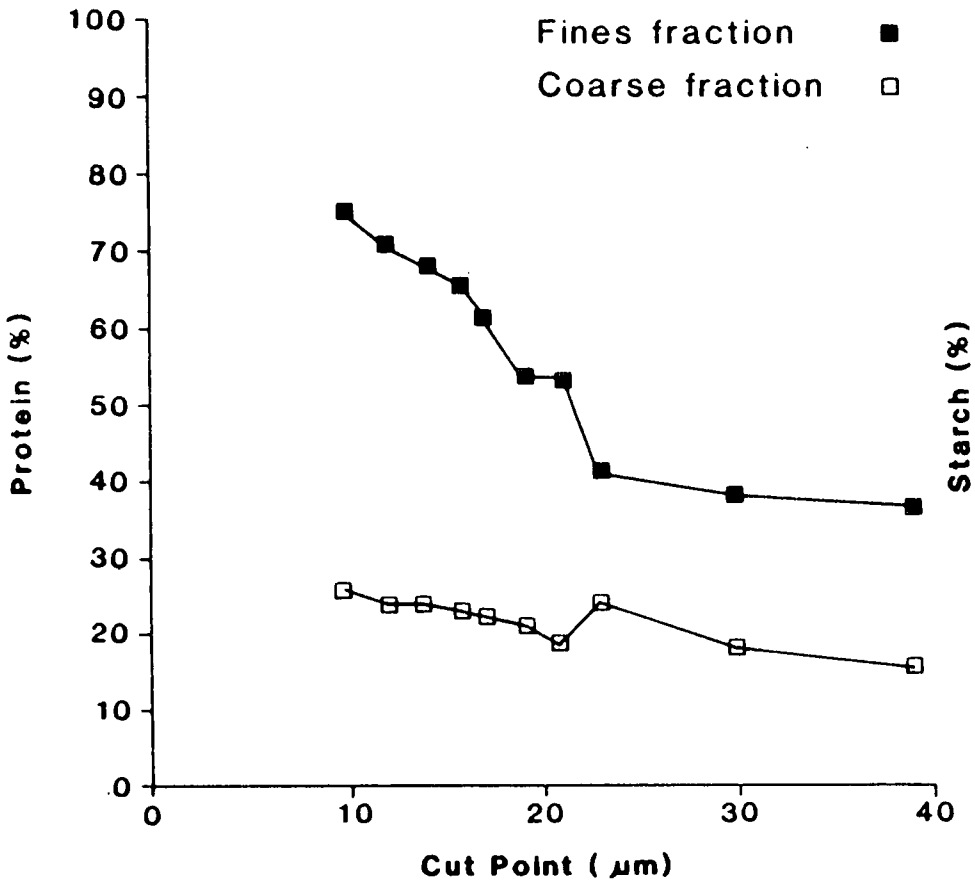
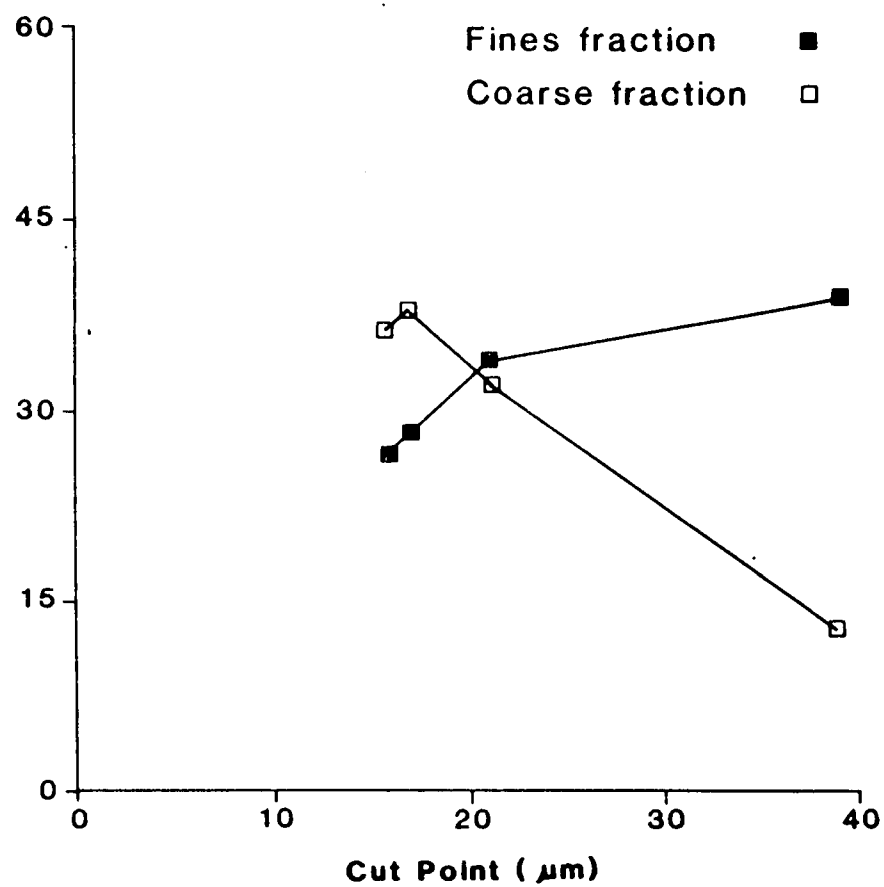


Figure 4. Relationship between cut point and a) percentage protein and b) percentage starch of air-classified fractions of IVS-G flour.





of the fines fraction increased with increasing cut point to a maximum at 30 μm for Maris Bead. Starch content of the coarse fraction of Maris Bead increased with increasing cut point to a maximum at 17 μm, and then decreased. Similar patterns were observed for IVS-G, but fewer data points were collected.

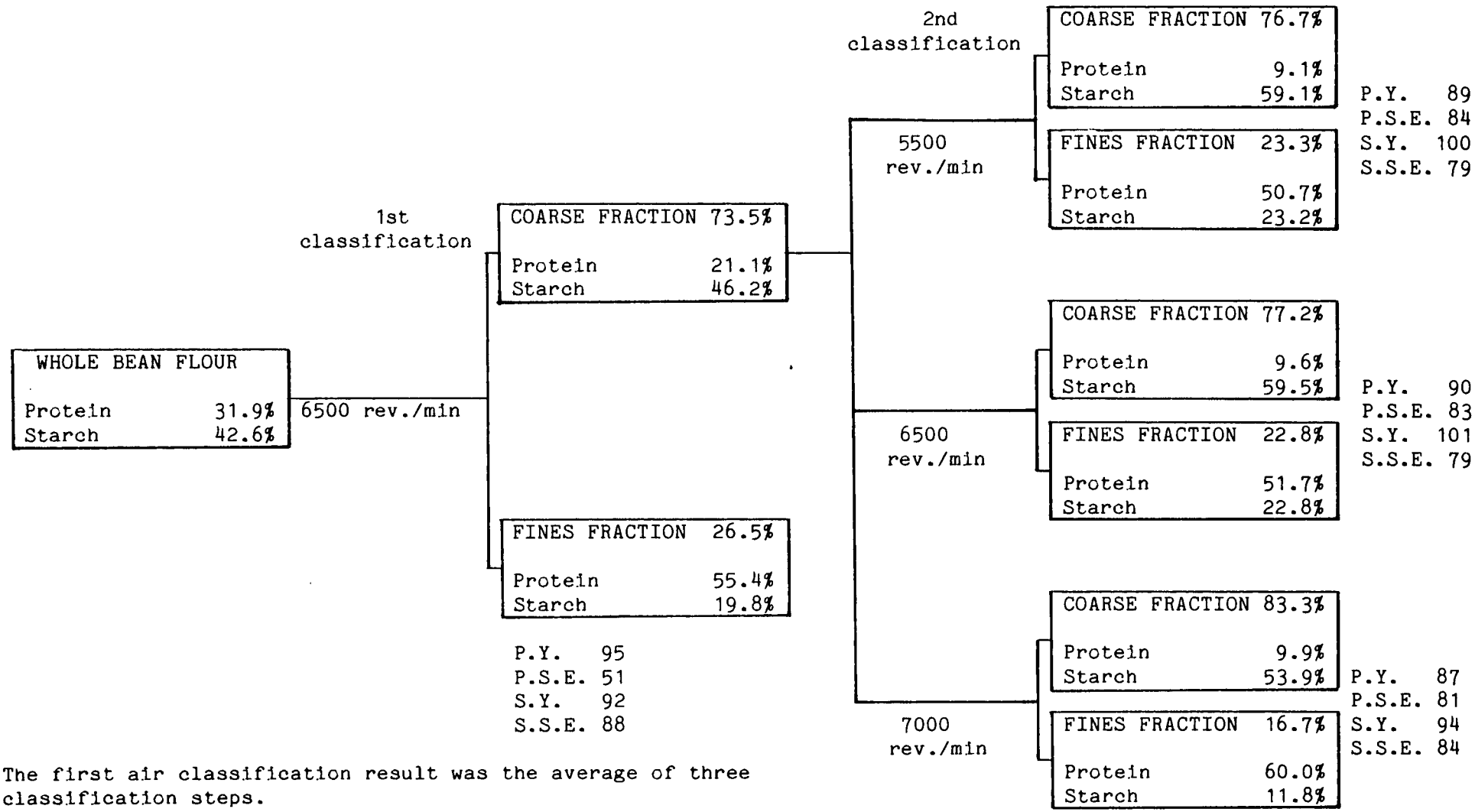
Reclassification of Maris Bead flour subjected to a second pass through a Kolloplex mill.

Results of the initial classification at 6500 rev./min and the reclassification of the coarse fraction are shown in Figure 5. Reclassification yielded coarse fractions with up to 59.5% starch and fines fractions with up to 60% protein.

Usually the initial and reclassified fines fractions are combined to give a protein concentrate. The remaining coarse fraction is a starch concentrate. The yields and compositions of the concentrates are shown in Table 7. Protein and starch concentrates with up to 57% protein and 73% starch respectively were produced. Low levels of protein remained in the starch fraction, however, approximately 20% starch remained in the protein concentrate. P.S.E. was approximately 70%, and S.S.E. was almost 80%.

Starch and protein concentrates produced at different reclassification speeds did not vary greatly in yield or composition. Generally starch content decreased and protein content increased in both starch and protein concentrates as the reclassification speed was increased.

FIGURE 5. Reclassification of the Maris Bead coarse fraction obtained at 6500 rev./min.



The first air classification result was the average of three classification steps.
The coarse fraction was remilled prior to the second air classification step.

TABLE 7. Yield and content of protein and starch concentrates produced by reclassification of the coarse fraction obtained at 6500 rev./min.

	Reclassification speed rev./min x 1000		
	5.5	6.5	7.0
PROTEIN CONCENTRATE			
Yield %	43.6	43.3	38.8
Protein %	53.6	53.9	56.9
Starch %	21.1	21.0	17.3
STARCH CONCENTRATE			
Yield %	56.4	56.7	61.2
Protein %	9.1	9.6	9.9
Starch %	59.1	59.5	53.9
P.S.E.	73.2	72.9	69.2
S.S.E.	78.2	79.5	77.4

Sequential classification of Maris Bead and IVS-G flours.

Maris Bead and IVS-G flours were classified sequentially and the results are shown in Table 8. Coarse percentage at speeds greater than 4000 rev./min was 85 - 97%. However, at 4000 and 3000 rev./min it was approximately 50%. Maris Bead and IVS-G gave similar results. Protein percentages of the air classified fractions are shown in Table 9. Protein percentage generally decreased with decreasing speed for both coarse and fines fractions. Protein percentage of the fines fraction decreased from 75% at 11000 rev./min to approximately 10% at

TABLE 8. Sequential classification of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	No. of samples	Average Coarse (%)		Average Fines (%)		(%) of Initial Flour: Average Coarse		Average Fines	
		Maris Bead	IVS-G	Maris Bead	IVS-G	Maris Bead	IVS-G	Maris Bead	IVS-G
11	3	86.1	84.4	13.9	15.6	86.1	84.4	13.9	15.6
9	2	94.9	95.6	5.1	4.4	81.7	80.7	4.4	3.7
8	2	97.1	97.8	2.9	2.2	79.3	78.9	2.4	1.8
7	2	95.7	96.0	4.3	4.0	75.9	75.7	3.4	3.2
6.5	2	95.3	96.3	4.7	3.7	72.3	72.9	3.6	2.8
6	2	94.9	92.8	5.1	7.2	68.6	67.7	3.7	5.2
5.5	2	89.9	89.9	10.1	10.2	61.7	60.8	6.9	6.9
5	1	86.2	86.8	13.8	13.2	53.2	52.8	8.5	8.0
4	1	54.4	55.7	45.6	44.4	28.9	29.4	24.3	23.4
3	1	47.0	59.5	53.0	40.5	13.6	17.5	15.3	11.9

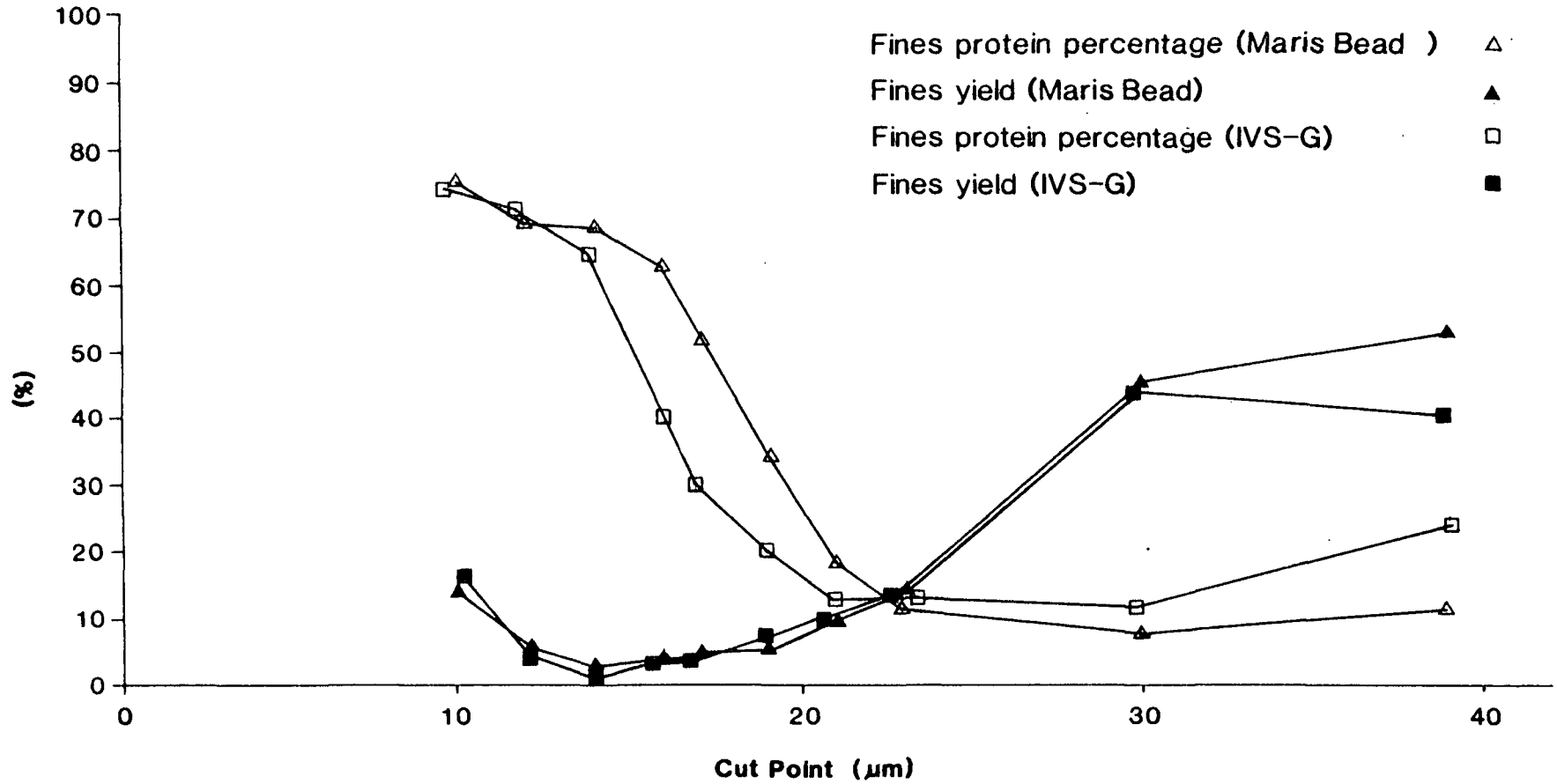
TABLE 9. Percentage protein in sequentially classified fractions of Maris Bead and IVS-G flours.

Classifier Speed rev./min x 1000	Protein (%)			
	Coarse		Fines	
	Maris Bead	IVS-G	Maris Bead	IVS-G
11	21.6	19.9	75.1	74.6
9	19.3	18.9	69.3	71.0
8	18.0	17.1	68.9	63.8
7	14.3	15.9	62.7	44.3
6.5	12.9	14.2	51.5	30.5
6	11.3	14.9	34.3	20.1
5.5	10.7	13.5	18.1	13.5
5	15.8	16.1	11.3	13.3
4	10.2	16.9	7.6	12.0
3	6.9	9.1	11.7	24.3

4000 rev./min for both Maris Bead and IVS-G. IVS-G gave generally lower protein percentages in the fines fractions than Maris Bead, however, at 3000 rev./min a relatively high protein percentage was observed for IVS-G.

Fines yield (%) and protein in the fines fraction (%) were plotted against cut point in Figure 6. Fines yield was less than 16% at cut points below 30 μm, but protein content increased with decreasing cut point from a minimum of approximately 10% at 30 μm to 75% at 10 μm. Maris Bead and IVS-G gave similar results.

Figure 6. Relationship between cut point and a) fines yield and b) fines protein percentage of sequentially air-classified fractions from Maris Bead and IVS-G flours.



4.2 EVALUATION OF ISOLATES, PROTEINATES AND MICELLAR PROTEINS.

Isolates produced from Maris Bead and IVS-G flours were soft, creamy coloured powders. Proteinates were darker and more fibrous. The micellar proteins were creamy white powders of greater density than the isolates. Isolates, proteinates and micellar proteins were analysed by SDS-PAGE (Figure 7), and were shown to contain all the major protein bands present in the respective flours. Full-fat and defatted products were shown to have similar protein patterns on SDS-PAGE.

Homogeneity of isolates, proteinates and micellar proteins was tested by using a gel filtration column of Superose 6 (Figure 8). Elution profiles of Maris Bead 11S and 7S proteins are shown in Figure 11. The samples were shown to differ with respect to protein aggregation, as determined by the amount of high molecular weight material (retention time of approximately 20 min), and the amount of low molecular weight material (retention time greater than 50 min). The defatted isolate and micellar proteins had the largest and smallest peaks designated to contain material of an approximate Mr. of 3000 x 10^3 respectively. Isolates and proteinates both showed large peaks of material with a Mr. of < 5000, but only small corresponding peaks were observed for the micellar proteins.

Protein contents of isolates, proteinates and micellar proteins are shown in Table 10. Full-fat samples had protein contents 2 - 4% lower than defatted samples. Micellar proteins had the highest protein content, and proteinates the lowest protein content. Using a nitrogen to protein conversion factor of 6.25 gives very high values of protein percentage as it is an average value for all proteins. A factor of 5.7 is generally used for Vicia faba proteins, and gives

Figure 7. SDS-PAGE of proteins extracted from flours, isolates, proteinates and micellar proteins.

Track	Sample
1	Standards
2	IVS-G flour
3	Maris Bead proteinate
4	Maris Bead isolate
5	Maris Bead micellar protein
6	Maris Bead flour

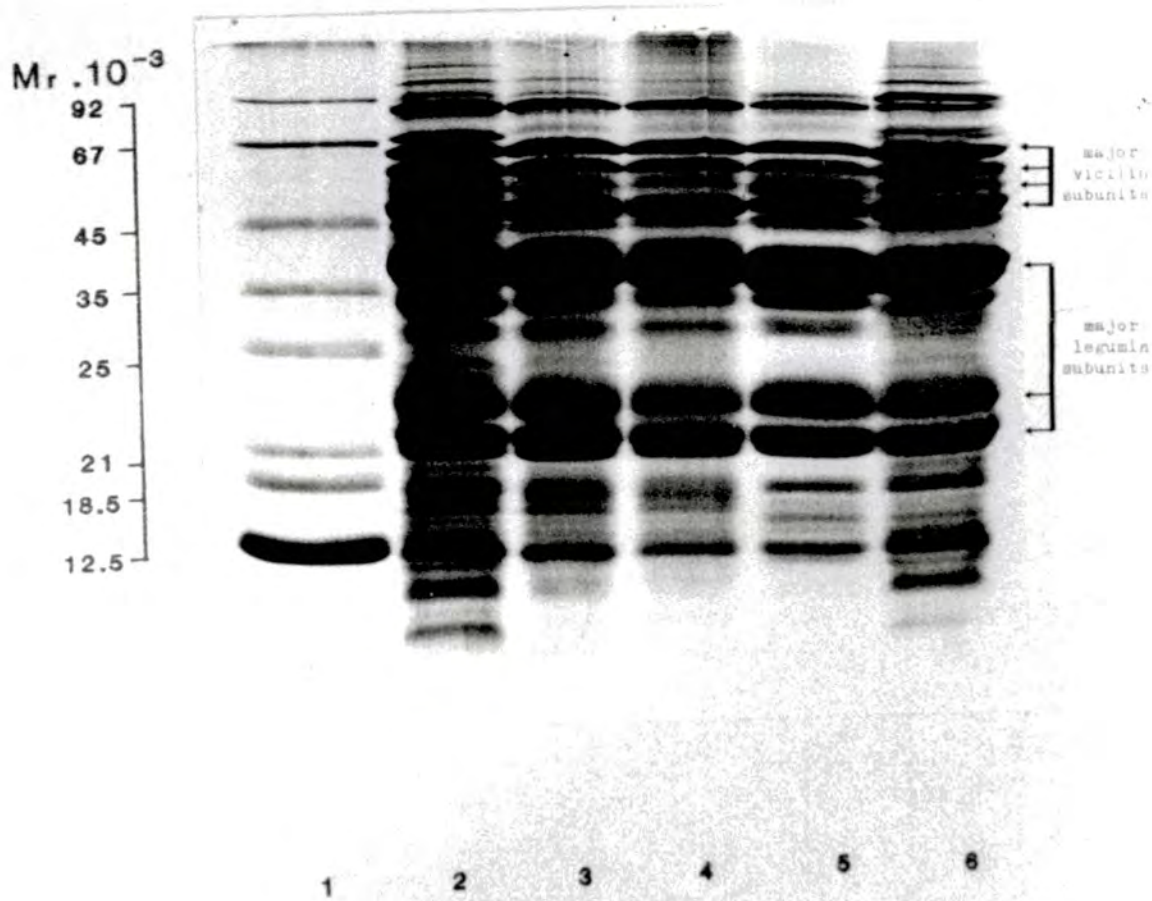
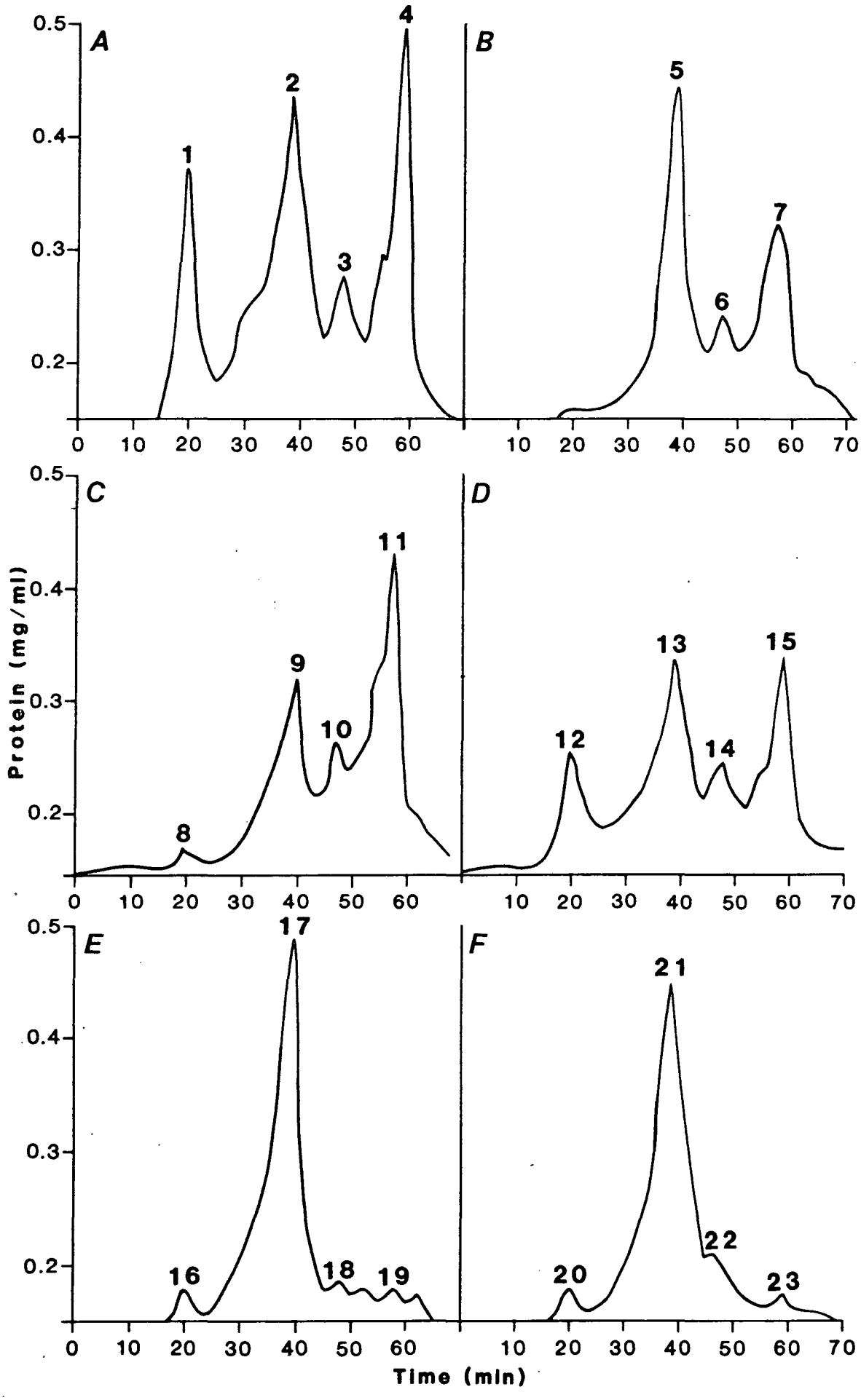


Figure 8. Elution profiles of isolates, proteinates, and micellar proteins from Maris Bead after gel filtration chromatography on Superose 6.



SAMPLE

A. Defatted isolate
B. Full-fat isolate
C. Defatted proteinate
D. Full-fat proteinate
E. Defatted micellar protein
F. Full-fat micellar protein

From a calibration graph the peaks were designated to contain material of molecular weights as indicated (Lambert, 1986).

Peak No.	Retention Time (min)	Mr. x 10^{-3}
1	20	3000
2	39	200
3	47	16
4	59	<5
5	39	200
6	47	16
7	57	<5
8	20	3000
9	40	150
10	47	16
11	57	<5
12	20	3000
13	39	200
14	48	15
15	58	<5
16	20	3000
17	39	200
18	48	15
19	58	<5
20	20	3000
21	38	350
22	47	16
23	58	<5

Loading was 1 mg in 100 µl.
Micellar proteins were produced by ultrafiltration.

TABLE 10. Protein contents of isolates, proteinates and micellar proteins.

Sample	Protein (%) N x 6.25
Maris Bead	
Full-fat proteinate	86.6
Defatted proteinate	90.6
Full-fat isolate	89.8
Defatted isolate	94.2
Full-fat micellar protein	105.8
Defatted micellar protein	107.8
IVS-G	
Full-fat proteinate	89.7
Defatted proteinate	91.6
Full-fat isolate	93.2
Defatted isolate	96.9
Defatted micellar protein	107.8

average protein contents of 83.1%, 87.1% and 98.3% for the defatted proteinates, isolates and micellar proteins respectively. However, to facilitate comparison with the literature a value of 6.25 has been used.

4.3 PURIFICATION AND ANALYSIS OF PROTEINS.

Separation of globulins on HA-Ultrogel yielded the elution profile shown in Figure 9. Legumin was represented by the first, larger peak and vicilin by the second and broader peak. A region where both proteins occurred was found between. The fractions were analysed by SDS-PAGE (Figure 10). Major protein bands were observed

Figure 9. Elution profile of crude globulins from Maris Bead on HA-Ultrogel.

Elution was performed using a linear concentration gradient of potassium phosphate buffer, pH 7.8 (50mM - 0.75M, 500 ml + 500 ml).

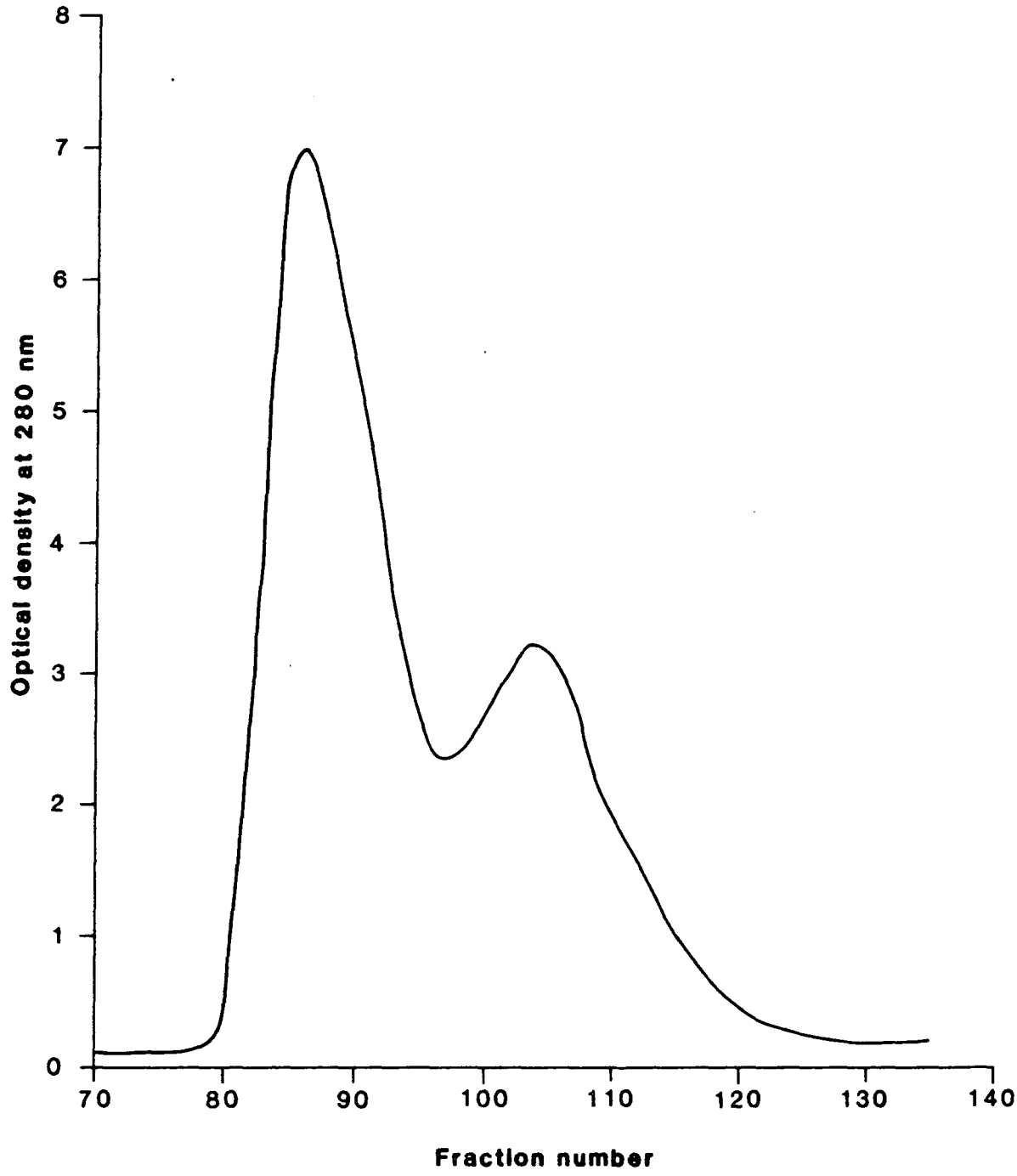
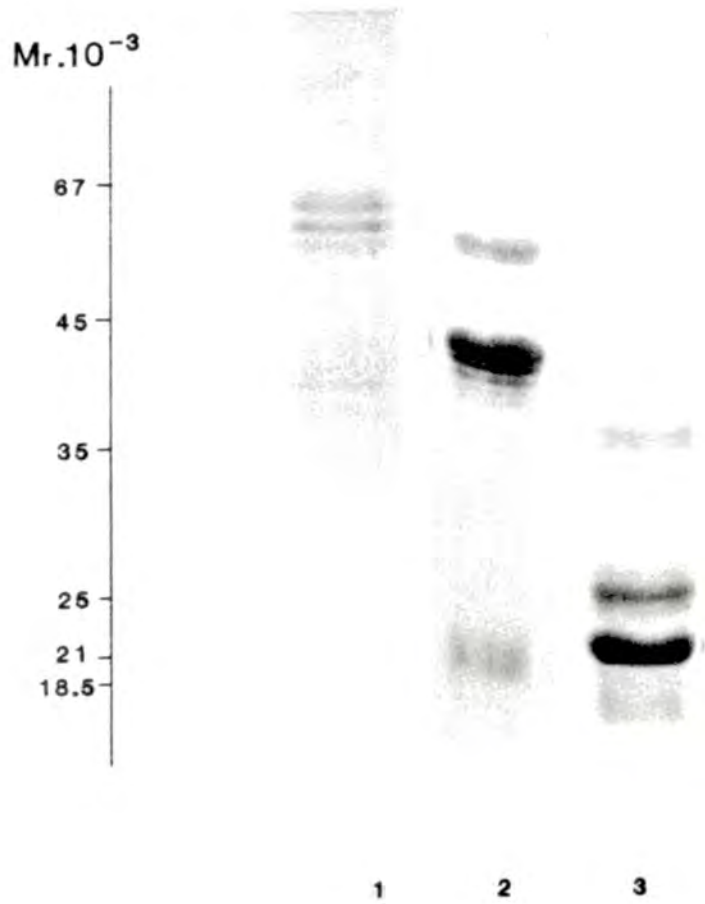


Figure 10. SDS-PAGE of legumin and vicilin from Maris Bead seeds.

Track	Sample
1	Vicilin
2	Legumin
3	Legumin (+ mercaptoethanol)



corresponding to molecular weights of 56 000 and 40 000 on non-reduced gels and 36 000, 23 000 and 21 000 on reduced gels for 11S proteins, and 66 000, 60 000, 55 000 and 38 000 for 7S proteins. Maris Bead and IVS-G proteins gave similar patterns on SDS-PAGE. Fractions showing legumin without contaminating vicilin, or vice versa, were kept for subsequent experiments. The proteins were tested for homogeneity on a gel filtration column of Superose 6 and using an analytical scanning ultracentrifuge (Figures 11 and 12 respectively). On Superose 6 vicilin gave a single peak with a retention time of 42 min. It was designated to contain material of an approximate Mr. of 100 x 10^3 . Legumin yielded three peaks designated to contain material of approximate Mr. values of 3 million, 600 000 and 300 000. The former peak was larger if 11S proteins were dialyzed against distilled water than if they were dialyzed against ammonium hydrogen carbonate. Using the analytical ultracentrifuge the 11S protein yielded a small peak with an Sobs value of 14 - 16 and a larger peak. Vicilin gave a single, broad peak. S^0_{20},w values are shown in Table 11.

TABLE 11. Sedimentation coefficients of 11S and 7S proteins.

Sample	S^0_{20},w	
	Maris Bead	IVS-G
11 S	11.3	11.7
7 S	7.4	7.3

SAMPLE

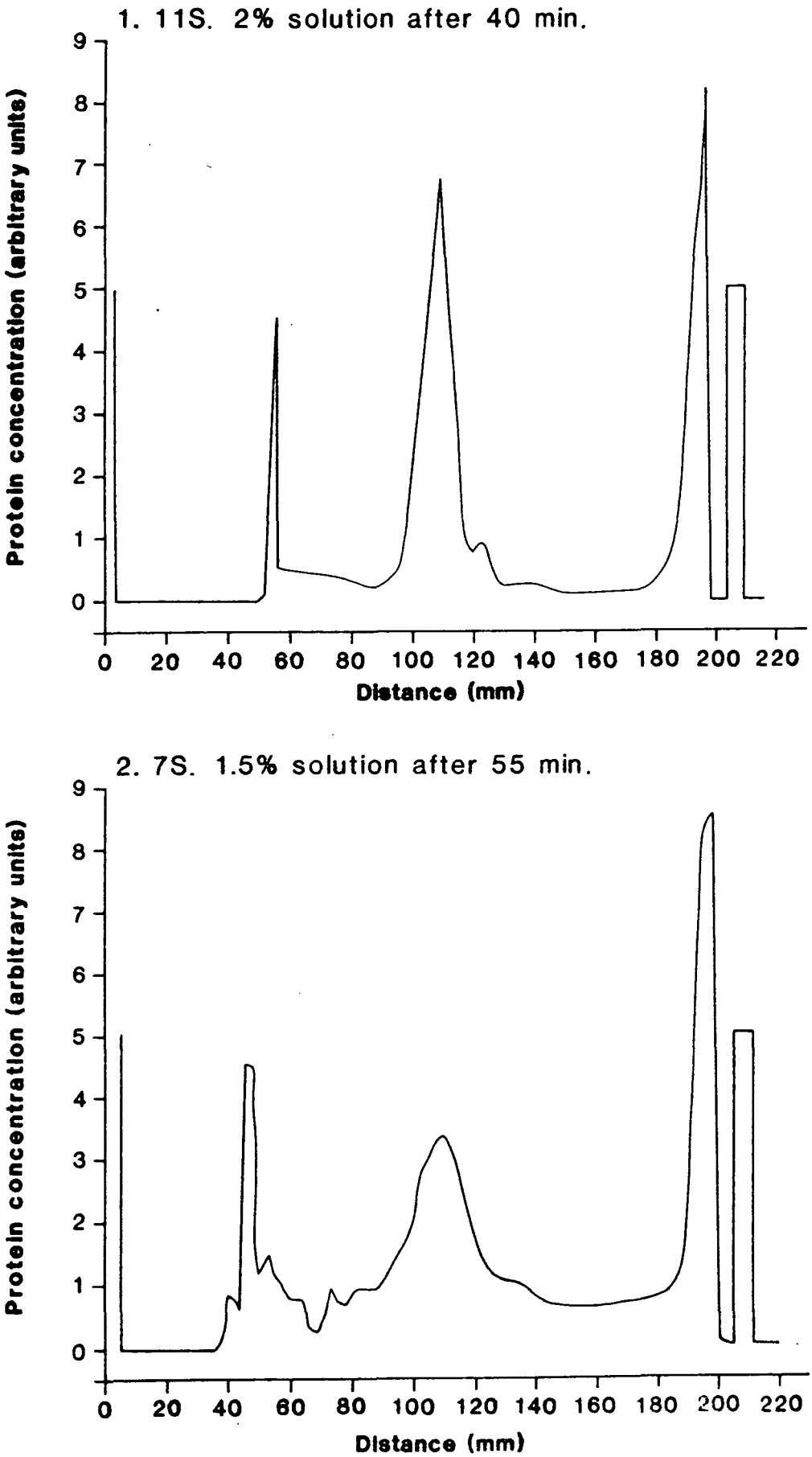
A. Maris Bead vicilin

B. Maris Bead legumin - dialyzed against distilled water

C. Maris Bead legumin - dialyzed against ammonium hydrogen carbonate

Peak No.	Retention Time (min)	Mr. x 10^{-3}
1	42	100
2	20	3000
3	34	600
4	38	300
5	20	3000
6	34	600
7	38	300

Figure 12. Analytical scanning ultracentrifugation of 11S and 7S proteins from Maris Bead.



4.4 DISPERSIBILITY AND GELLING CHARACTERISTICS OF ISOLATES, PROTEINATES AND MICELLAR PROTEINS.

Dispersibility and gelling characteristics of Maris Bead and IVS-G proteinates and micellar proteins with respect to pH are shown in Figures 13 - 18. Protein solubility and turbidity of the same samples are shown in Figures 19 - 22. Sediment volume was used as a measure of dispersibility, and non-dispersed material was assumed to be composed of protein aggregates.

Sediment volumes of proteinates were generally highest at pH 3.5 and 6, and lowest at pH values of <3 and >6. The full-fat Maris Bead proteinate did not give a peak at pH 6 but as fewer data points were collected this may have been missed. Micellar proteins gave similar results but the volumes of sediment were lower. This did not reflect a greater dispersibility, as can be seen from the protein solubility graphs for defatted IVS-G proteinate and micellar protein (Figures 19 and 22). The proteinate had approximately 10% more protein in solution despite having an original protein content of 15% less than the micellar protein. Turbidity was also lower for the micellar protein. Volume of sediment can not be taken as an absolute measure of dispersibility as it is affected by the nature of the sample and pH value. These results show sediment volume is negatively correlated with protein content of the supernatant and turbidity over a pH range for each sample tested. It is, therefore, a quick and useful assessment of dispersibility of a particular sample at various pH values, but of less use for comparing dissimilar samples.

Gels were formed from 10% solutions of defatted and full-fat proteinates at pH values of 2 - 3.5 and 6 - 7, and at 3.5 - 4 and >7 for micellar proteins. The 20% defatted IVS-G proteinate gelled over a

Figure 13. Dispersibility and gelling characteristics of defatted Maris Bead proteinate (10% solution).

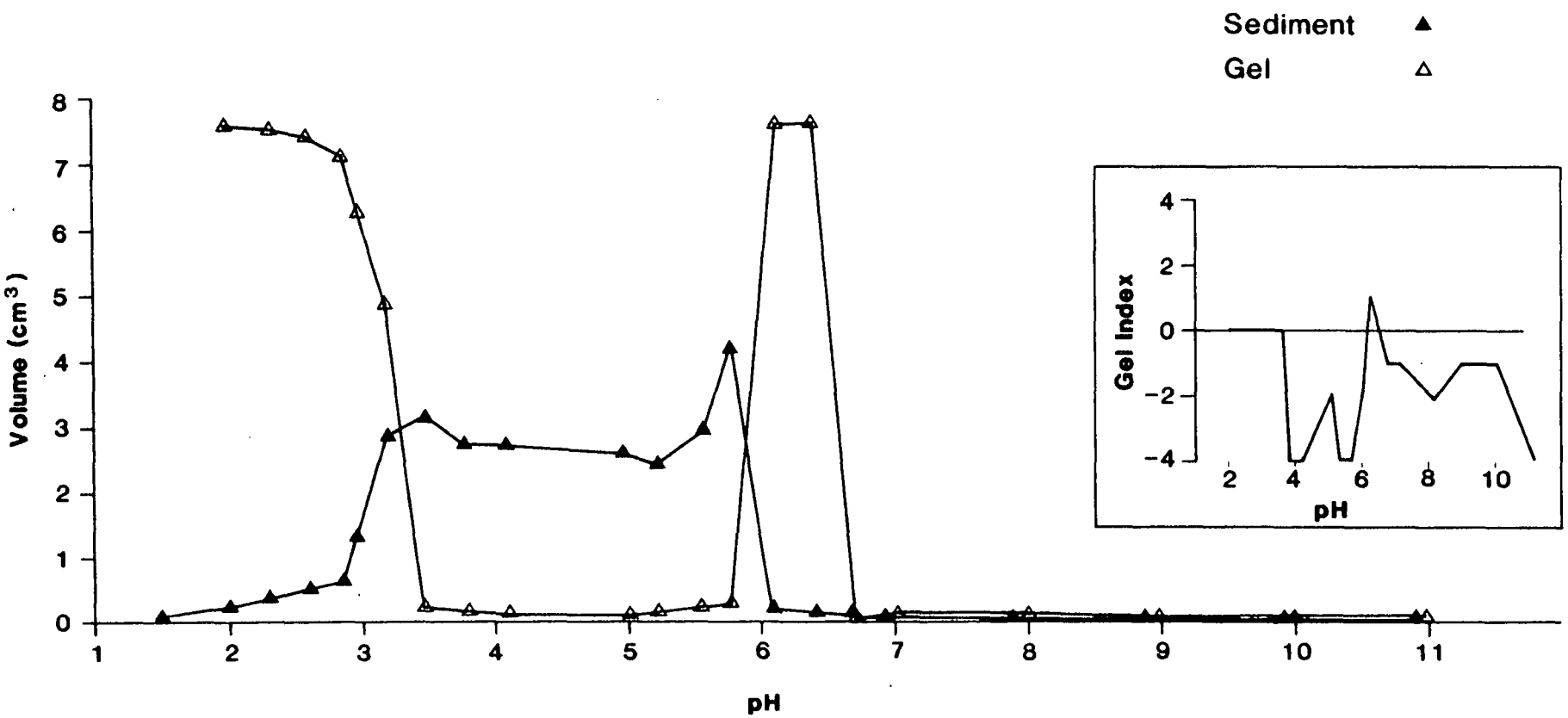


Figure 14. Dispersibility and gelling characteristics of defatted IVS-G proteinate (10% solution).

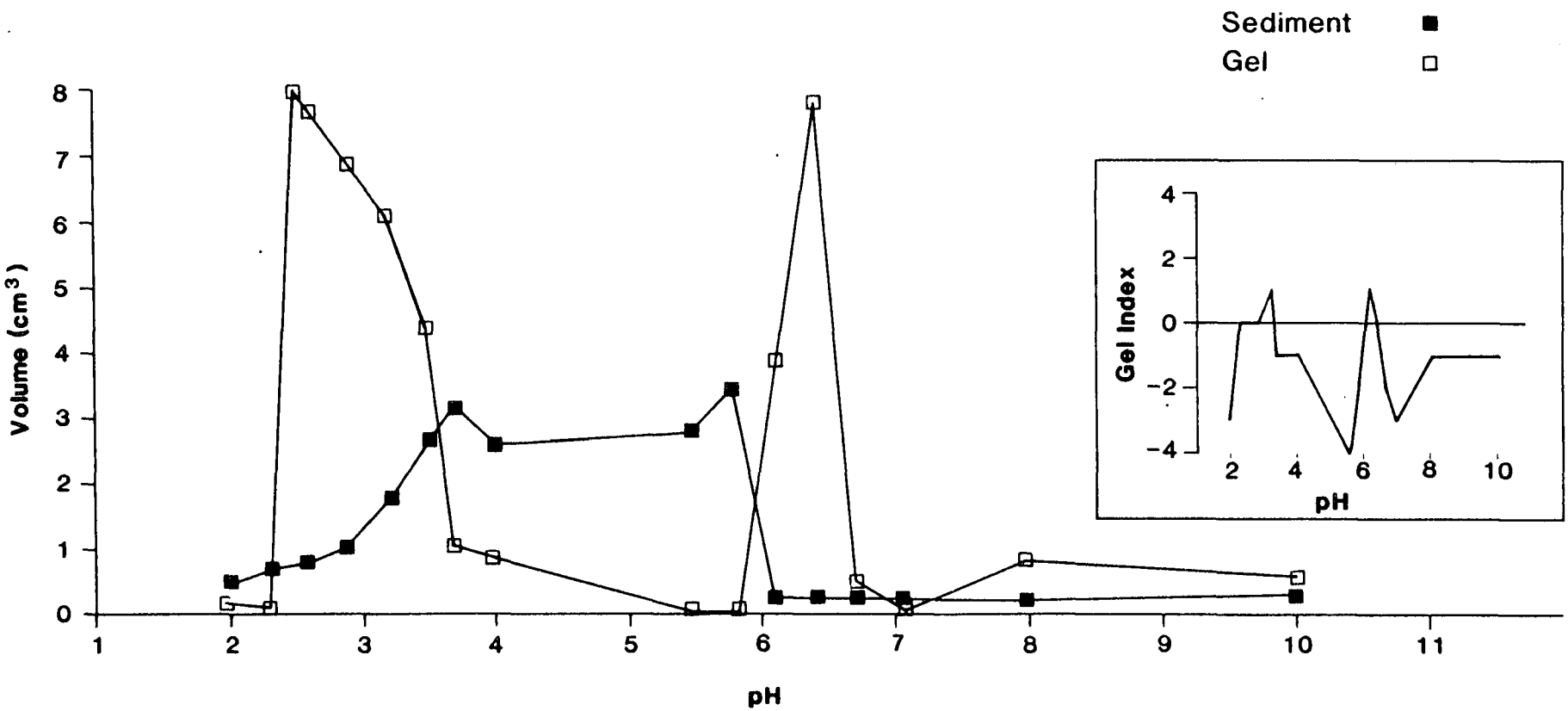


Figure 15. Dispersibility and gelling characteristics of defatted IVS-G proteinate (20% solution).

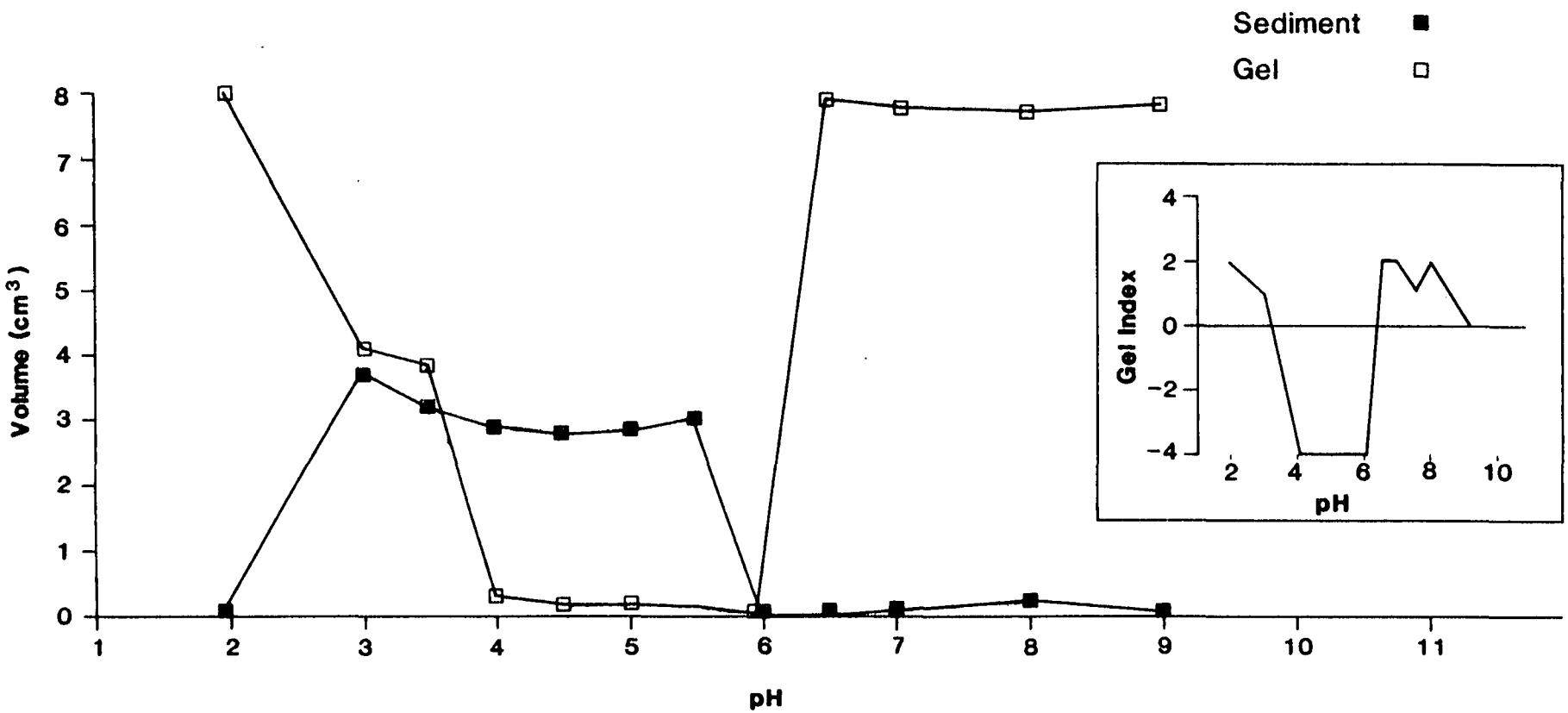


Figure 16. Dispersibility and gelling characteristics of full-fat Maris Bead proteinate (10% solution).

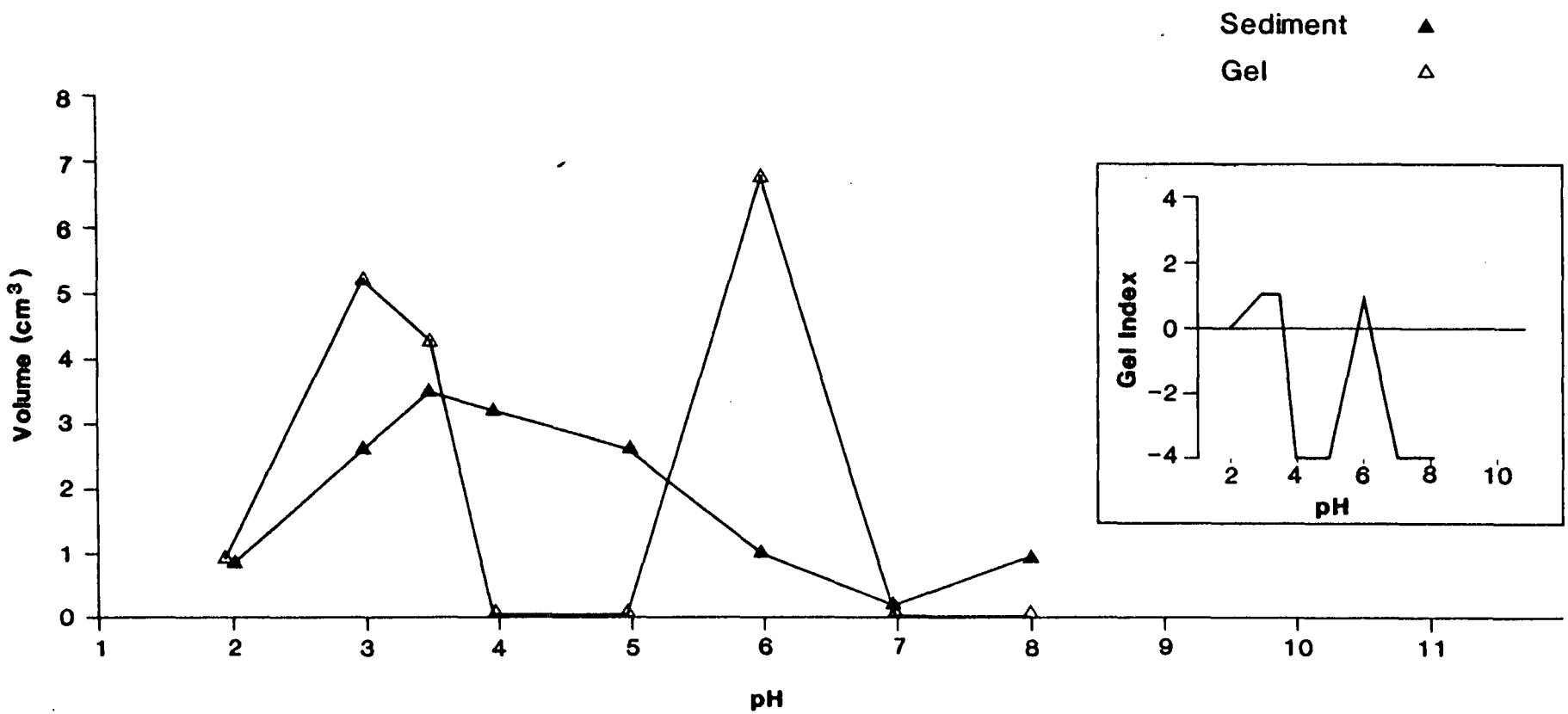


Figure 17. Dispersibility and gelling characteristics of defatted Maris Bead micellar protein (10% solution).

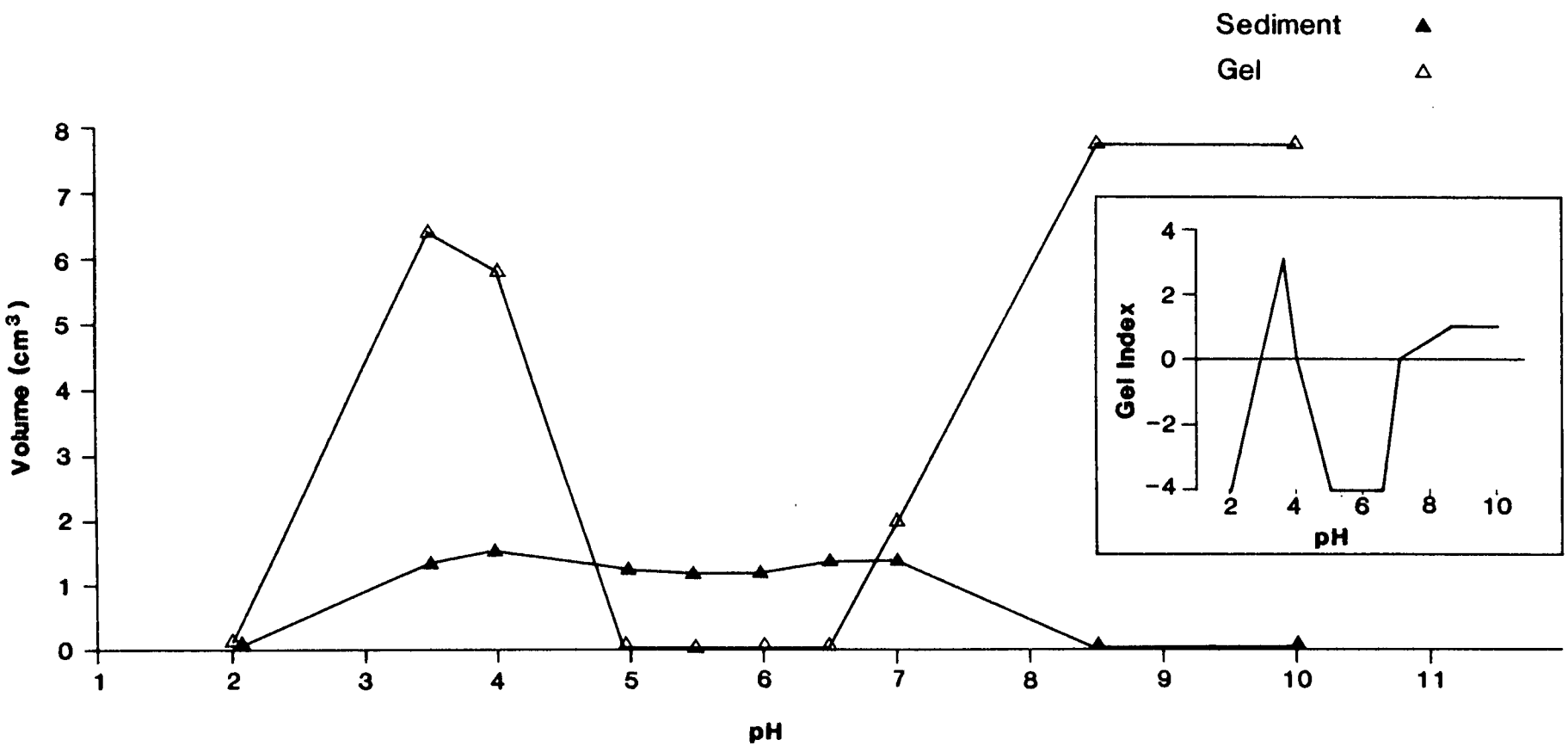


Figure 18. Dispersibility and gelling characteristics of defatted IVS-G micellar protein (10% solution).

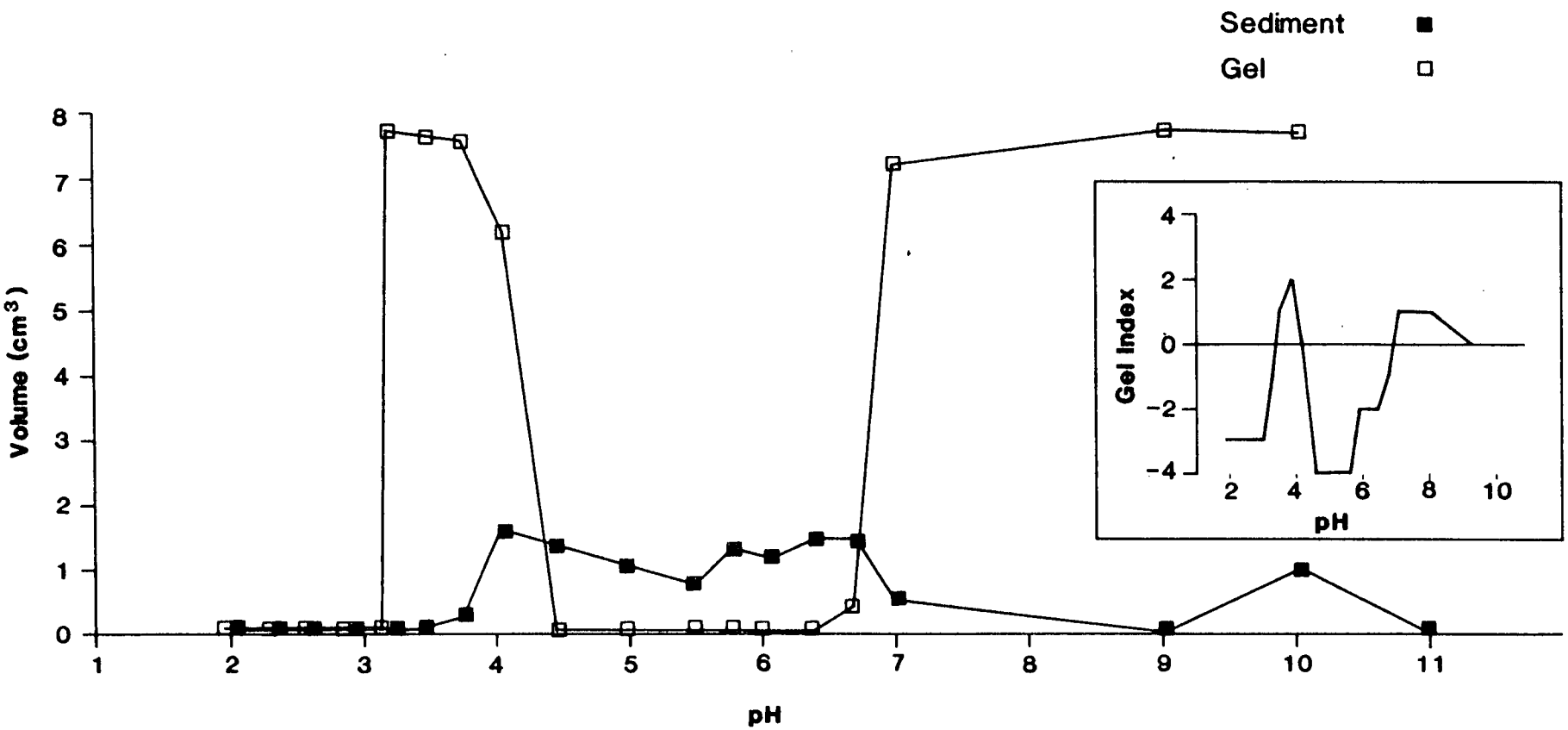


Figure 19. Protein solubility of defatted IVS-G proteinate (10% solution).

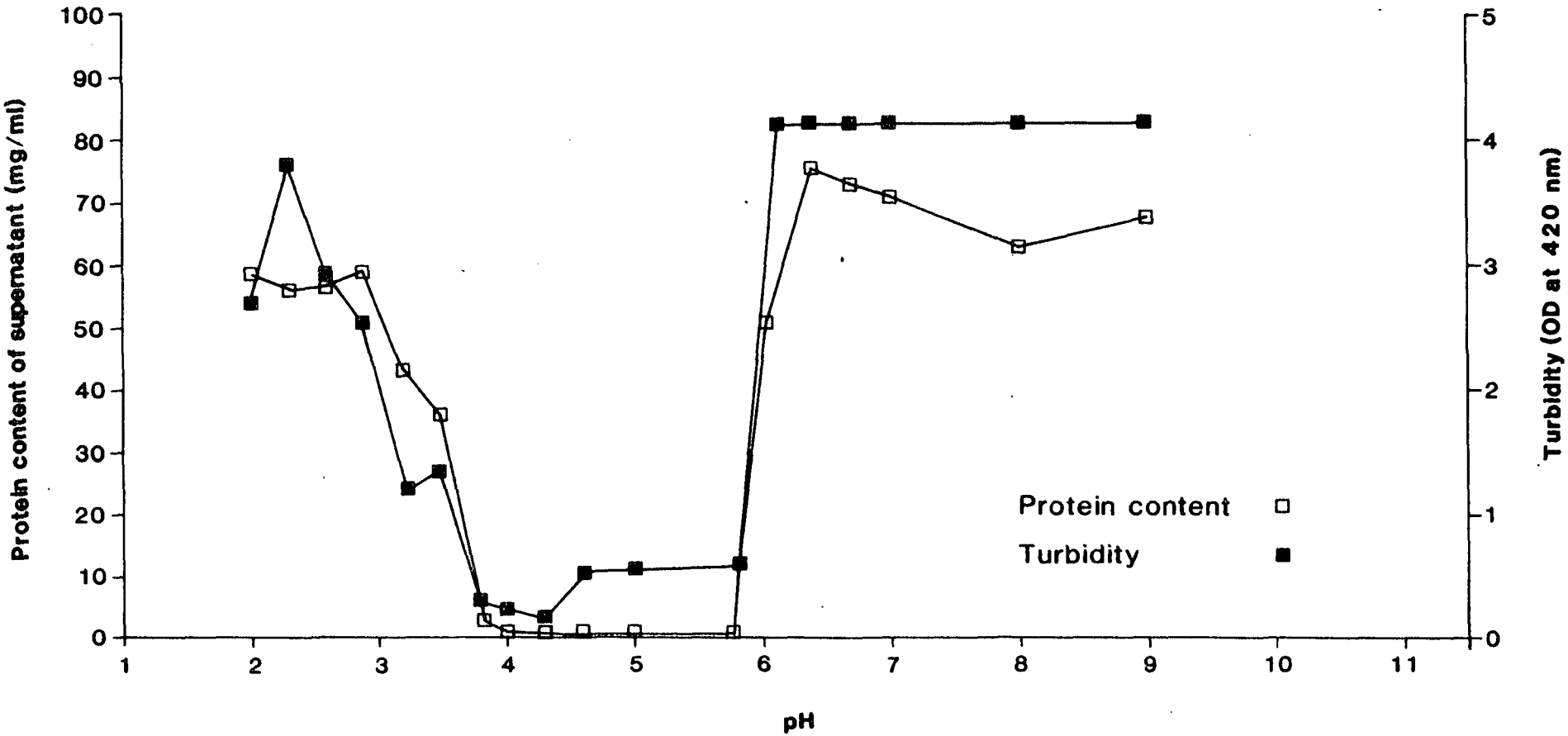


Figure 20. Protein solubility of full-fat Maris Bead proteinate (10% solution).

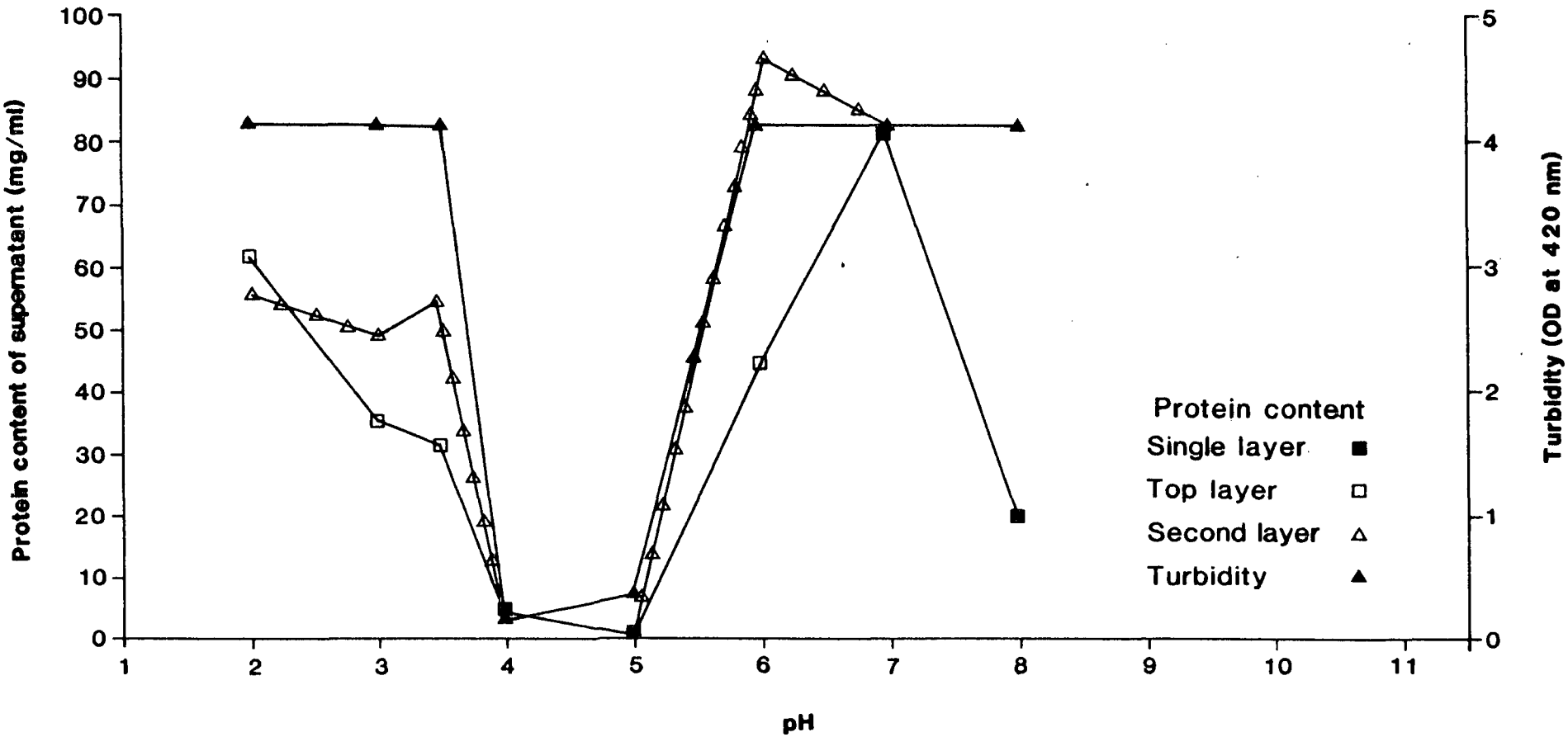


Figure 21. Protein solubility of defatted Maris Bead micellar protein (10% solution).

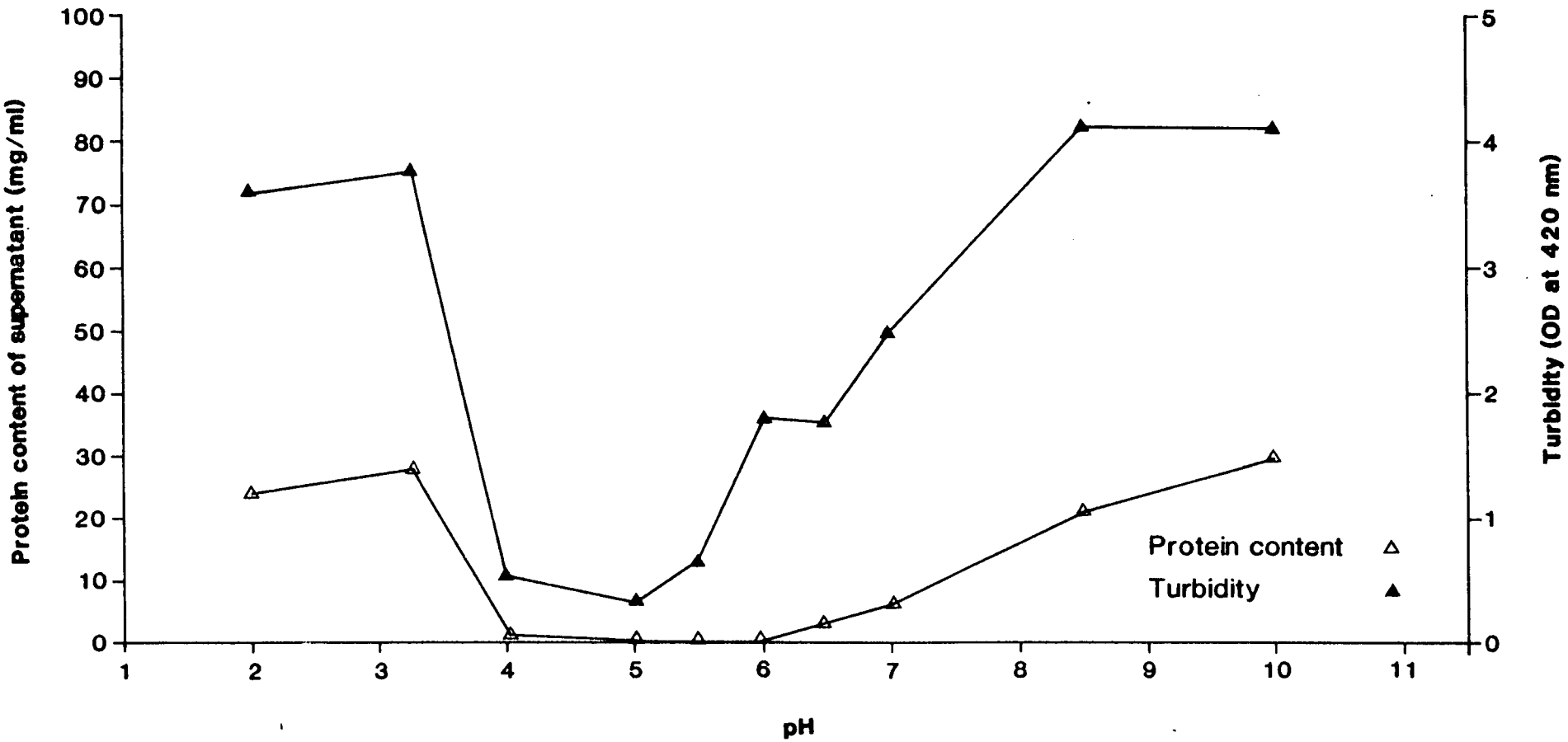
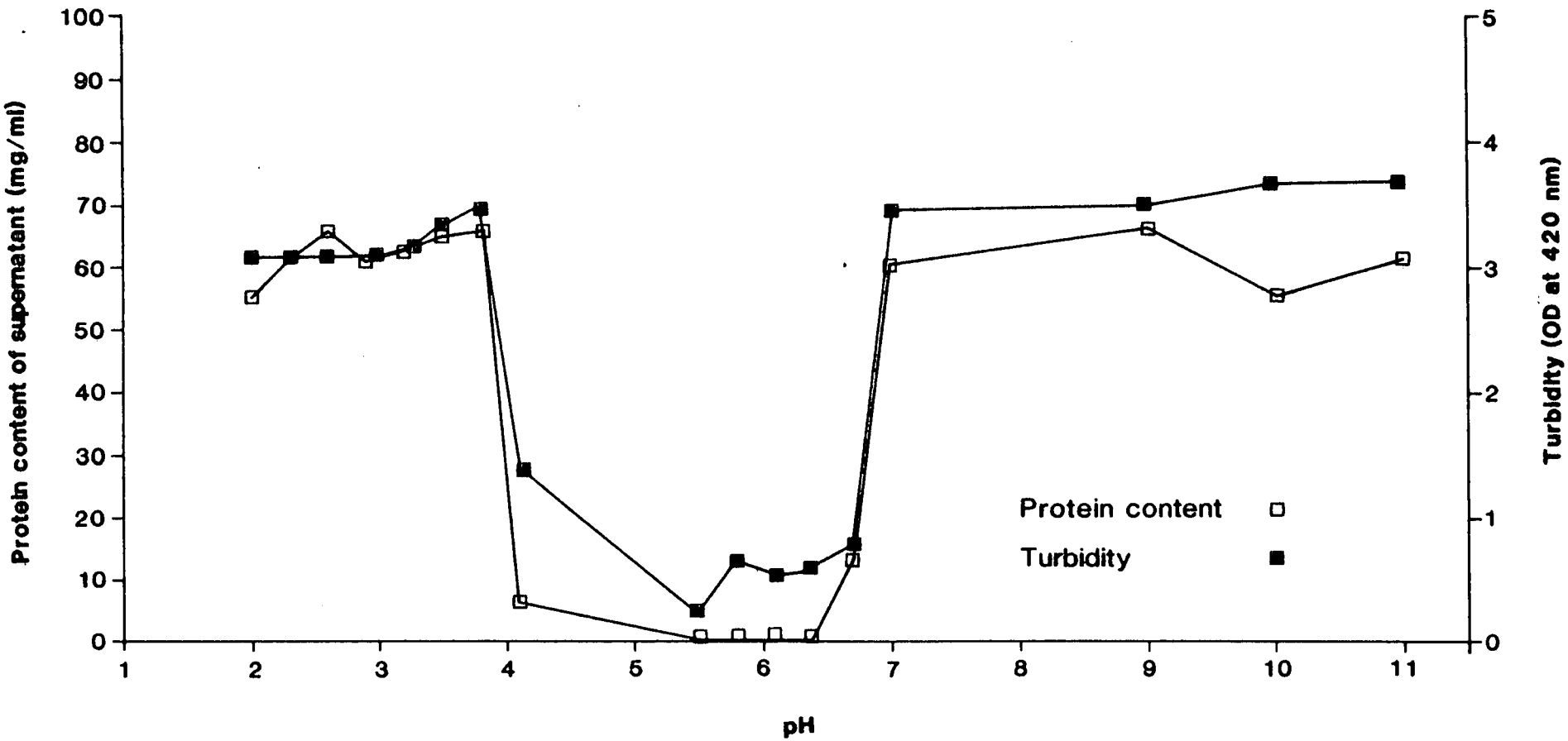


Figure 22. Protein solubility of defatted IVS-G micellar protein (10% solution).



broader pH range, i.e. 2 - 3.5 and 6.5 - 9. Gel volume is also affected by the nature of the sample and pH. Structural differences were reflected in the varied physical appearance of gels with respect to pH. At pH values of 3.5 and 6 - 7 gels were generally opaque and pasty. At other pH values they were less granular and often translucent. The former attained higher values on the gel index.

Protein solubility and turbidity were generally well correlated (Figures 19 - 22), and negatively correlated with volume of sediment. They were high at pH values between 2 - 3.5 and >6. Protein contents at the above pH values were approximately 80% for proteinates, 65% for IVS-G micellar protein and <35% for Maris Bead micellar protein.

The full-fat Maris Bead proteinate behaved in a different manner to the other samples, forming three layers in the test-tube after the first centrifugation at some pH values. The bottom layer was a solid precipitate, the second layer an opaque colloid accounting for 50 - 75% of the total volume, and the third layer a clear solution accounting for 7 - 20% of the total volume. After heating at 95°C two distinct regions of gel were observed. The top layer was translucent whilst the bottom layer was opaque and very granular. They exhibited similar gel indices. Prior to gelling a sample of solution was taken from both layers and analysed for nitrogen content. Both values are plotted in Figure 20. The turbidity values refer only to the colloidal layer at those pH values where three layers were formed. A colloid was not formed at pH values of 4, 5, 7 or 8.

The effect of salt on the gel index, dispersibility and gelling characteristics of defatted Maris Bead proteinate (pH 7.7) is shown in Figure 23. Protein solubility and turbidity are plotted

against salt content in Figure 24. Sediment volume was low at all salt concentrations, but a small increase with increasing salt content was observed. Weak gels were formed at salt concentrations between 0.5 and 1.5%. Protein content of the supernatant generally decreased with increasing salt content. The supernatants were dark in colour at all pH values, which interfered with the turbidity readings. However, the supernatants were translucent at all salt concentrations with the exception of 1.5 and 1.75%.

Compression and stress relaxation properties of gels.

Values of G, n and rupture strength obtained from uniaxial compression experiments on 20% gels of Maris Bead and IVS-G proteinates, isolates and micellar proteins are shown in Table 12. Values of G ranged from 0.29 - 1.76 $kgmm^{-2}$ x 10^{-4}. Defatted proteinates (pH 7) generally gave a lower value of G than defatted micellar proteins (pH 7). G was large for samples gelled at pH 4. Values of n ranged from 0.13 - 1.50. IVS-G proteinates gave higher values of n than Maris Bead proteinates. At pH 4 lower values of n were observed compared to equivalent samples at pH 7. Rupture strength was greatest for the full-fat Maris Bead isolate (pH 4.6). Defatted IVS-G micellar protein and proteinate (pH 7) gave similar values, as did defatted Maris Bead micellar protein and proteinate (pH 7).

Protein contents of the 20% solutions after centrifuging and pH correction, but prior to gelling are shown in Table 13. Protein concentrations were 15 - 16% for proteinates, 11 - 12% for full-fat isolates and 8.5 - 12.5% for defatted micellar proteins. The samples prepared for scanning electron microscopy had lower protein contents.

Figure 23. Effect of salt on the dispersibility and gelling characteristics of defatted Maris Bead proteinate (10% solution at pH 7.7).

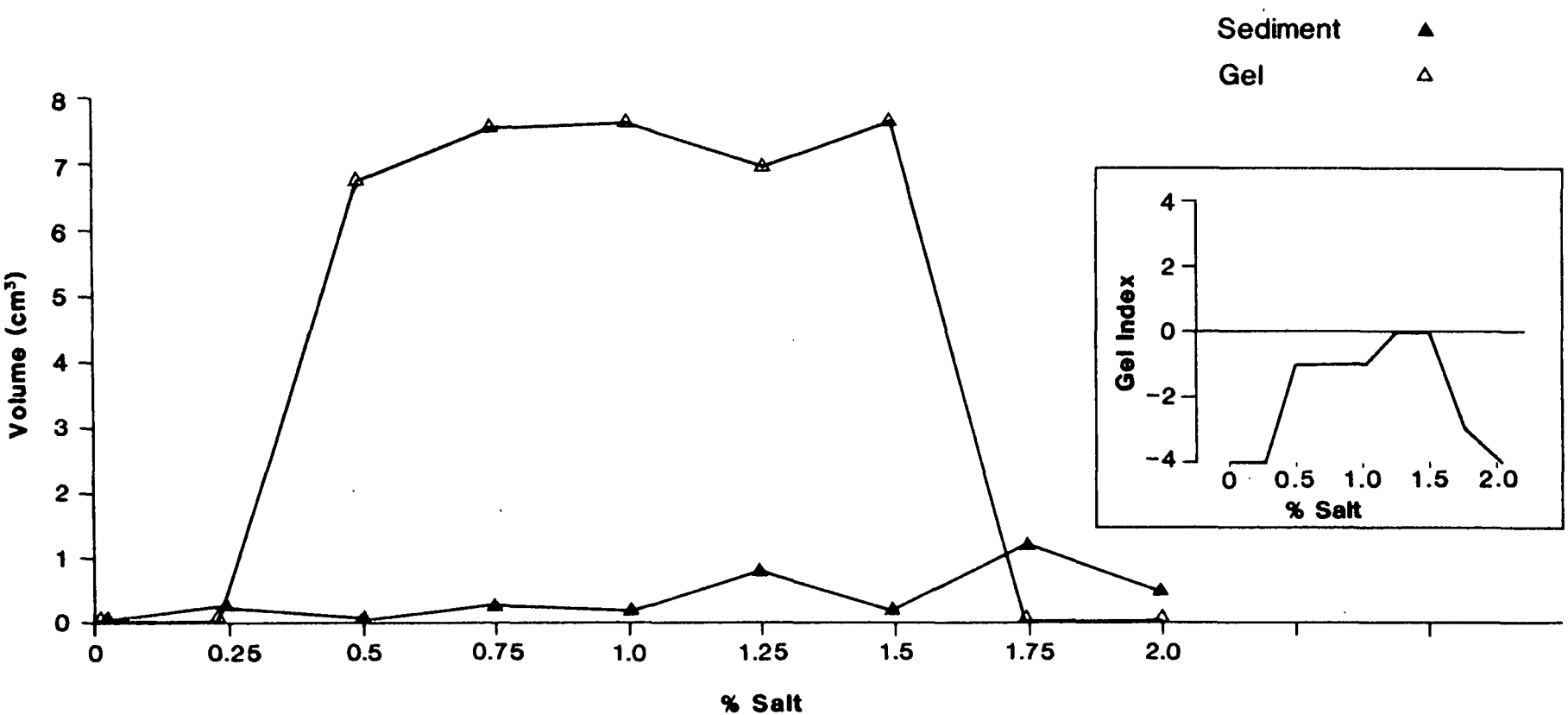


Figure 24. Effect of salt on protein solubility of defatted Maris Bead proteinate (10% solution).

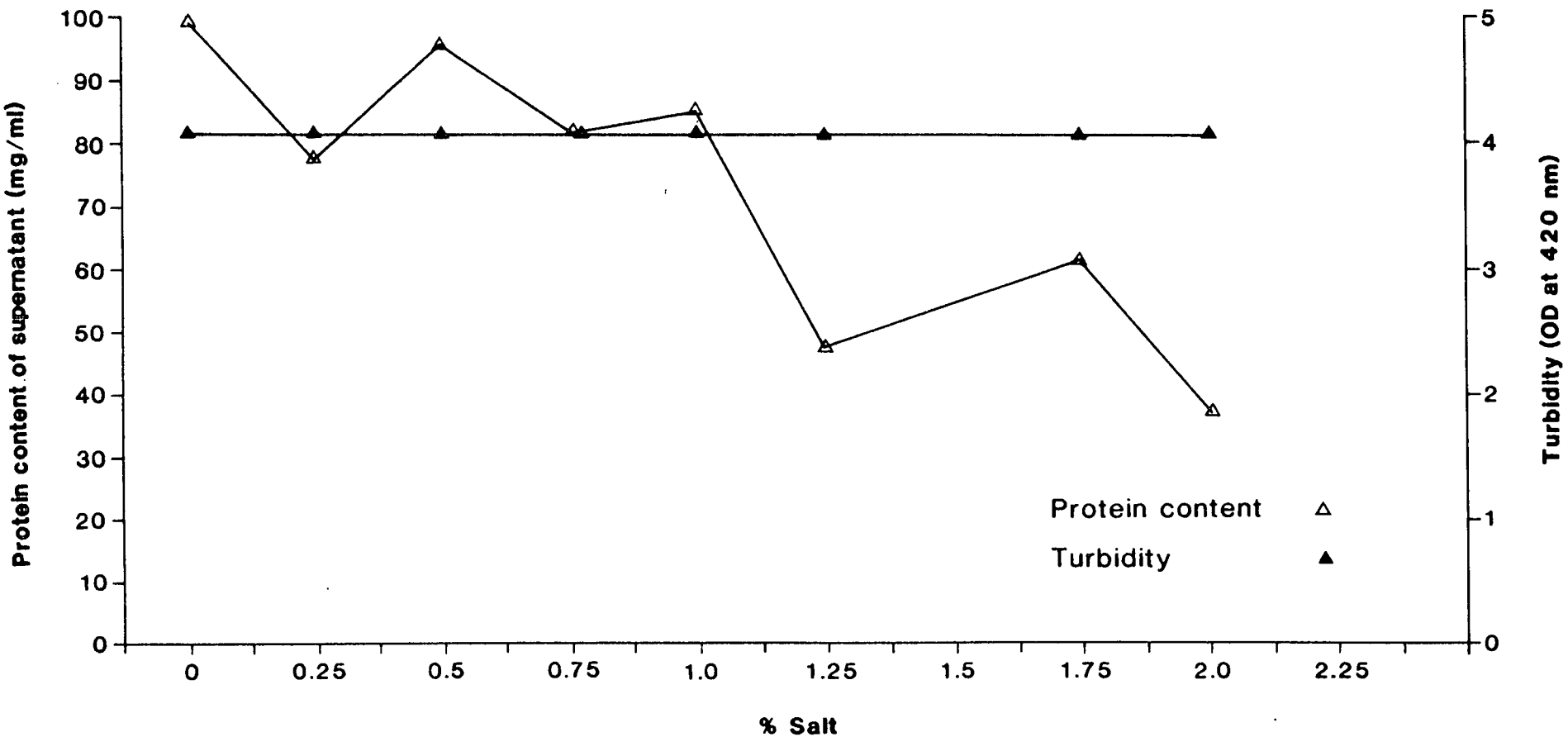


TABLE 12. Uniaxial compression of isolate, proteinate and micellar protein gels.

Sample	pH	No. of Samples	G Kg mm^{-2} x 10^{-4}	n	Rupture strength Nm^{-2}
MB proteinate - full-fat	7	4	1.76 ±0.21	0.83 ±0.28	1500 ±130
MB proteinate - defatted	7.4	2	0.57 ±0.21	0.96 ±0.07	600 ±90
IVS-G proteinate - defatted	7	4	0.66 ±0.08	1.50 ±0.14	840 ±70
IVS-G proteinate - defatted	7.7	2	1.07 ±0.25	1.35 ±0.36	1040 ±140
MB isolate - full-fat	7	3	0.29 ±0.07	1.19 ±0.06	290 ±20
MB isolate - full-fat	4.6	4	1.62 ±0.06	0.78 ±0.09	2130 ±830
MB micellar protein - full-fat	7	4	1.12 ±0.39	0.66 ±0.21	830 ±220
MB micellar protein - defatted	4	2	1.70 ±0.08	0.13 ±0.24	933 ±50
MB micellar protein - defatted	7	6	0.61 ±0.16	0.92 ±0.42	610 ±250
IVS-G micellar protein - defatted	7	6	1.62 ±0.21	0.92 ±0.19	1080 ±370

MB = Maris Bead

The gels varied greatly in appearance. At pH 7 proteinates and isolates generally formed dark coloured, semi-translucent gels that were springy to the touch. At pH 4.6 the isolate formed a lighter coloured, pasty gel which exhibited syneresis. The micellar proteins formed gels with the most pasty appearance, particularly at pH 4 where syneresis was also observed. Scanning electron micrographs of 20% gels of defatted Maris Bead isolate, proteinate and micellar protein are

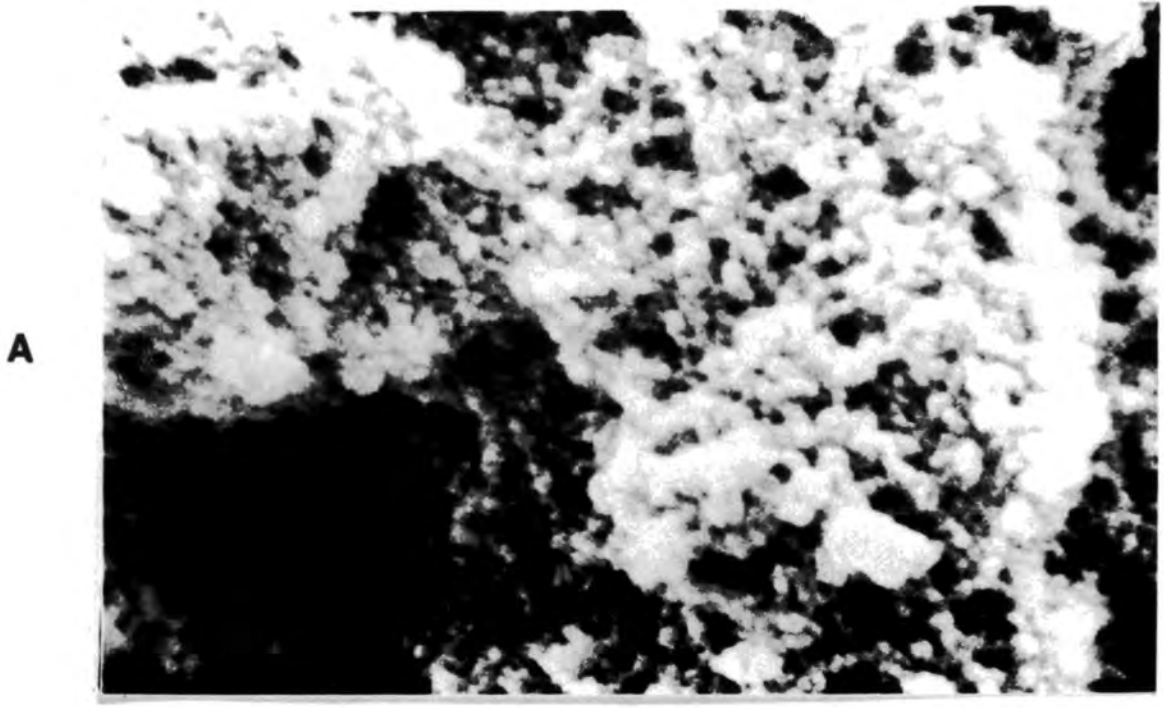
TABLE 13. Protein contents of 20% solutions prior to gelling.

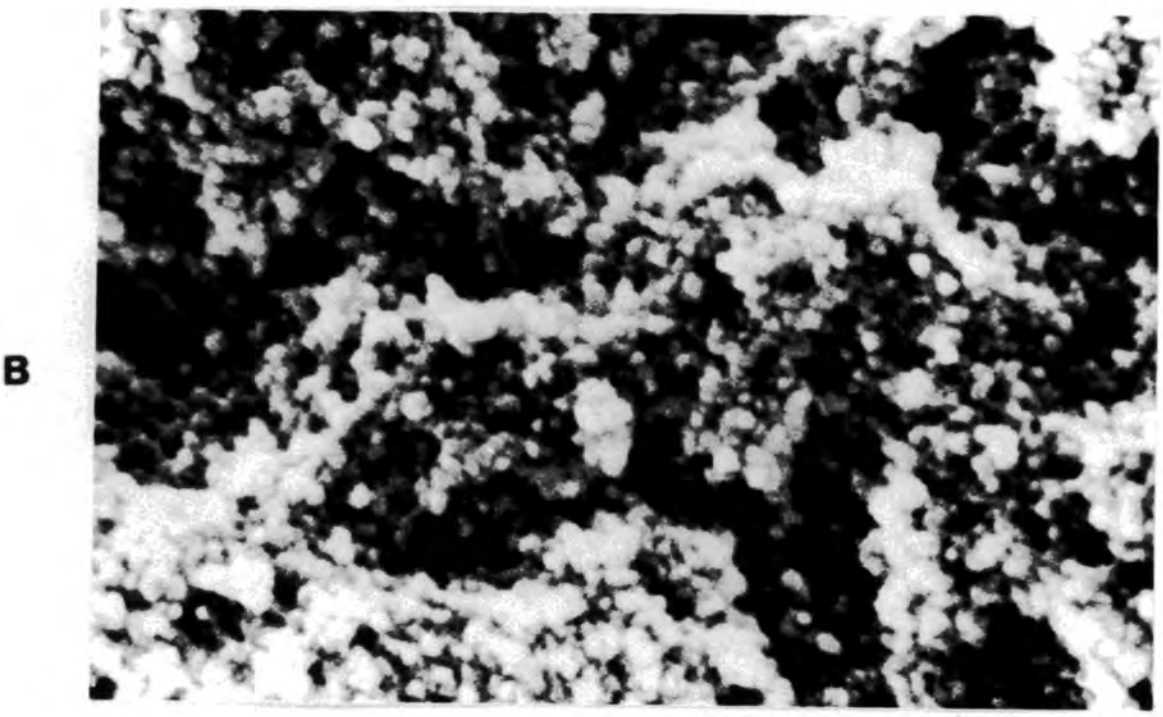
Sample	Protein Content (%)
MB proteinate - full-fat - pH 7	15.6
MB proteinate - defatted - pH 7.4	15.9
IVS-G proteinate - defatted - pH 7	15.8
MB isolate - full-fat - pH 7	11.2
MB isolate - full-fat - pH 4.6	12.0
MB micellar protein - defatted - pH 4	8.6
MB micellar protein - defatted - pH 7	10.4
IVS-G micellar protein -defatted pH 7	12.4
SEM Samples	
MB proteinate - defatted - pH 7	10.8
MB isolate - defatted - pH 7	11.3
MB micellar protein - defatted - pH 7	8.6

MB = Maris Bead

shown in Figure 25. Differences in structure were observed. The proteinate and isolate had coarser internal structures than the micellar protein, reflecting a greater extent of protein aggregation.

Relaxation times and peak heights from stress relaxation experiments on 20% gels are shown in Table 14. Relaxation times were of the order 0.8 s, 8 s and 90 s for proteinates, 0.3 s, 3 s and 25 s for isolates and 0.8 s, 7 s and 50 s for micellar proteins. Generally the slowest relaxation time gave the largest peak height (A_3) for the proteinates, although this was not much larger than A_2. A_1 was approximately 65% of either of the other two peak heights. The peak heights of the slowest relaxation time for the isolates and micellar


A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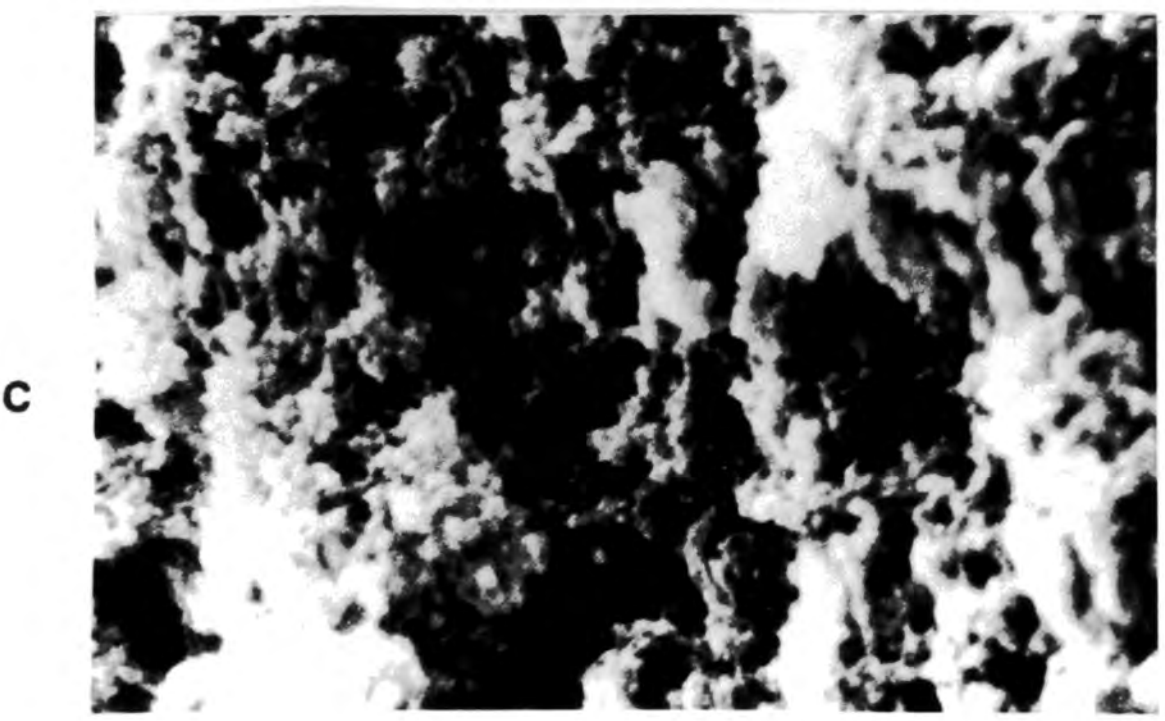

C

Figure 25. Scanning electron micrographs of gels of Maris Bead isolate, proteinate and micellar protein.

A. Defatted Maris Bead isolate

B. Defatted Maris Bead proteinate

C. Defatted Maris Bead micellar protein

Magnification factor was x20 000.

All gels were produced from 20% solutions at pH 7.

TABLE 14. Relaxation times of isolate, proteinate and micellar protein gels.

* results of only one sample tested

Sample	pH	Strain %	Relaxation time s			Peak Height x 10^{-3} N		
			1	2	3	A_1	A_2	A_3
MB proteinate - full-fat	6.9	10	0.49	4.2	35	41	56	63
		20	0.72	5.7	70	122	177	196
		30	0.74	6.6	74	195	246	261
MB proteinate - full-fat	7.0	10	0.54	5.0	45	47	69	73
		20	0.94	8.9	89	151	201	202
		30	0.76	7.7	96	211	276	276
MB proteinate - defatted	6.9	10	0.69	6.9	70	53	81	81
		20	0.79	7.2	70	93	184	229
		30	1.00	9.8	106	171	230	263
IVS-G proteinate - defatted	6.6	10	0.58	5.7	52	56	79	75
		20	0.71	6.5	66	110	143	149
IVS-G proteinate - defatted	7.0	10	0.65	6.4	33	32	34	36
		20	0.64	6.0	69	91	121	120
		30	0.79	7.7	81	116	152	233
MB isolate - full-fat	7.0	10*	0.41	3.0	25	62	86	98
		30	0.32	2.0	17	167	215	115
MB isolate - full-fat	4.6	10*	0.31	3.3	33	199	192	154
		15*	0.31	2.9	29	178	184	156
		20*	0.25	2.0	18	100	105	99
MB micellar protein - full-fat	7.0	10	0.66	6.0	50	17	24	21
		20	0.76	6.2	57	52	65	51
MB micellar protein - defatted	4.0	10*	0.71	7.5	8.8	97	125	139
		20*	0.48	2.9	16.9	119	189	305
MB micellar protein - defatted	7.0	10*	1.0	9.4	68	41	43	21
		20*	0.43	3.1	27	105	158	196
		30*	0.49	4.5	54	151	206	237
IVS-G micellar protein - defatted	7.0	10	0.43	5.0	53	34	50	37
		20	0.77	7.4	77	99	139	146
		30	0.85	7.9	106	160	205	254

MB = Maris Bead

proteins were proportionally less in some instances. From these samples there was no clear pattern.

Co-operative flow analysis of the same samples yielded the results shown in Table 15. Co-ordination number, z, generally increased with increasing compressive strain. For proteinates the co-ordination number was higher (3 - 4) than for isolates (approximately 2).

Samples were tested in duplicate or triplicate unless otherwise indicated.

TABLE 15. Co-ordination number of the first flow process in gels subjected to stress relaxation experiments.

Sample	pH	Strain %	Co-ordination Number z	Intercept with F/F$_0$ axis
MB proteinate - full-fat	6.9	10	3.2	0.25
		30	3.9	0.22
MB proteinate - full-fat	7.0	20	2.3	0.39
		30	3.2	0.24
MB proteinate - defatted	7.0	10	3.4	0.35
		20	2.5	0.45
		30	4.3	0.25
IVS-G proteinate - defatted	6.6	10	1.4	0.47
		20	1.8	0.42
IVS-G proteinate - defatted	7.0	10	2.2	0.01
		20	3.9	0.12
		30	2.9	0.01
MB isolate - full-fat	7.0	10	1.8	0.29
MB isolate - full-fat	4.6	10	1.3	0.45
		15	1.5	0.43
		20	2.2	0.28

MB = Maris Bead

4.5 ASSESSMENT OF FOAMING ABILITY

Foaming ability was assessed by two contrasting methods: a) a large scale whipping method and b) a small scale conductivity method. The results are summarised in Table 16. BSA, hemoglobin, sodium caseinate and soy whey protein produced the highest values of F.E. and F.V.S. obtained by the whipping method, whilst α-casein, sodium caseinate, soy whey protein, spray dried egg white and BSA produced the highest initial conductivities in the sparging experiments. The two methods did not produce the same ranking order for foam expansion or foam stability. In the whipping experiments lysozyme, full-fat soy flour and Filby micellar protein produced the lowest values of F.E. and F.V.S.. However, flours and air classified fractions were the poorest foaming agents as judged by initial conductivity and the foam stability index ($Co.\Delta t/\Delta c$) in the sparging experiments.

There was little variation in F.E. determined by the sparging experiments, despite a ten fold variation observed in the whipping experiments. F.V.S. from the sparging experiments varied from 7.5 for lysozyme to 74.8 for defatted soy flour.

Values of F.L.S. determined by both methods were low. The bulk of the solution incorporated in the foam during whipping or sparging drains very quickly after foaming is completed. Subsequent changes in liquid volume are small. This makes F.L.S. a relatively insensitive measure of foam stability. γ-globulin had the greatest and myoglobin the lowest foam expansion as determined by log (initial rate of fall in conductivity). Foam volume stability (determined by dividing conductivity after 3 min by initial conductivity) was high for sodium caseinate, BSA and soy whey protein, and low for lysozyme and myoglobin.

TABLE 16. Foaming ability from whipping and sparging experiments.

SAMPLE	WHIPPING			SPARGING
	F.E.	F.V.S.	F.L.S.	Cl $mS\ cm^{-1}$
Lysozyme	52 ± 0	14.2 ± 2.2	11.2 ± 0	0.75 ± 0.02
Hemoglobin	575 ± 19.8	82.8 ± 3.3	15.8 ± 7.4	1.13 ± 0.13
γ-globulin	252 ± 23.4	76.9 ± 3.1	18.4 ± 4.0	0.94 ± 0.02
Myoglobin	236 ± 0	49.1 ± 1.6	10.4 ± 2.3	1.49 ± 0.12
BSA	588 ± 13.9	90.1 ± 0	32.0 ± 6.9	1.62 ± 0.17
Ovalbumin				1.21 ± 0.12
Sodium Caseinate	522 ± 4.0	85.3 ± 3.1	21.3 ± 1.2	2.06 ± 0.15
β-lactoglobulin				0.77 ± 0.02
α-casein				2.13 ± 0.01
Maris Bead 11 S				1.06 ± 0.1
Maris Bead 7 S				1.40 ± 0.01
IVS-G 11 S				1.23 ± 0.08
IVS-G 7 S				0.87 ± 0.04
Birte 7 S				1.19 ± 0.06
Birte globulins				1.48 ± 0.05
Birte 11 S				1.49 ± 0.01
Renwhites	414 ± 19.8	81.0 ± 1.5	18.0 ± 0	1.67 ± 0.13
Filby whey protein				1.22 ± 0.02
Soy whey protein	548 ± 0	79.2 ± 0.6	15.2 ± 1.1	1.80 ± 0.05
Soy concentrate	204 ± 0	69.8 ± 0.09	12.2 ± 0.3	1.13 ± 0.06

SPARGING					
$Co.\Delta t / \Delta c$	F.E.	F.V.S.	F.L.S.	$\log \left[\frac{\Delta c}{t}\right]^*$	$\frac{C\ 3min}{Ci}$
0.4	146	7.5	7.9	-0.14	0.03
5.1	143	64.3	27.6	-0.26	0.24
				-0.44	0.43
4.3	154	42.0	11.8	-0.01	0.10
6.7	147	65.3	22.4	-0.29	0.42
7.3				-0.20	0.46
7.9	136	72.6	25.0		
10.7	149	73.6	28.1		
9.3	137	73.0	26.3		
8.1	154	71.4	35.1		
10.0	145	69.4	22.4		
8.1					
7.6	150	70.0	26.3		
8.8	150	71.1	28.9		
7.6				-0.31	0.41
8.3					
6.8	153	72.0	30.3	-0.26	0.42
5.7	161	66.7	22.8	-0.23	0.24

* t is 0 - 0.9 min.

TABLE 16 continued.

SAMPLE	WHIPPING			SPARGING
	F.E.	F.V.S.	F.L.S.	Ci mS cm^{-1}
Soy meal - defatted	426 ± 2.8	85.1 ± 0.5	23.4 ± 0.8	1.19 ± 0.06
Soy meal - full-fat	42 ± 2.8	20.0 ± 0	4.0 ± 0	1.14 ± 0.2
Soy isolate	446 ± 8.5	84.3 ± 1.3	21.0 ± 1.4	1.21 ± 0.05
Maris Bead proteinate - full-fat	156 ± 5.7	63.5 ± 0.5	11.6 ± 0.6	1.23 ± 0.03
Maris Bead proteinate - defatted	314 ± 2.0	80.0 ± 1.4	21.2 ± 0.6	1.22 ± 0.03
Maris Bead isolate - full-fat	122 ± 2.8	57.8 ± 0.1	7.2 ± 0	0.87 ± 0.03
Maris Bead isolate - defatted	335 ± 39.9	79.8 ± 3.0	15.6 ± 2.1	1.14 ± 0.03
IVS-G proteinate - full-fat	150 ± 2.8	53.0 ± 0.3	4.6 ± 0.8	0.91 ± 0
IVS-G proteinate - defatted	254 ± 19.8	74.9 ± 2.1	13.4 ± 0.3	1.19 ± 0
IVS-G isolate - full-fat	221 ± 1.4	65.7 ± 0.7	5.0 ± 0.3	0.91 ± 0.01
IVS-G isolate - defatted	262 ± 8.2	75.2 ± 0.8	10.9 ± 1.2	1.16 ± 0.02
Maris Bead micellar protein - full-fat	178 ± 8.5	69.4 ± 0.4	15.0 ± 1.4	1.15 ± 0.03
Maris Bead micellar protein - defatted	275 ± 14.0	76.8 ± 0.3	13.7 ± 2.1	1.21 ± 0.04
IVS-G micellar protein - defatted				0.50 ± 0.03
Filby proteinate - full-fat	164 ± 0	49.6 ± 2.2	3.0 ± 0.3	1.13 ± 0.04
Filby isolate - full-fat	152 ± 2.8	50.7 ± 4.9	5.8 ± 2.5	1.02 ± 0.04
Filby micellar protein - full-fat	76 ± 0	31.7 ± 0.8	4.8 ± 0	0.81 ± 0
Progreta Proteinate - defatted	310 ± 8.5	78.7 ± 0.9	17.8 ± 1.4	1.14 ± 0.02
Progreta isolate - full-fat	239 ± 4.6	70.1 ± 1.8	10.7 ± 2.3	1.09 ± 0

Micellar proteins were prepared by ultrafiltration.

SPARGING					
$Co\Delta t/\Delta c$	F.E.	F.V.S.	F.L.S.	$\log\left[\frac{\Delta c}{t}\right]^*$	$\frac{C\ 3min}{Ci}$
7.4	168	74.8	23.7	-0.35	0.41
5.7	140	59.9	16.3	-0.24	0.25
7.6	154	68.9	21.1	-0.34	0.41
7.2				-0.34	0.40
7.8				-0.35	0.41
6.6				-0.41	0.32
7.4				-0.37	0.40
6.9	147	67.6	17.1	-0.37	0.32
5.6				-0.27	0.27
5.2	146	60.4	5.2	-0.21	0.15
6.6	149	66.7	13.2	-0.31	0.35
7.7	146	72.7	36.8	-0.40	0.43
7.6	149	70.6	23.0	-0.34	0.40
5.3	138	42.5	15.8	-0.18	0.20
5.2	154	24.9	12.5	-0.24	0.21
5.8	150	57.9	13.2	-0.32	0.22
7.4	147	67.3	21.9	-0.34	0.39
6.1	155	70.1	21.1	-0.34	0.33

* t is 0 - 0.9 min.

TABLE 16 continued.

SAMPLE	WHIPPING			SPARGING
	F.E.	F.V.S.	F.L.S.	Cl $mS\ cm^{-1}$
Progreta isolate - defatted	452 ± 11.3	85.3 ± 0.3	18.6 ± 0.3	1.37 ± 0.06
Field bean proteinate - defatted	379 ± 4.2	83.4 ± 1.1	22.6 ± 7.6	1.31 ± 0.08
Maris Bead flour - full-fat	162 ± 2.8	46.3 ± 1.3	4.4 ± 0.6	0.29 ± 0.02
Maris Bead flour - defatted	236 ± 5.7	72.8 ± 1.8	10.8 ± 1.7	0.84 ± 0.11
IVS-G flour - full-fat	160 ± 5.7	55.6 ± 1.0	3.4 ± 0.8	0.38 ± 0
IVS-G flour - defatted	181 ± 4.2	66.1 ± 0.3	6.8 ± 2.3	0.66 ± 0
Filby pea flour - defatted	205 ± 9.8	68.8 ± 1.7	8.9 ± 1.8	0.77 ± 0.04
Filby pea flour milled twice - defatted	236 ± 8.5	67.4 ± 0.1	5.6 ± 1.7	0.41 ± 0.02
AIR-CLASSIFIED FRACTIONS				
Maris Bead 3 C	14 ± 2.8	6.3 ± 0.8	2.2 ± 0.3	0.15 ± 0.07
IVS-G 3 C	146 ± 25.5	42.0 ±12.7	4.3 ± 0.6	0.2 ± 0
Maris Bead 6.5 C	125 ± 1.4	50.7 ± 1.4	4.0 ± 1.1	0.2 ± 0
IVS-G 7 C	148 ± 0	47.3 ± 1.4	1.2 ± 0.6	0.22 ± 0.03
Maris Bead 7 F	162 ± 2.8	57.1 ± 1.5	4.6 ± 1.4	0.47 ± 0.03
IVS-G 7 F	179 ± 9.9	59.0 ± 0.2	2.6 ± 0.3	0.39 ± 0.03
Maris Bead 11 F	171 ± 1.4	59.4 ± 0.8	6.0 ± 0	0.41 ± 0.01
Maris Bead S11 F	170 ± 2.8	60.6 ± 1.9	4.6 ± 0.3	
IVS-G 11 F	210 ± 2.8	63.4 ± 2.4	4.4 ± 0	0.46 ± 0

C = Coarse Fraction F = Fines Fraction S = Sequentially classified speed

Speed = rev./min x 1000

SPARGING					
Co.Δt /Δc	F.E.	F.V.S.	F.L.S.	log $\left[\frac{\Delta c}{t}\right]$*	$\frac{C\ 3min}{C1}$
6.4	143	68.1	19.7	-0.23	0.33
7.5	150	72.6	26.3	-0.36	0.43
5.4	128	49.1	6.6	-0.65	0.14
6.6	145	64.2	14.9	-0.36	0.29
5.0	138	46.4	14.5	-0.56	0.11
6.7	150	64.2	11.8	-0.39	0.24
6.3	141	62.8	11.8	-0.33	0.22
6.3				-0.51	0.15
7.1				-0.91	0.13
4.3	133	24.3	1.3	-0.78	0.05
6.5	128	52.0	7.9	-0.81	0.15
5.6	141	27.3	2.6	-0.70	0.09
5.5	163	57.5	2.6	-0.51	0.17
5.2	140	57.1	5.3	-0.56	0.13
5.2	143	60.5	6.6	-0.57	0.12
5.0	140	56.6	5.3	-0.49	0.13

* t is 0 - 0.9 min.

Purified 11S and 7S proteins from bean and pea were tested by the sparging method only. They had lower initial conductivities than α-casein, sodium caseinate, soy whey protein and BSA. However, their foam stability index was high. They had superior functionality compared to flours, isolates, proteinates and micellar proteins.

Full-fat flours, isolates, proteinates and micellar proteins gave lower values of F.E., F.V.S., F.L.S. (from whipping experiments), Ci and $Co.\Delta t/\Delta c$ than their equivalent defatted samples. F.E., F.V.S. and Ci were similar for isolates and proteinates, but Maris Bead had higher values of F.E. and F.V.S. than IVS-G. Soy isolate was superior to other isolates, but defatted soy flour had equivalent functionality with respect to foaming power and foam stability. IVS-G and Filby micellar proteins were inferior to isolates and proteinates.

With the exception of defatted soy flour, flours had poor foaming properties. Coarse fractions from air classification experiments were inferior to fines fractions. Foaming ability of the fines fractions increased with classifier speed and protein content of the fraction.

Foam decay curves of standard proteins are shown in Figure 26. Both α-casein and BSA had high initial conductivity and relatively flat curves. This suggests good foam expansion and stability. Myoglobin and lysozyme had steep initial slopes indicative of the brittle foams they produce. The similar foam decay curves produced by bean and pea 11S and 7S proteins (Figure 27) suggest a similarity in their foaming properties. The curves are relatively flat which is indicative of good foam stability.

Figure 26. Foam decay curves of selected protein standards.

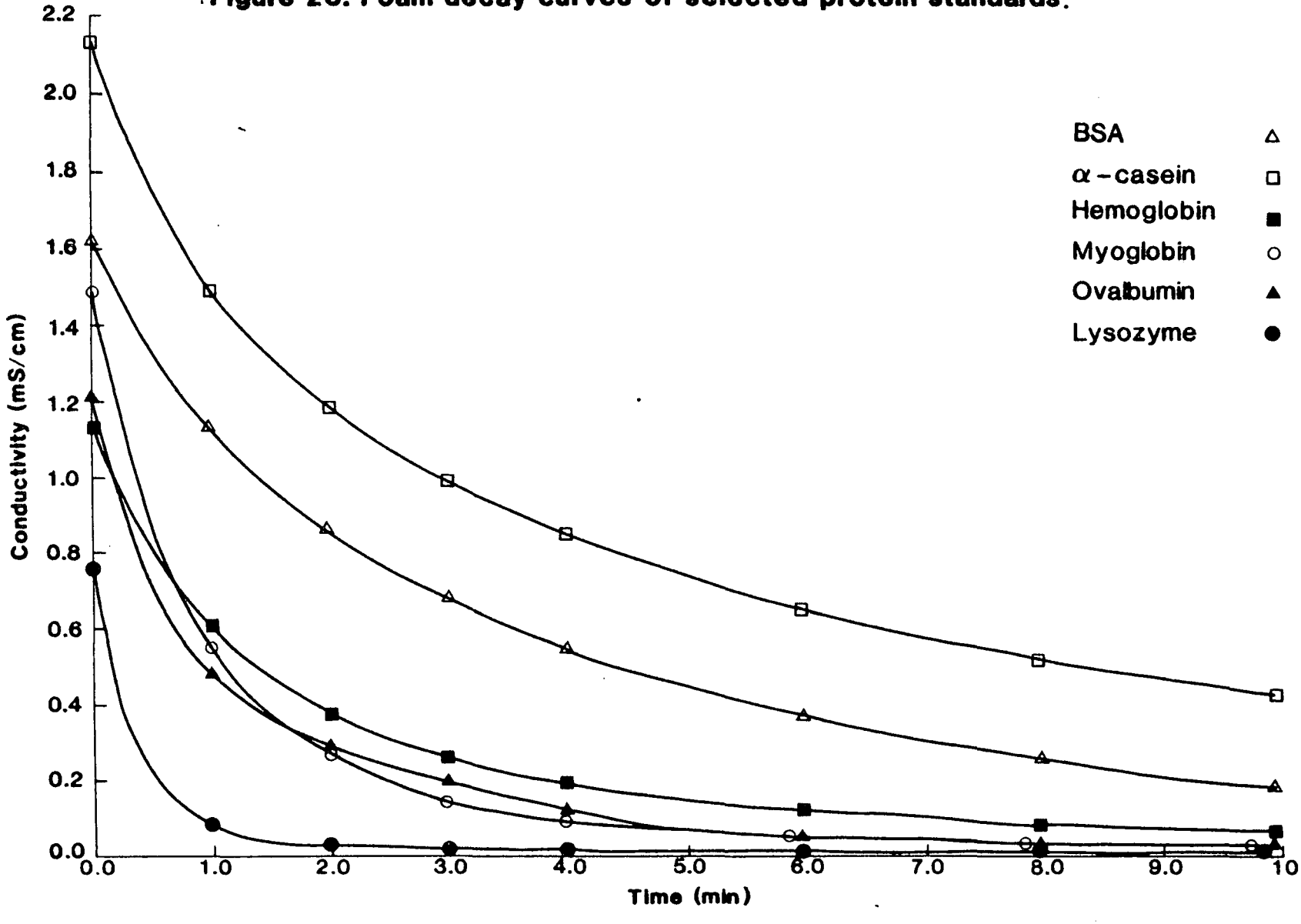
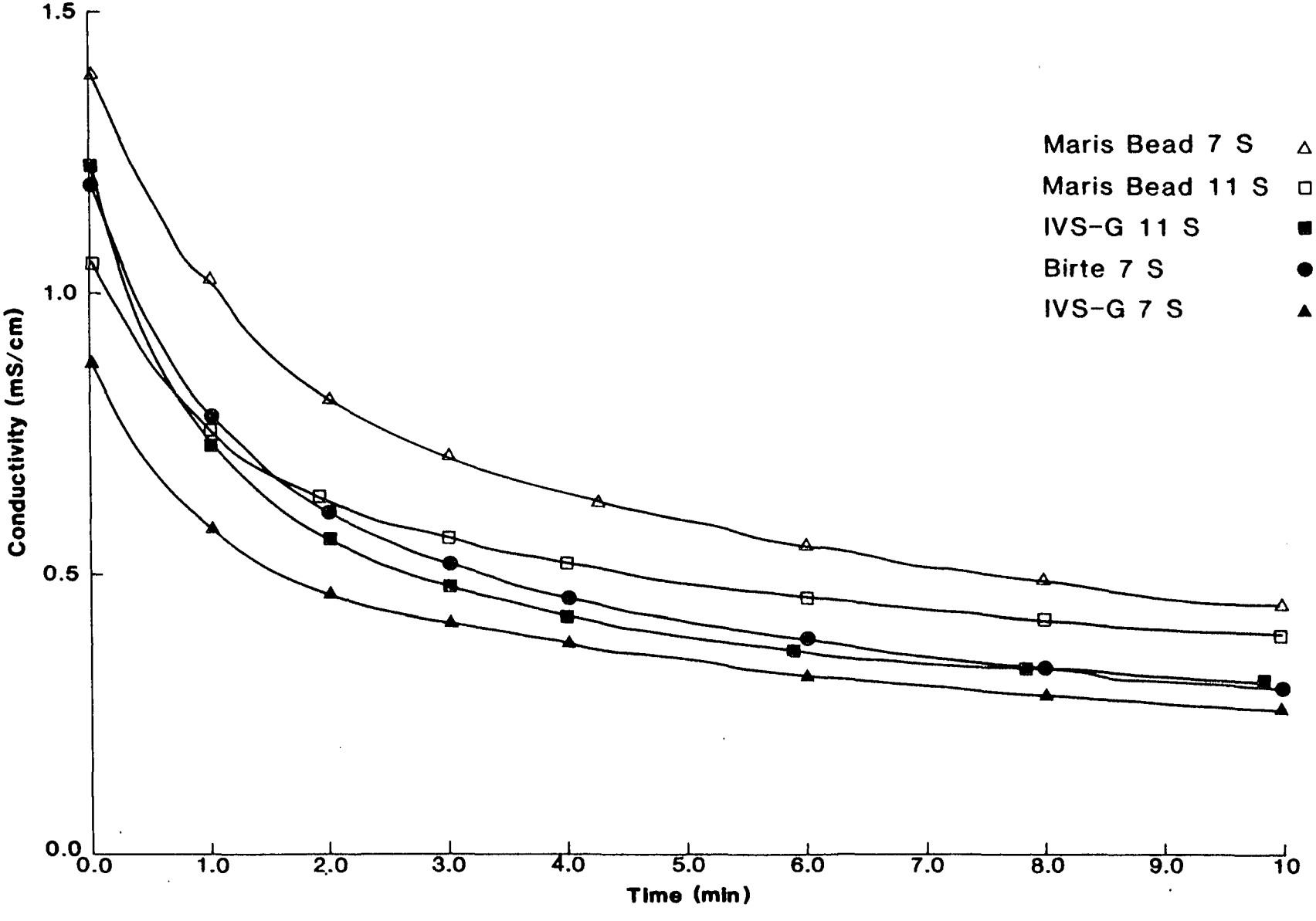


Figure 27. Foam decay curves of purified bean and pea proteins.



Foam decay curves of selected soy bean products are shown in Figure 28. Soy whey protein had superior properties to other products. The isolate and defatted soy flour had very similar curves, as did the full-fat soy flour and the soy concentrate.

Foam decay curves of sodium caseinate, spray dried egg white, and Filby pea whey protein (Figure 29), selected flours (Figures 30 and 31), defatted and full-fat isolates (Figures 32 and 33), defatted and full-fat proteinates (Figures 34 and 35) and defatted micellar proteins (Figure 36) are shown. Defatted and full-fat soy flour were shown to be superior to bean and pea flours. However, bean and pea isolates produced similar curves to soy isolate (Figure 32). Generally all defatted and full-fat isolates and proteinates tested were similar with respect to initial conductivity. However, full-fat products showed a greater initial loss of conductivity.

Micellar proteins exhibited greater variation in their foam decay curves. Defatted and full-fat Maris Bead micellar proteins had similar curves to isolates and proteinates. The Filby and IVS-G curves had lower initial conductivities and a steeper initial drop in conductivity. The Maris Bead micellar protein used in this experiment was prepared using a dialyzer/concentrator to reduce the supernatant volume. The IVS-G and Filby micellar proteins had been prepared by the method of Murray et al. (1978).

The whipping and sparging methods were compared, with respect to foam expansion and foam stability, by linear regression analysis of a) all the data points and b) standard proteins. Correlation coefficients are shown in Table 17. F.E. determined by the whipping method did not correlate strongly with any of the measures of foaming power employed for the sparging method. The best correlation occurred

Figure 28. Foam decay curves of soy products.

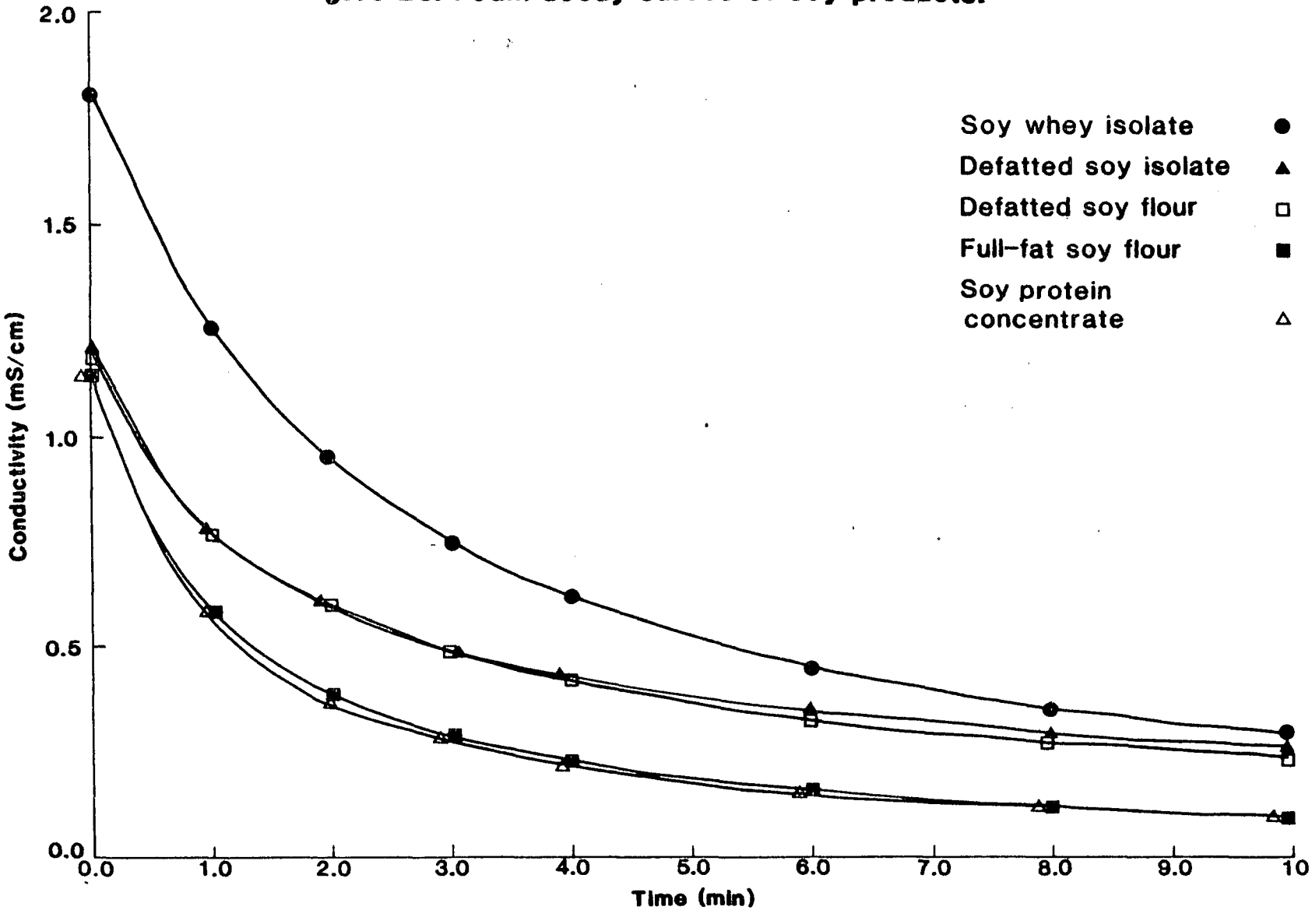


Figure 29. Foam decay curves of sodium caseinate, spray dried egg white and Filby whey protein.

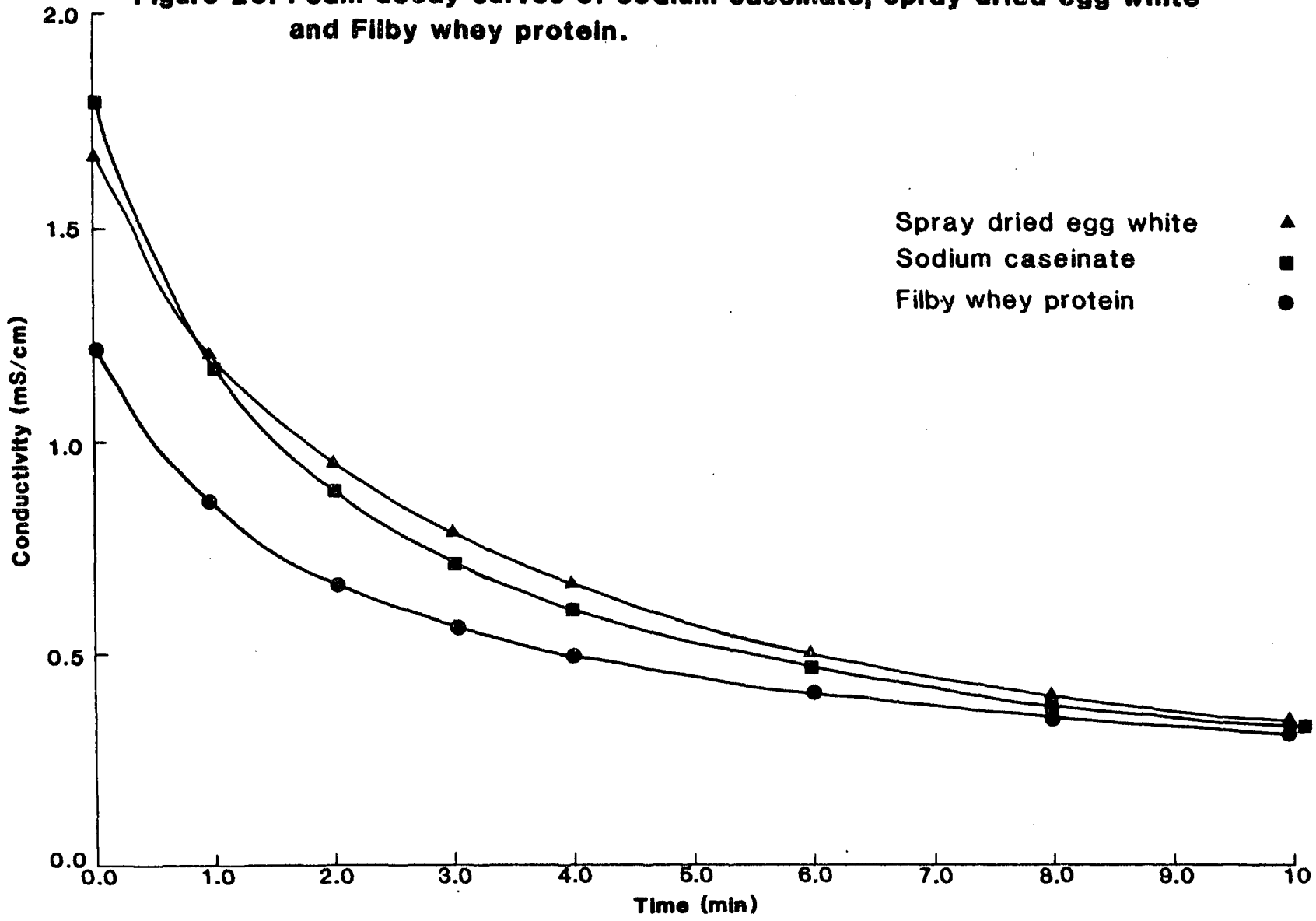


Figure 30. Foam decay curves of defatted bean and pea flours.

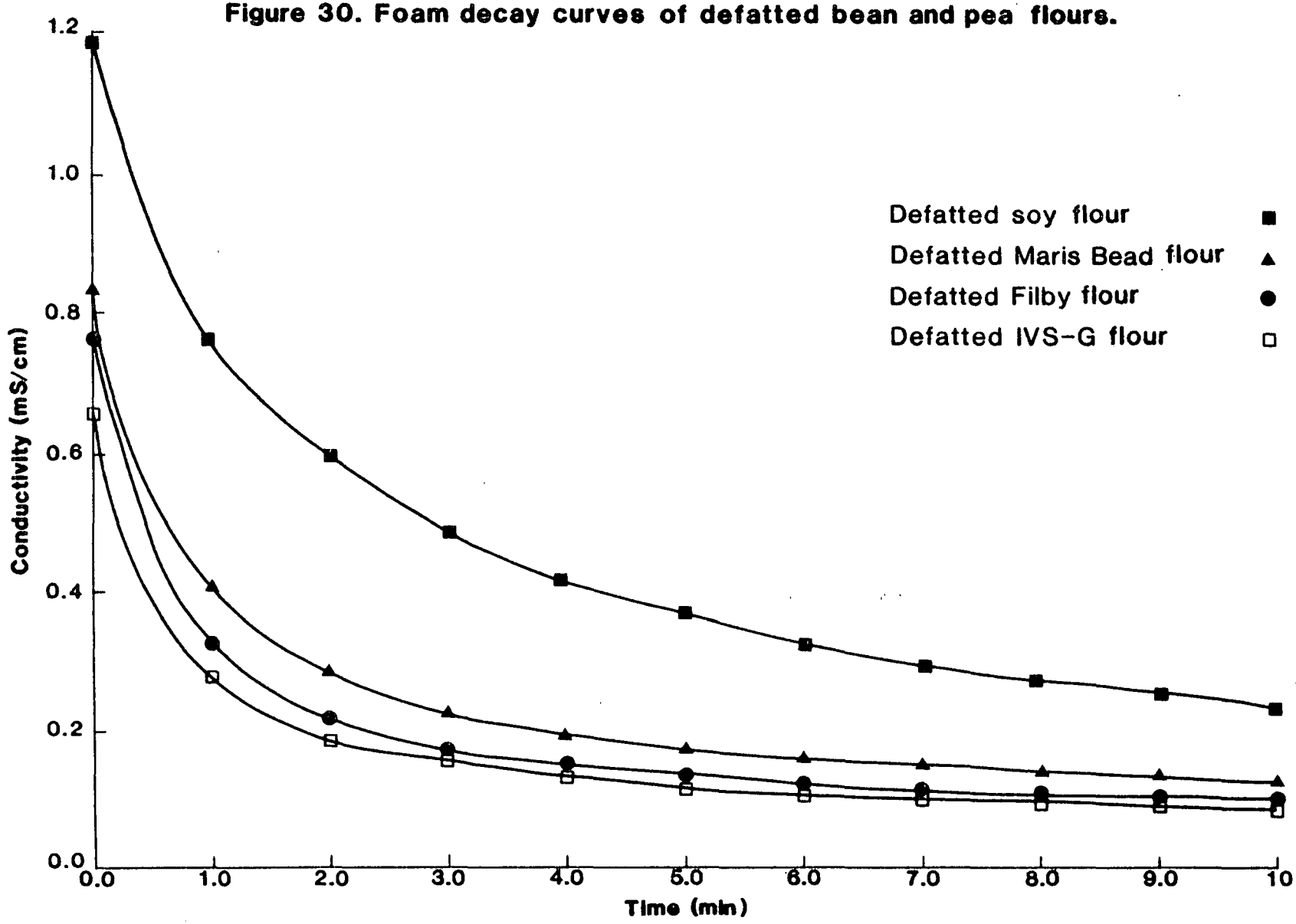


Figure 31. Foam decay curves of full-fat bean and pea flours.

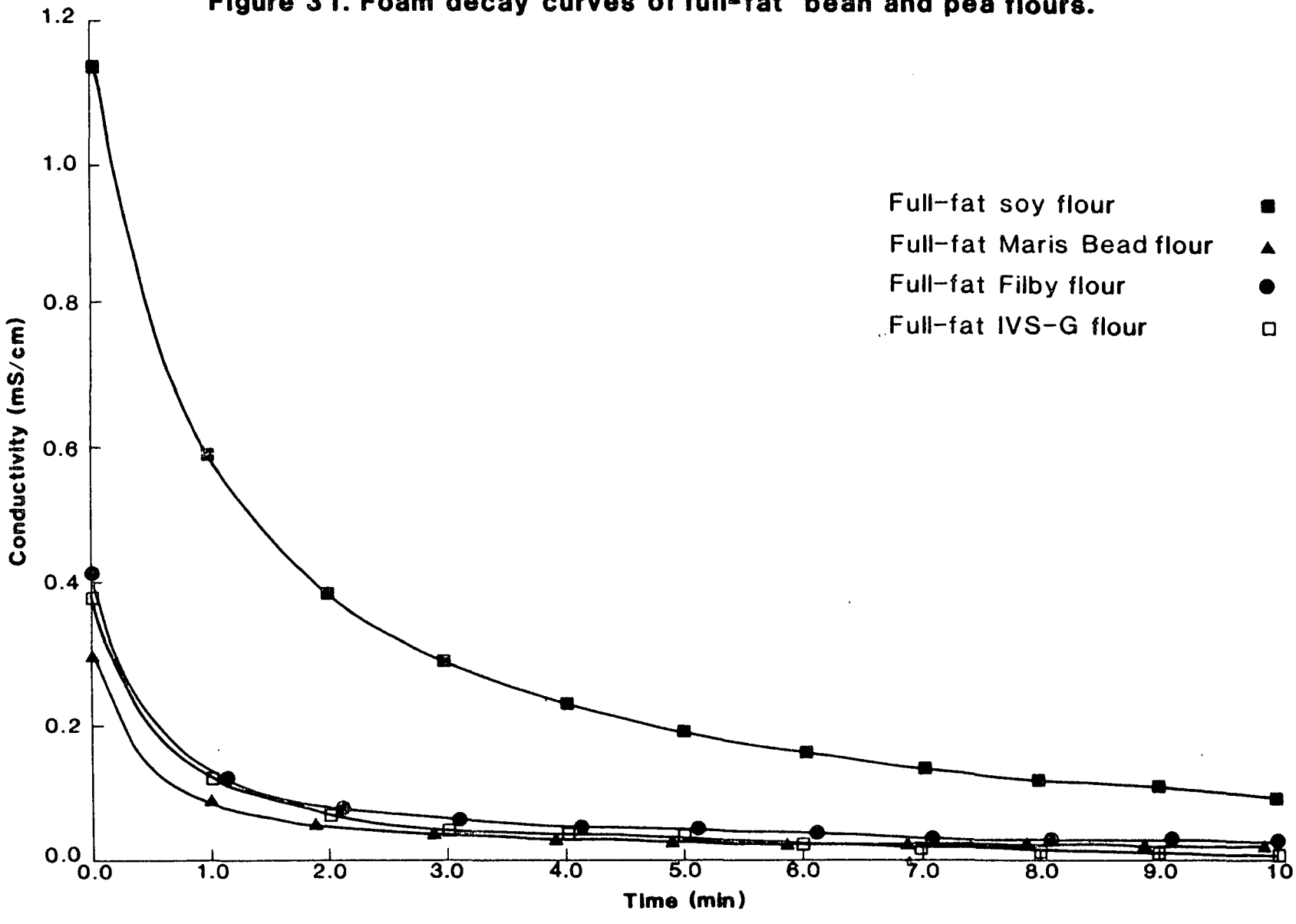


Figure 32. Foam decay curves of defatted bean and pea isolates.

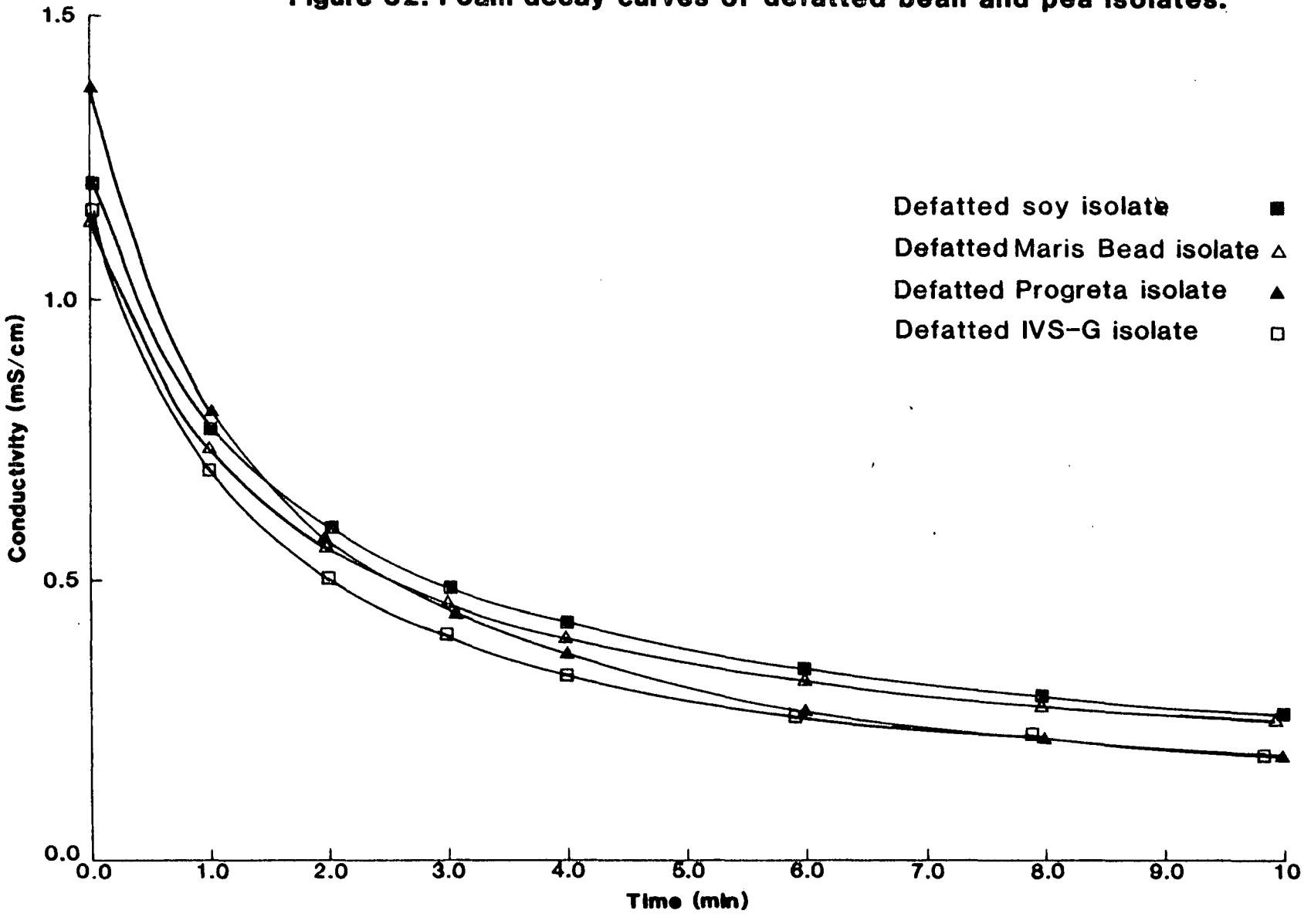


Figure 33. Foam decay curves of full-fat bean and pea isolates.

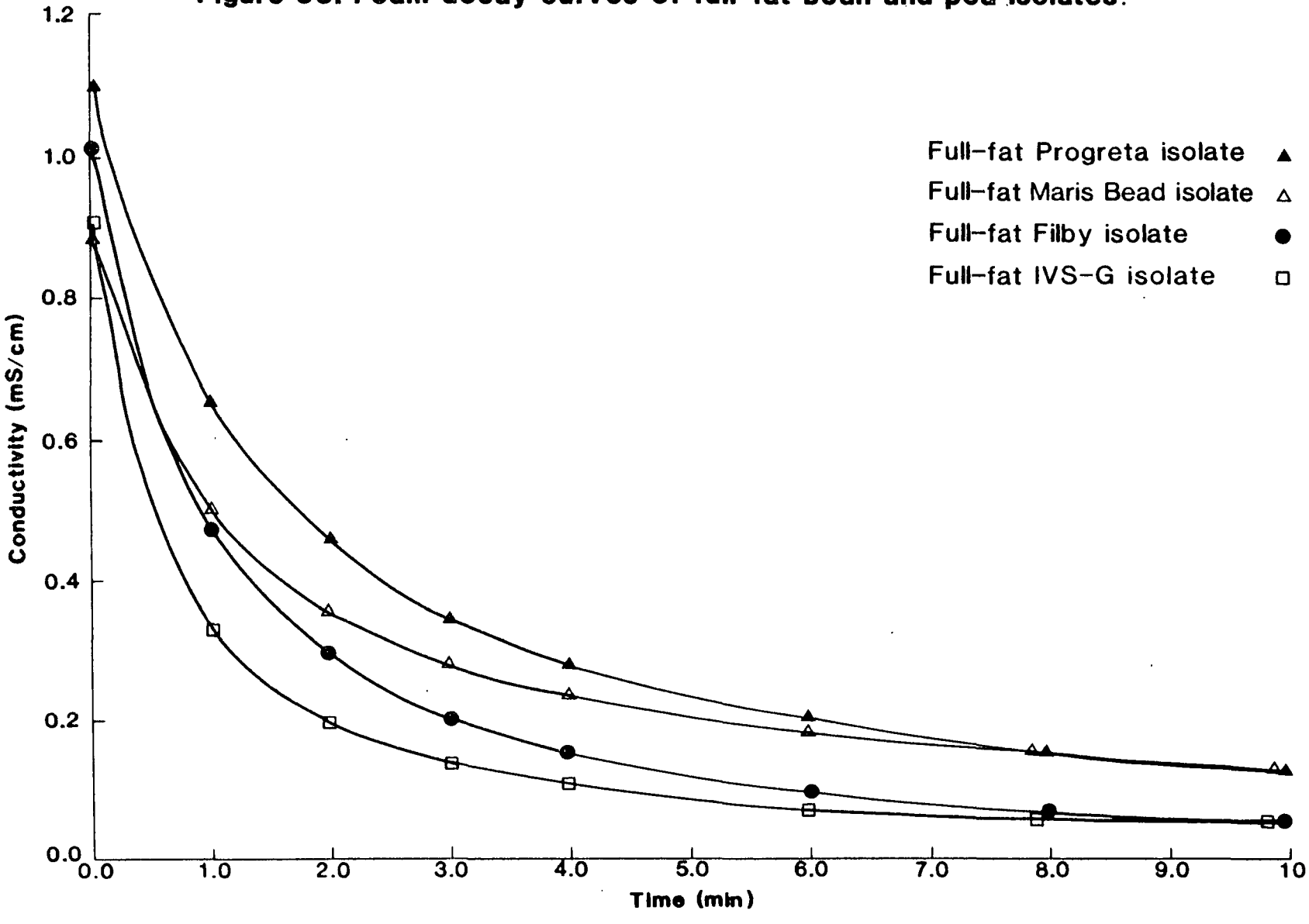


Figure 34. Foam decay curves of defatted bean and pea proteinates.

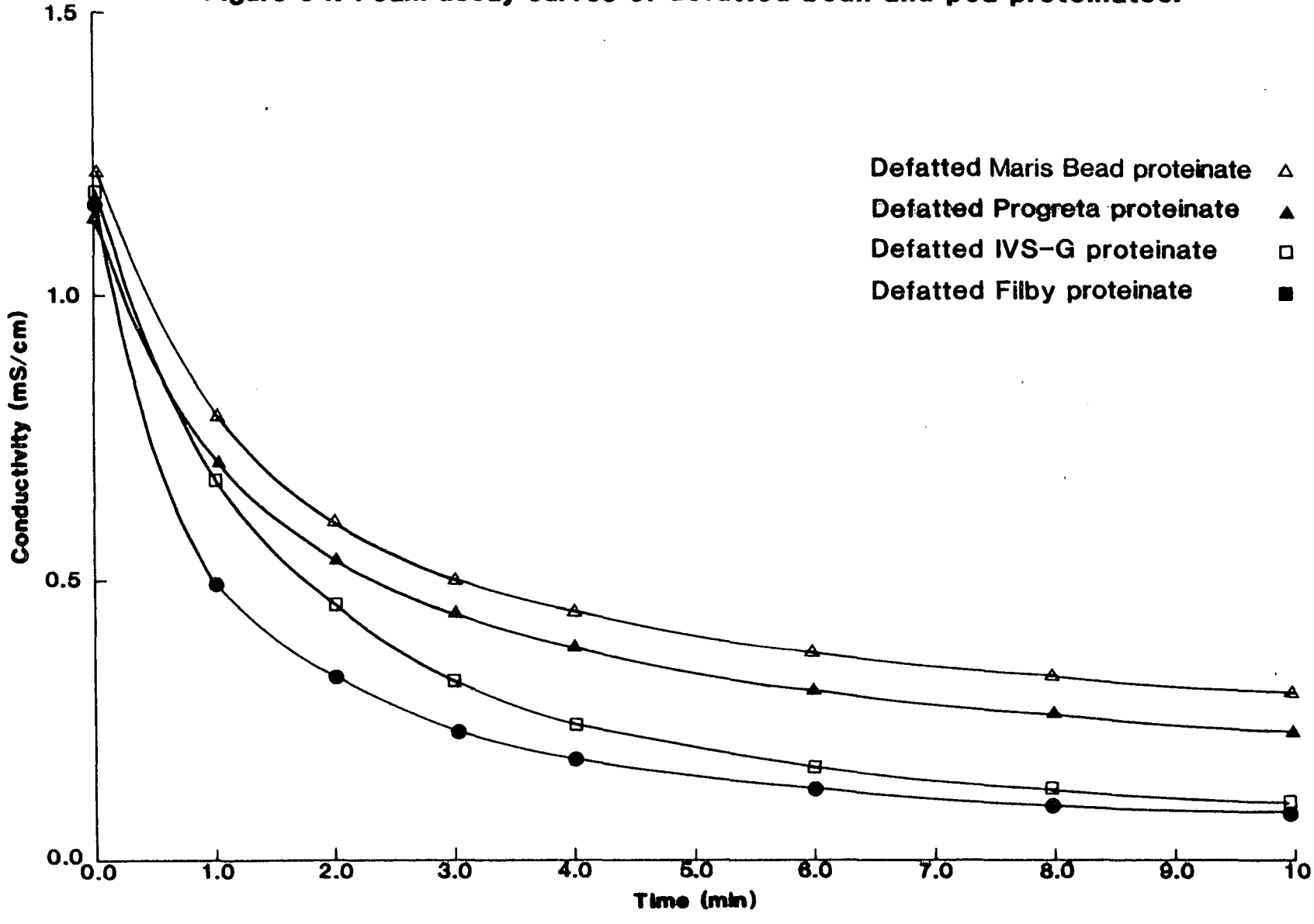


Figure 35. Foam decay curves of full-fat bean and pea proteinates.

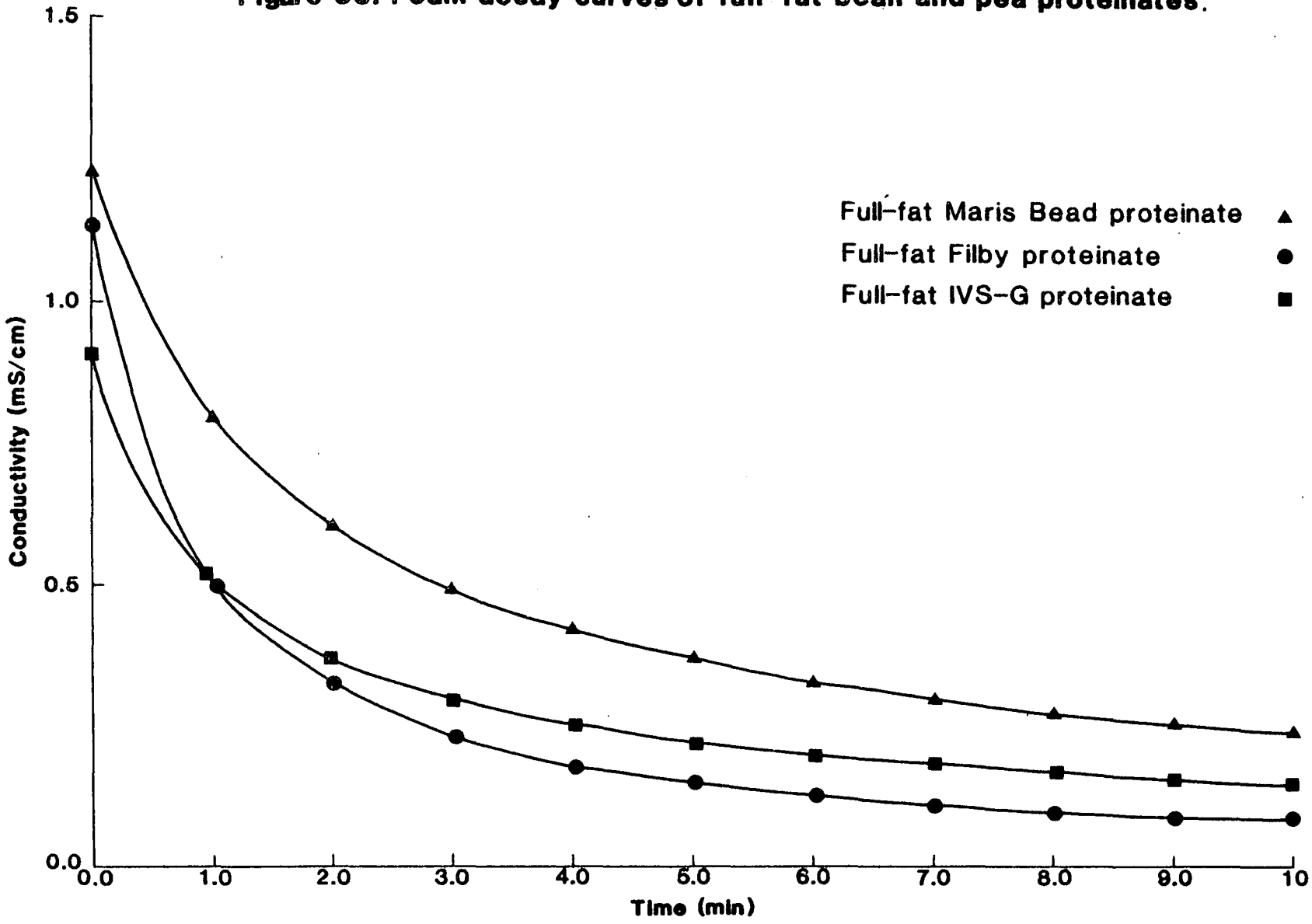


Figure 36. Foam decay curves of bean and pea micellar proteins.

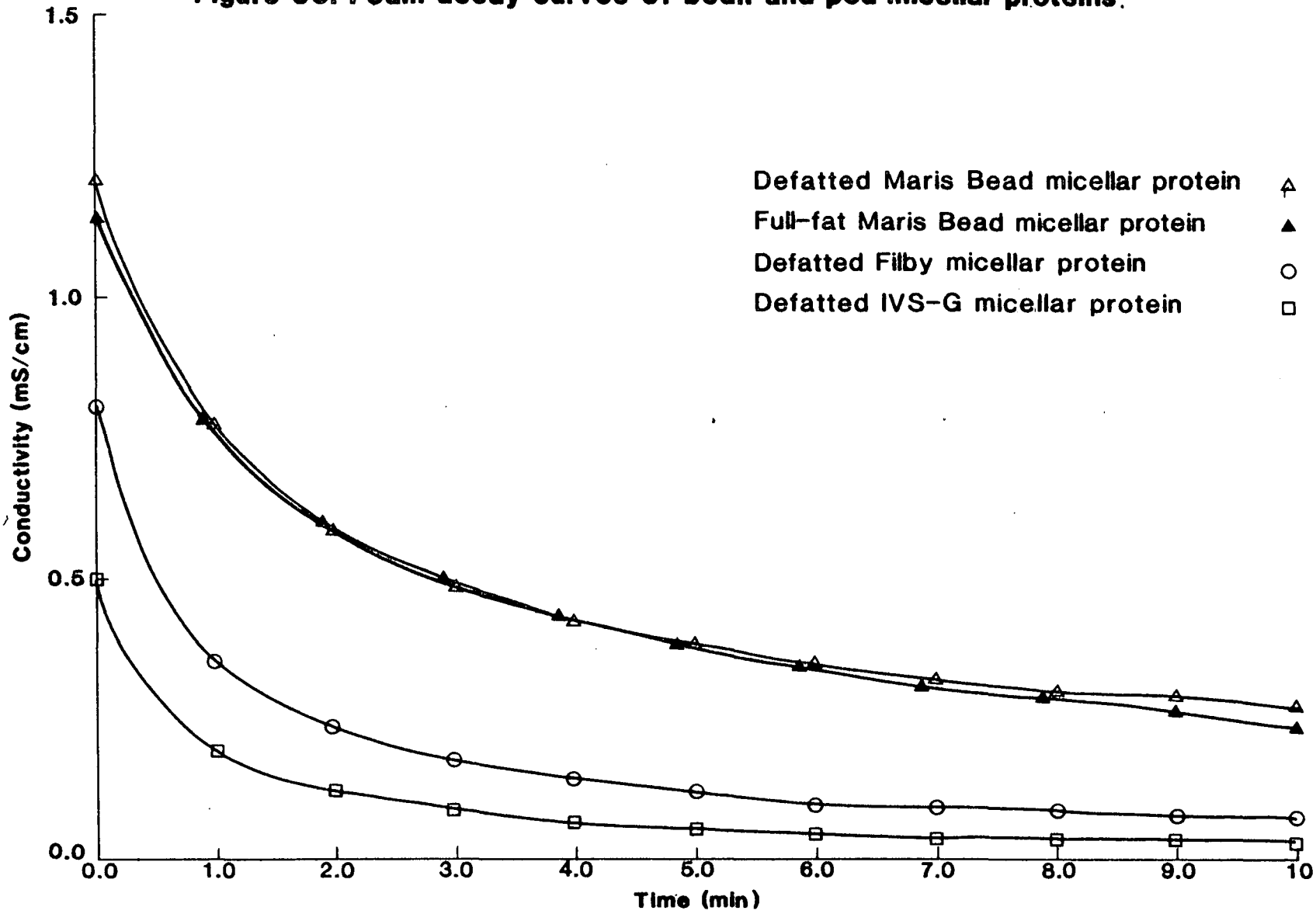


TABLE 17. Comparison of whipping and sparging methods for determining foam expansion and foam stability.

		CORRELATION COEFFICIENTS					
		SPARGING					
		ALL SAMPLES			STANDARD PROTEINS		
WHIPPING	F.E.	Ci $mS\ cm^{-1}$	F.E.	$\log \left[\frac{\Delta c}{t}\right]$	Ci $mS\ cm^{-1}$	F.E.	$\log \left[\frac{\Delta c}{t}\right]$
		0.71	0.30	0.38	0.67	0.39	-0.30
	F.V.S.	Co . Δt / ΔC	F.V.S.	C 3 min / Ci	Co . Δt / ΔC	F.V.S.	C 3 min / Ci
		0.70	0.73	0.70	0.95	0.99	0.88

with initial conductivity. However, F.V.S. determined by the whipping method did correlate with all three measures of foam stability employed for the sparging method when standard proteins were used.

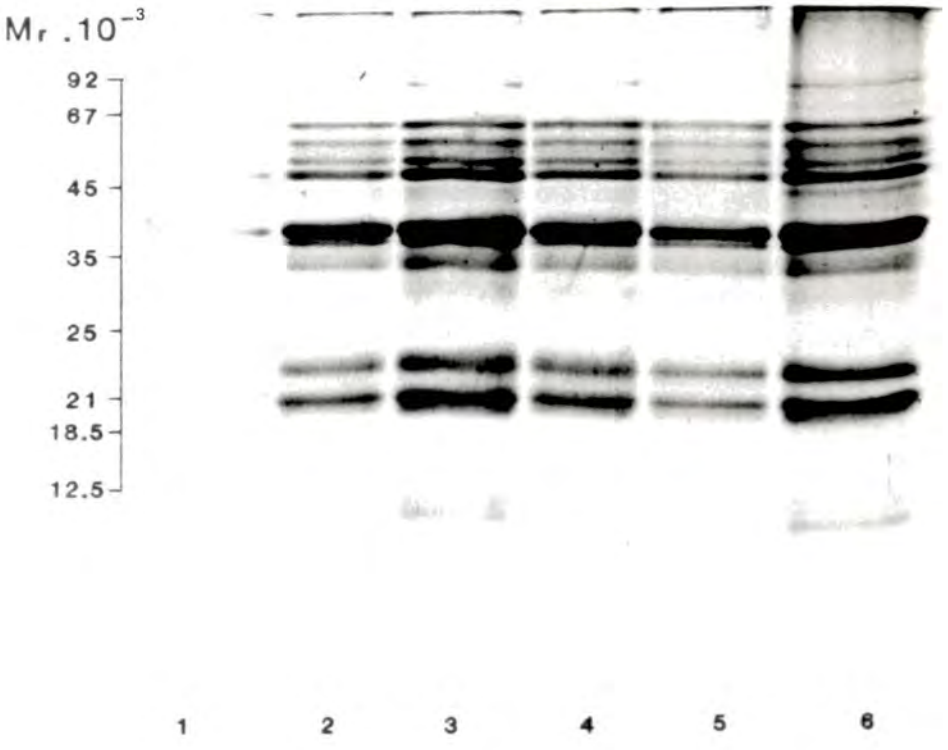
SDS-PAGE of liquid drained from a foam of 0.5% defatted Maris Bead isolate at selected time intervals is shown in Figure 37. No preferential retention of any protein species was observed.

The effect of sugar on the foaming ability of a 0.5% solution of defatted Maris Bead proteinate is shown in Table 18. Stirring the solution with sucrose prior to foaming greatly reduced F.E. and F.V.S. However, although F.E. and F.V.S. did decrease with increasing sucrose content, delaying the addition of sucrose enabled both F.E. and F.V.S. to be maintained close to their original values.

4.6 ASSESSMENT OF EMULSION ACTIVITY.

The results of tests on flours, concentrates, isolates, proteinates, micellar proteins and purified proteins to determine emulsifying properties are shown in Table 19. Maris Bead and IVS-G isolates and proteinates emulsified more oil than flours and air classified fractions, but slightly less than the soy concentrate and soy isolate which emulsified all the available oil. Defatted products generally emulsified less oil than equivalent full-fat products. Emulsion activity decreased with decreasing protein content of the air classified coarse fractions, however, protein content was not the only factor involved as emulsion activity was not correlated with ml oil/g protein. Purified 11S and 7S proteins emulsified more oil than casein.

FIGURE 37. SDS-PAGE of drained liquid from a defatted Maris Bead isolate.



TRACK	SAMPLE
1	Liquid drained over-night.
2	Liquid drained after 1 h.
3	Liquid drained after 30 min.
4	Liquid drained after 15 min.
5	Unfoamed liquid.
6	Total (before foaming).

TABLE 18. The effect of sucrose on the foaming ability of a defatted Maris Bead proteinate.

	Time sucrose was added after start of whipping								
	0 MIN			2 MIN			4 MIN		
Sucrose %	F.E.	F.V.S.	F.L.S.	F.E.	F.V.S.	F.L.S.	F.E.	F.V.S.	F.L.S.
0	314 ± 2	80 ± 1.4	21 ± 0.6						
6				300	80	20	312	83	19
12				292	80	24	316	78	12
15	156 ± 11	68 ± 3	18 ± 3						
24				284	77	12	292	77	12
30	123 ± 33	60 ± 5	12 ± 0						
60	16 ± 0	24 ± 0	12 ± 0						

TABLE 19. Emulsion activities of flours, concentrates, isolates, proteinates, and micellar proteins.

SAMPLE	Emulsion Activity	
	ml oil/g sample	ml oil/g protein
Soy concentrate	5.95 ± 0.07	9.0
Soy isolate - defatted	6.0 ± 0	7.1
Maris Bead flour - defatted	5.2 ± 0	16.0
IVS-G flour - defatted	5.1 ± 0	15.4
Filby pea flour - defatted	4.2 ± 0	19.4
IVS-G flour - full-fat	4.8 ± 0.7	15.0
Maris Bead flour - full-fat	4.6 ± 0.8	14.4
Filby pea flour - full-fat	5.3 ± 0.3	23.8
AIR CLASSIFIED FRACTIONS		
Maris Bead 3 F	5.2 ± 0	17.4
" " 7 F	2.8 ± 0.07	4.8
" " 11 F	4.1 ± 0	5.5
" " 3 C	0.2 ± 0	2.9
" " 5 C	5.3 ± 0	23.8
" " 6.5 C	3.0 ± 1.3	17.8
" " 9 C	4.9 ± 0	22.5
IVS-G 3 F	5.4 ± 0	14.6
" 5 F	5.4 ± 0	13.1
" 7 F	5.8 ± 0	8.8
" 9 F	5.0 ± 0	7.1
" 3 C	4.4 ± 0	27.7
" 6.5 C	4.1 ± 1.3	10.2
SEQUENTIALLY CLASSIFIED FRACTIONS		
Maris Bead 9 F	4.1 ± 0.4	5.9
" " 7 F	3.3 ± 1.5	5.2
" " 5 F	3.7 ± 1.1	16.5
" " 11 C	3.5 ± 0.4	16.2
" " 9 C	2.3 ± 0.4	11.9
" " 7 C	1.7 ± 0.8	11.9
" " 5 C	0.8 ± 0	5.1
" " 3 C	0.2 ± 0	2.9

F = Fines Fraction C = Coarse fraction Speed = rev./min x 1000

SAMPLE	Emulsion Activity	
	ml oil/g sample	ml oil/g protein
SEQUENTIALLY CLASSIFIED FRACTIONS		
IVS-G 11 F	5.5 ± 0.1	7.4
" 9 F	5.5 ± 0.1	7.7
" 7 F	5.4 ± 0.5	12.2
" 5 F	2.9 ± 0.1	21.8
" 3 F	4.3 ± 1.1	17.6
" 11 C	3.9 ± 0.6	19.6
" 9 C	4.0 ± 0	21.2
" 7 C	2.8 ± 0.3	17.6
" 5 C	2.4 ± 0.5	14.9
" 3 C	0.2 ± 0	2.2
MB proteinate - full-fat	5.8 ± 0	7.0
IVS-G proteinate - full-fat	5.95 ± 0	6.6
MB proteinate - defatted	5.6 ± 0.4	6.2
MB isolate - full-fat	5.7 ± 0	6.3
IVS-G isolate - full-fat	5.8 ± 0.3	6.2
Progreta isolate - full-fat	4.3 ± 0.1	5.1
MB isolate - defatted	5.6 ± 0	5.9
MB micellar protein-full-fat	5.0 ± 0	5.0
MB micellar protein-defatted	5.6 ± 0	5.6
PROTEINS 0.1 g/10 ml		
Maris Bead 11 S		29.5 ± 0
IVS-G 11 S		25.0 ± 0
Maris Bead 7 S		23.8 ± 3.2
IVS-G 7 S		24.0 ± 0
Casein		1.8 ± 1.5
Casein 0.5 g/10 ml		5.5 ± 0

F = Fines Fraction C = Coarse fraction Speed = rev./min x 1000

MB = Maris Bead

All results were the average of at least two tests. Micellar proteins were prepared by ultrafiltration.

4.7 DIFFERENTIAL SCANNING CALORIMETRY.

A differential scanning thermogram of Maris Bead flour is shown in Figure 38. There were two major transitions and a third lying between them. The isolates, proteinates and micellar proteins did not have the first low temperature transition, therefore this was attributed to starch gelatinization. The other two peaks were assigned to legumin and vicilin after comparison with the Tm and Tmax values of the purified proteins. Purified 11S and 7S proteins produced only one peak. Transition temperatures of flours, isolates, proteinates, micellar proteins and purified proteins are shown in Table 20. Peaks 2 and 3 from Maris Bead flour had higher Tmax values than equivalent peaks from isolates, proteinates and micellar proteins. Average Tmax values for the latter were 361.9 $\pm$ 0.74 K and 369.8 $\pm$ 0.74 K for peaks 2 and 3 respectively. Purified 11S and 7S proteins had the lowest Tmax values, 368.4 K and 357.4 K respectively. There were fewer values of Tm or Tmax recorded for peak 2 as the shape of the peak made determination of these values difficult.

ΔH values for denaturation of the samples varied with the type of sample. Proteinates had the lowest ΔH values (3.34 $\pm$ 0.53 cal g^{-1}) and micellar proteins the highest (5.00 cal g^{-1} for flocculated samples and 4.54 cal g^{-1} for the sample prepared with the dialyzer/concentrator); isolates had intermediate values (3.91 $\pm$ 0.05 cal g^{-1}). Aging the proteinate sample for two years was shown to slightly reduce ΔH. The proportion of total protein represented by legumin in flours, isolates, proteinates and micellar proteins was 66.0 $\pm$ 3.9%, where total protein is defined as legumin plus vicilin only, and ΔH values of 5.4 cal g^{-1} and 4.6 cal g^{-1} were used for legumin and vicilin respectively (Wright and Boulter, 1980).

Figure 38. DSC thermogram of Maris Bead flour.

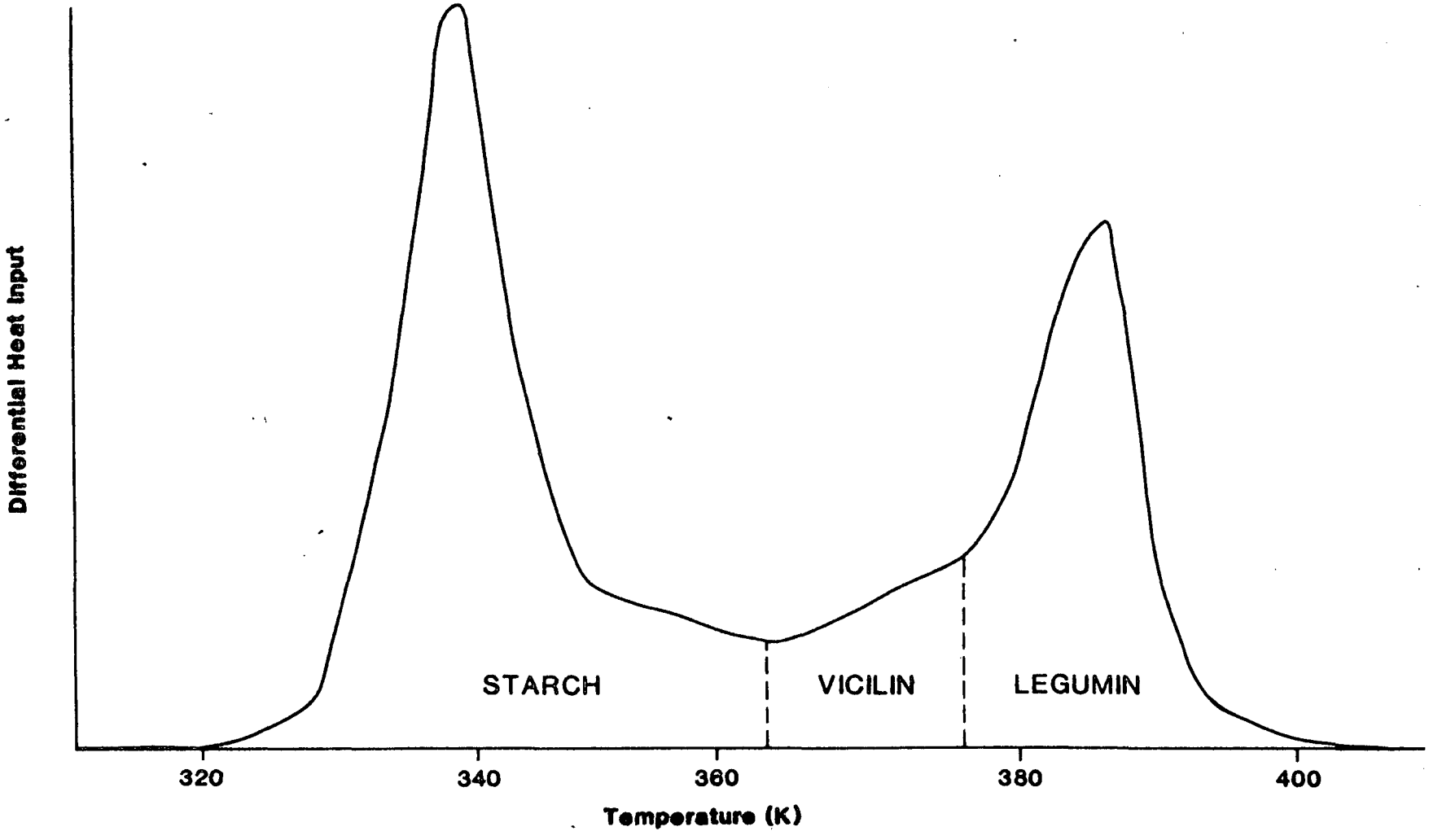


TABLE 20. Transition temperatures of Maris Bead and IVS-G flours, isolates, proteinates, micellar proteins and purified proteins.

SAMPLE	1		2		3		ΔH
	Tm (K)	T max (K)	Tm (K)	T max (K)	Tm (K)	T max (K)	Cal g^{-1}
Maris Bead flour	330.4	337.7 ±0.3		364.3 ±0.2		374.0 ±0.3	
Maris Bead proteinate						369.5 ±0.3	2.98 ±0.2
Maris Bead proteinate aged 2 years				361.5 ±1.0		369.8 ±0.32	2.80 ±0.1
IVS-G proteinate 1983 sample				361.9 ±0.2		368.8 ±0.3	3.76 ±0.2
IVS-G proteinate 1985 sample						368.7 ±0.32	3.83 ±0.3
Maris Bead isolate						370.0 ±0.9	3.55 ±0.1
IVS-G isolate				363.0 ±0.4		370.2 ±0.5	4.26 ±0.3
Maris Bead micellar protein U/F						370.6 ±0.2	4.54 ±0.1
Maris Bead micellar protein (diluted)				361.0 ±0.6		370.9 ±0.3	5.09 ±0.3
IVS-G micellar protein (diluted)				362.1 ±0.8		369.8 ±0.4	4.92 ±0.3
Maris Bead 11S					360.8 ±0.6	368.9 ±0.3	5.02 ±0.4
IVS-G 11S					360.5 ±1.1	367.9 ±1.1	5.43 ±0.4
Maris Bead 7S			347.8 ±0.9	355.8 ±0.6			4.83 ±0.3
IVS-G 7S			349.8 ±0.2	359.0 ±0.2			5.64 ±0.2

All the temperatures are the average of six samples.
U/F = Prepared by ultrafiltration.

CHAPTER 5

DISCUSSION

Leguminous seeds contain a number of constituents which are of potential interest to the food manufacturing industry, ie. oil, protein, starch and fibre. Available technology can be used to produce a range of products with varying physical and chemical properties in an attempt to meet the requirements of the food processing industry. In this thesis a number of processing methods were used, and the samples were assessed for functionality in some model tests.

Flours.

Protein contents of Maris Bead and IVS-G flours were approximately 32% on a dry weight basis (Table 1). This compares with previously reported values for Maris Bead of 32.8% and 34.7% (Bhatty, 1974). Generally spring sown varieties of Vicia faba minor, such as Maris Bead, have protein contents ranging from 25.5 - 35.4%, and winter sown varieties have slightly lower protein contents, ranging from 24.3 - 29.9% (Eden, 1968). These values also include free amino acids which may account for 0.73-1.5% of the flour and consist chiefly of arginine residues (Barratt, 1982). Varieties with a high protein content also have high levels of free amino acids. Eden (1968) reported true protein levels of 22.3 - 31.7% in 104 spring sown

varieties. This compares with typical values for crude protein of 22% and 40% for peas and soy beans respectively.

The starch content of IVS-G flour was 7% lower than that of Maris Bead (Table 1). Bhatty (1974) reported starch contents of 35.0 and 37.8% on a dry weight basis for Maris Bead. These values are lower than those found in the present study. However, he did report slightly higher values for protein content for Maris Bead, and protein and starch contents have been shown to be inversely correlated (Bhatty, 1974; Barratt, 1982). Starch contents of 28 - 40% and 35 - 53% were reported for a number of varieties of field beans by Bhatty (1974) and Barratt (1982) respectively. Typical starch contents of peas and soy beans are 47% and 0 respectively.

A comprehensive analysis of field bean composition was conducted by Eden (1968), and field bean carbohydrates were discussed by Pritchard et al. (1973).

The composition and potential uses of full-fat soy flour have been discussed by Pringle (1974). Generally full-fat products have limited market potential because of poor functionality and storage problems associated with high lipid contents (Wolf, 1975; St. Angelo and Ory, 1975; St. Angelo and Graves, 1986). This is particularly relevant to oil seeds, which store oil in preference to starch, eg. soy and peanut. Soy beans have a lipid content of 22%, compared to 1-2% in field bean (Eden, 1968).

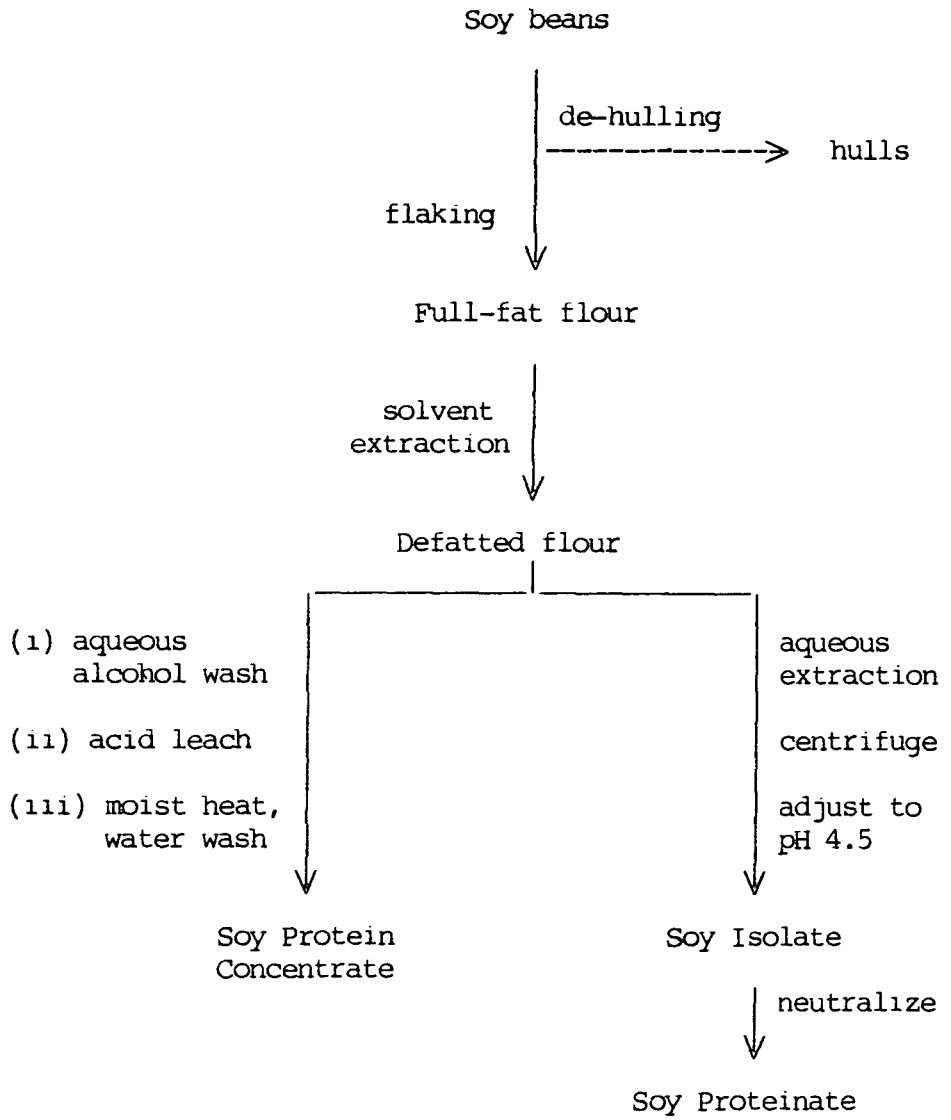
Defatting the flours marginally increased their protein contents to approximately 33% (Table 1). Defatted soy flours typically have a protein content of 56%. Extraction of lipids with hexane only removes the non-polar lipids. In pea this accounts for only 40% of the total lipid (Wright and Bumstead, 1984). The remaining fraction

consists primarily of polar phospholipids and glycolipids which can be removed by subsequent extraction in various solvent systems. However, this usually results in a loss of solubility (Wu and Inglett, 1974). Eldridge et al. (1971) showed that a mixture of 20% hexane and 80% 2-propanol caused the least subsequent insolubilization of soy bean flours. Defatted soy flours and grits form the largest part of the market for soy products in the United States, and a wide range of products differing in composition and physical properties are commercially available (Kellor, 1974).

Production of Protein and Starch Concentrates.

The technology developed for the processing of soy beans can be successfully applied to grain legume crops. The processes involved are summarized in Figure 39 (Wright and Bumstead, 1984). However, an alternative method is available for the production of high protein and high starch fractions from legumes; namely air classification. The basis of separation is the internal composition and organization of cereal and grain seeds. In legumes storage proteins are located in discrete protein bodies with a diameter of approximately 3 μm (Weber and Neumann, 1980). Protein bodies of Vicia faba cotyledons have been studied by Briarty et al. (1969), and compared to those of pea cotyledons by Weber et al. (1981). The starch is located in much larger granules, ranging in size from 25–40 μm (Vose et al., 1976). Early reports suggested that legumes contained a single population of starch granules (Biliaderis et al., 1981). However, more recent evidence suggests that some legumes contain significant populations of starch granules with a diameter of less than 10 μm (Ring, 1983). The size differential between protein and starch constituents facilitates their separation during air classification to yield protein and starch

Figure 39. Processing of soy beans.



enriched fractions.

Prior to air classification the seeds are split and milled. For the air classification process to be efficient the milling process must effectively separate the flour into its constituent particles, ie. starch granules and protein bodies.

It was noted that IVS-G seeds took longer to split on the end-runner mill than those of Maris Bead. Therefore, it is likely that seeds of IVS-G are harder than those of Maris Bead. Seed hardness, as measured by grinding time, was shown to be negatively correlated with

moisture content for field peas and faba beans by Tyler and Panchuk (1982). However, Kosmolak (1978) and Obuchowski and Bushuk (1980) reported longer grinding times for softer cultivars of wheat, and for a single cultivar as moisture content was increased. This probably reflects differences in internal structures between cereals and grain legumes. The observed differences in grinding time between Maris Bead and IVS-G seeds could not be attributed to differences in moisture content (Table 1), so must reflect a genuine difference in structure between the seeds.

Moisture contents of the flours were 11 - 11.5%. This is higher than the optimum values reported for air classification by Tyler and Panchuk (1982). They classified samples of field peas and faba beans with moisture contents ranging from 3.8 - 14.3%. Seed moisture contents of greater than 9% significantly affected both yield and composition of the air classified fractions. Seeds with low moisture contents produced fractions with decreased starch fraction yield, protein content of the starch and protein fractions and starch separation efficiency (SSE), and increased protein fraction yield, starch content of the coarse and fines fractions and protein separation efficiency (PSE). This was attributed to more efficient particle size reduction during milling in seeds with a low moisture content. During pin milling seeds are shattered as they strike a hard surface at speed. More brittle seeds would presumably undergo a greater particle size reduction. The increased starch content of the fines fraction was probably due to starch damage during the milling process, although an increased proportion of damaged starch granules was not detected by their assay.

The coarse fractions obtained from IVS-G flour below a classifier speed of 7000 rev./min were significantly larger than those obtained from Maris Bead (Table 2). PSE was also comparatively low (Table 5). This suggests that the pin milling process was less efficient for IVS-G seeds, and is contrary to expectations as IVS-G seeds appeared to be harder than those of Maris Bead. An efficient separation is dependent on complete cellular disruption during milling, causing a release of protein and starch storage organelles as separate entities. Differences in impact milling efficiency may result from differences in a) the amount of cell wall material (CWM), b) the thickness and structural rigidity of the cell wall, c) the degree of adhesion between the cell contents and the cell wall, d) the degree of adhesion between proteinaceous material and starch granules, e) the extent to which proteinaceous material is broken into unit particles, and f) the degree of adhesion between individual cells (Tyler, 1984). An increase in any of the above characteristics would cause an increase in the energy input required to achieve complete cellular disruption.

After a second pass through the pin mill the IVS-G coarse fraction was significantly reduced at all classifier speeds (Table 2a), and there was a concomitant decrease in the protein content of the fines fraction and increase in PSE (Tables 3a and 5a respectively). Protein content of the fines fraction presumably decreased due to the concentration of other cellular components in the fines fraction, eg. starch and CWM (Tyler and Panchuk, 1982). A high proportion of starch was found in the fines fraction and SSE was low at 6500 and 7000 rev./min compared to values obtained for Maris Bead (Tables 4 and 6). This may have been attributable to starch damage during the second milling step.

A number of studies have shown that repeated milling steps reduce the protein content of the starch fraction (Vose et al., 1976; Reichert and Youngs, 1978; Tyler et al., 1981; Tyler, 1984; Wright et al., 1984). The latter authors observed that repeated milling produced an increased proportion of particles with a diameter of less than 20 μm, largely at the expense of particles in the 25-30 μm diameter range. The latter were assumed to be protein body agglomerates and starch-protein body agglomerates. These have been observed using SEM (Reichert and Youngs, 1978). However, total separation of starch and protein components is not easy to achieve as a proportion of the total protein remains attached to the starch granules despite four or five milling steps. After five milling steps northern beans, field peas and cowpeas had coarse fractions with protein contents of 3.1, 2.7 and 3.3% respectively (Tyler, 1984). Colonna et al. (1980) reported coarse fractions with 2% protein from faba beans and smooth seeded peas after two and three milling steps respectively. This residual protein has been shown by SEM to consist of starch granules embedded in a protein matrix (Reichert and Youngs, 1978). After milling and air classification of peas which retained their green colour at maturity the agglomerates were shown to be chiefly composed of starch granules and chloroplast remnants. The adherent protein can be explained by consideration of the physiology of the developing and mature pea cotyledon (Bain and Mercer, 1966). From 10 - 20 days after fertilization a single starch granule is initiated in most pea chloroplasts in the embryo. The starch granule grows rapidly from days 20 - 45, disrupting the structure of the plastid and compressing the lamellae and grana against the limiting membrane of the chloroplast. Membrane remnants persist around the starch granules in the mature pea cotyledon. Similarly, Barlow et al. (1973) have demonstrated the existence of membrane remnants surrounding starch granules in wheat.

It is possible to obtain almost pure starch fractions by repeated water washings to remove the residual protein. Vose et al. (1976) obtained starch fractions with 0.15 and 0.05% nitrogen from horse beans and field peas respectively. The isolation of pure starch by conventional wet processing methods is also difficult due to the presence of a highly hydrated fine fibre fraction, probably derived from the cell wall (Vose et al., 1976).

However, there are disadvantages in using repeated milling steps. As mentioned previously the protein content of the fines fraction tends to decrease with successive milling steps. This is accompanied by an increase in starch content, attributable to damage of starch granules during milling (Vose, 1977; Reichert and Youngs, 1978). Vose (1977) observed that the proportions of starch granules susceptible to enzymic hydrolysis were 21.8, 5.8 and 23.9% after two milling steps, and 39.6, 8.8 and 32.6% after four milling steps for pea, corn and wheat starches respectively. SEM demonstrated that some starch granules had been shattered whilst others had superficial exfoliation of the surface. Reclassification of the flour results in some of this starch being classified into the fines fraction, causing a decreased protein content in the fines fraction and SSE, and an increased starch content of the fines fraction.

Damaged starch granules were shown to exhibit increased viscosity during pasting compared to native starch granules.

At 3000 and 4000 rev./min the major component of the coarse fractions from both Maris Bead and IVS-G was visibly hull material. This was reflected in the low protein and starch contents of these fractions (Tables 3 and 4). Wright et al. (1984) observed that at 3000 rev./min the coarse fraction from pea had a high cellulose,

hemicellulose and pectin content. Furthermore, the balance of sugars in this fraction was similar to that of isolated hull material. Hull material has been shown to account for approximately 13% of seed weight in various cultivars of Vicia faba (Griffiths, 1981). Partition of hull material into the coarse fraction has also been observed by Vose et al. (1976), Kon et al. (1977), and Sosulski and Youngs (1979). It is apparent that pin milling is not an effective method for grinding the testa.

Hull material from peas has found a commercial outlet as a fibre supplement in white bread. It has a significantly higher fibre content (75%) than wheat bran (46%), (Wright et al., 1984). A similar use of Vicia faba hulls may be limited by the significant quantities of tannins that have been reported for some varieties (Griffiths and Jones, 1977; Martin-Tanguy et al., 1977; Moseley and Griffiths, 1979). Tannin content has been positively correlated with coloured flowers in Vicia varieties (Griffiths, 1981), and with seed coat colour in Phaseolus beans (Elias et al., 1979). Maris Bead and IVS-G flowers are off-white in colour with some darker petal markings. Furthermore, Maris Bead seeds are dark brown in colour. IVS-G seeds vary in colour from light green to buff. It is probable that both contain significant tannin levels, although IVS-G may have lower levels than Maris Bead. Tannins have been shown to a) have an astringent taste, b) adversely affect protein solubility, and c) to inhibit digestive enzymes including trypsin, α-amylase and lipase (Griffiths and Jones, 1977; Griffiths, 1979). Whether these properties are significant at the appropriate inclusion rates for hull materials into processed foods needs further investigation. The use of white seeded or white flowering types would circumvent these problems.

Hull material does not contribute to the functional or nutritional properties of flours or protein concentrates. Dehulling has been shown to improve the functional properties of Phaseolus vulgaris flours (Deshpande et al., 1982), and the nutritional properties of Vicia faba flours when fed to animals (Nitsan, 1971; Marquardt and Campbell, 1973; Martin-Tanguy et al., 1977; Moseley and Griffiths, 1979; Fowler, 1980).

Air classification of Maris Bead and twice-milled IVS-G flours generally gave similar results, with the exception of at 3000 rev./min where IVS-G flour produced a significantly larger coarse fraction (Table 2). This could be attributed to less efficient size reduction of either hull material or starch protein agglomerates. Air classification results for Maris Bead and twice-milled IVS-G flours were compared with those of Wright et al. (1984) for the air classification of Filby pea flour performed under similar conditions. The major differences observed were as follows:

1) at 3000 rev./min the coarse fractions of Maris Bead and IVS-G were larger. This can be attributed to a larger proportion of hull material in the Vicia faba beans. Griffiths (1981) reported hull contents of 8.2 and 13% for Filby pea and Vicia faba varieties respectively.

2) from 4000 - 6500 rev./min Filby pea produced larger yields of coarse fraction, attributable to the higher starch content of peas.

3) protein percentages were higher in both coarse and fines fractions for Maris Bead and IVS-G, reflecting the higher initial protein content. The higher protein content of the coarse fraction was presumably due to protein body agglomerates and starch-protein body agglomerates.

4) Starch percentages were higher in both coarse and fines fractions for Filby pea. This was attributed to its higher initial starch content. The higher starch content of the fines fraction was probably due to either a population of small starch granules or a larger number of damaged starch granules.

5) PSE was higher for Maris Bead and IVS-G flour. The higher starch content of Filby flour would result in an increased proportion of protein of chloroplast origin associated with the starch granules. This protein is more difficult to separate during milling.

6) SSE was higher for Filby pea flour.

The highest starch and lowest protein containing fractions for both Maris Bead and IVS-G seeds were obtained in the coarse fraction produced at 6500 rev./min. This corresponds to a cut point of 18.5 μm. Wright et al. (1984) analysed particle size distributions of a) pea flour produced by a single pass through a Kolloplex mill, b) pure pea starch, and c) fines and coarse fractions obtained by air classification, using a Coulter counter. They found 90% of particles had a diameter of less than 40 μm in the milled flour. The peak for pure starch was at 22 μm, and a peak in the whole flour was observed at 22.5 μm. Analysis of the coarse and fines fractions showed that cut point was not sharply defined during air classification. However, plotting cut point against fines fraction yield does give some information on particle size distribution (Figure 2). 82 and 74% of Maris Bead and IVS-G particles respectively had a diameter of less than 40 μm. There was a bulge in the graphs showing a large proportion of particles between the cut points of 16 and 24 μm. Coarse fractions obtained between these cut points had the highest starch contents, indicating that the bulk of the starch granule population occurs in

this region (Figures 3 and 4). Protein content of the fines fraction declined steeply between 10 and 16 μm, indicating that most proteinaceous material had a particle diameter of less than 10 μm. Individual protein bodies have a diameter of approximately 3 μm (Weber and Neumann, 1980). However, protein body agglomerates would be of a greater size. Peaks corresponding to particle sizes of 2,4,7 and 11 μm were observed in whole pea flour by Wright et al. (1984). These were all attributed to protein body agglomerates. In the sequential classification experiment 14% of the flour had a particle diameter < 10 μm, and this fraction had a protein content of 75% (Table 8, Figure 6). Relatively small quantities of flour were obtained between the cut points of 10 and 18 μm. These fractions also had a high protein content and presumably consisted largely of protein body aggregates. Fines fraction size increased dramatically between cut points of 18 and 30 μm, and was accompanied by an equally dramatic fall in protein content. This is largely attributable to the fractionation of starch granules into the fines fraction. At a cut point of 40 μm the coarse fraction was composed predominantly of hull material.

A single milling and air classification yielded flours with up to 75% protein. However, this accounted for only 29 and 36% of the total protein in IVS-G and Maris Bead flours respectively. Comparative figures for Filby pea were 55% protein accounting for 29% of the total protein (Wright et al., 1984). Conventionally protein concentrates produced by the air classification method undergo an initial milling and air classification step, followed by remilling and classifying the coarse fraction. The two fines fractions are combined to give the protein concentrate. This results in a large yield of high protein product. The coarse fraction obtained at 6500 rev./min was used for

the reclassification experiment as it had the highest starch content, and the corresponding fines fraction had a reasonable yield and protein content (Tables 2,3 and 4).

The initial classification at 6500 rev./min of the reclassification experiment produced a PSE value significantly lower than in the previous experiment (Table 5; Figure 5). However, at 7000 rev./min in the original experiment PSE was similar to that obtained at 6500 rev./min in the reclassification experiment. The difference in cut point obtained at these two classifier speeds is very small, and so the results are not that surprising. Air classification does not produce a sharply defined cut point, and furthermore, the starch granule size peak (22 μm) is very close to the cut point used (19 μm). A small change in operating conditions could result in a different proportion of the starch granule population being classified into the fines fraction.

Remilling the coarse fraction shifted some of the proteinaceous material, presumably present as agglomerates of protein bodies or starch - protein bodies into the fines fraction. The three re-classification speeds used represent cut points of 18.5 - 22 μm, and were selected as they produce relatively large fines fractions with high protein contents. The small differences observed in the concentrate yields and compositions reflects the small differences in cut point used, and the proximity of these cut points to the peak in starch granule size (Table 7). Relatively few starch granules were shifted into the fines fraction even at the lowest classifier speed used. However, protein concentrates had a starch content of approximately 20%. A number of factors may have been responsible for this. They are: a) the overlap in particle size distributions obtained in the coarse and fines fractions by air classification (Wright et

al., 1984), b) a population of smaller starch granules (Ring, 1983), and c) a proportion of starch granules which sustained damage during the milling process (Vose, 1977). It should prove possible to reduce this starch content by reducing the cut point used. However, this will have a detrimental effect on the fraction yield.

Wright et al. (1984) produced protein concentrates from pea after three milling and air classification steps with yields of 35.1 and 26.6%, protein contents of 49.5 and 54.1%, and residual starch contents of 8.1 and 3.9% at 9000 and 11000 rev./min respectively. Starch concentrates with yields of 64.9 and 74.3%, starch contents of 65.4 and 61.3% and residual protein contents of 7.1 and 10.2% were produced at 9000 and 11000 rev./min respectively. The lower yield of protein concentrate with equivalent protein content reflects the lower protein content of the pea compared to faba beans. The higher yields of protein concentrate from bean flours should make their production more of a commercial viability, providing they have desirable functional and flavour characteristics.

The yields of starch and protein concentrates are slightly higher and lower respectively than those obtained for horse beans by Vose et al. (1976) using different operating conditions. They also achieved higher starch (69%) and protein (61%) contents in their starch and protein concentrates respectively, and lower amounts of residual protein (5%) and contaminating starch (11%). Tyler et al. (1981) produced a lower yield (37%) of protein concentrate with a higher protein content (70%) and low starch content (4%) from Vicia faba minor cv. Diana. They also produced a starch concentrate containing 81% starch, and only 8% protein.

A number of factors contribute in determining the yield and composition of starch and protein concentrates. They are:

a) Initial protein content. Generally higher yields and protein contents of protein concentrate are obtained from beans with a higher protein content. However, Vicia faba minor cv. Diana and Vicia faba equina L. had similar and lower protein contents respectively compared to Maris Bead and IVS-G seeds.

b) Moisture content. Both of the above studies used flour equilibrated to a moisture content of 8%. This may have improved impact milling efficiency (Tyler and Panchuk, 1982).

c) Milling procedure. Both of the other experiments used an Alpine Contraplex 250 CW pin mill. Wright et al. (1984) observed that a greater yield of fines fraction, and a higher protein recovery in the fines fraction were obtained during air classification of Filby pea flour when the Contraplex mill was used instead of the mill employed in this study. Particle size reduction was probably more efficient during milling in the other two studies.

d) Type of air classifier used.

e) Differences in impact milling efficiency,

and f) differences in starch granule size distribution.

Further experiments are needed to investigate the possibility of increasing the purity of starch and protein concentrates without significantly compromising yields, by optimizing the operating conditions. A change in the milling procedure would probably have the most significant effect on the yields and compositions of the concentrates.

Protein concentrates have been prepared commercially in Canada from navy beans and field peas, and compete with imported soy products (Reichert, 1982). Soy protein concentrates typically have a protein content of 72%. Their composition, nutritional, functional and quality characteristics have been assessed by Mattil (1974). The protein concentrates produced in this study are more equivalent in protein content to a defatted soy flour (56% protein). However, ultimately their use will depend on functional and organoleptic properties.

Starch concentrates have found applications in potash ore refining, as adhesives for corrugated board production, and as pressure sensitive microcapsule coatings for carbonless paper (Reichert, 1982), as well as applications in the food industry (Luallen, 1985).

Protein yield (PY) from all of the air classification experiments was generally less than 100% (Table 5). PY was lowest at high classifier speeds, where the fines fractions were small. Total protein recovery is not achieved in practice as very fine particles are carried through to the air filter by the classifying air stream (Wright et al., 1984). Values of PY may have been higher for IVS-G as particle size reduction was less efficient, resulting in fewer very fine particles. Values for starch yield (SY) were generally higher than those for PY. However, at 3000 rev./min low values of SY were obtained. This was attributed to sampling difficulties due to the heterogeneous nature of the coarse fraction.

Air classification has considerable advantages over the more conventional wet processing methods originally developed for the production of soy flours, protein concentrates and isolates (Figure 39). Damage to protein constituents may occur at several stages during

wet processing, eg. during solvent extraction, desolventizing or removal of soluble sugars. The effects of protein denaturation on functionality have been reviewed by Wu and Inglett (1974). The process is also comparatively expensive as energy input is required to dry the product after solvent extraction, sugar removal or isolate production, and effluent has to be disposed of. Furthermore, the activity of lipoxidases during processing may lead to the rapid development of rancid and painty odours or flavours (Kon et al., 1970). Heating the sample to control lipoxidase activity can result in further denaturation and loss of functionality. In contrast dry processing, by milling and air classification, leaves the protein in its native state inside the protein body. Such protein might be expected to have superior functional characteristics.

However, there are some problems associated with the air classification process. Reichert (1982) showed that the composition of air classified fractions was dependent on the initial protein content of the flour. For varieties which show significant variations in protein content it may prove difficult to produce concentrates with consistent starch and protein contents. A uniform product is of obvious importance to the food manufacturer. Also, as environmental factors affect the compositions of individual crops it may prove necessary to optimize processing conditions for each batch. This would be expensive in time and operator costs. For leguminous crops with low protein contents it may prove impossible to produce adequate yields of protein fractions with a sufficiently high protein content for commercial applications. However, starch concentrates produced from such crops would tend to be large and have low protein contents. The development of markets for the utilization of starch concentrates may prove important if leguminous grain crops are to compete with the oil

bearing seeds. The use of starch as a functional ingredient for the food industry has been recently reviewed by Luallen (1985). Its major uses are as thickeners, a source of carbohydrate, stabilizers, texturizers, water or fat binders, and emulsification aids.

Not all legume flours are suited to the air classification technique. Starchy legumes with high lipid contents are not effectively separated as the lipid causes the flour to agglomerate, eg. chickpea flour (Sosulski and Youngs, 1979). Also, the technique has no practical application for oil bearing seeds which have no differential particle size distribution, eg. soy beans (Ireland et al., 1986).

Various seed components have been shown to fractionate with the protein into the fines fraction. The most significant of these is the partition of lipid (Vose et al., 1976; Kon et al., 1977; Sosulski and Youngs, 1979; Tyler et al., 1981; Wright et al., 1984). Protein bodies of air-dried peas have been shown to be coated with a lipid rich structure (Swift and Buttrose, 1973). Lipid contents of up to 7% have been reported in pea protein concentrates. Problems associated with high lipid contents are discussed in the next section. Sugars also tend to fractionate into the fines fraction. These include sucrose, raffinose, stachyose, galactose, and arabinose (Kon et al., 1977; Wright et al., 1984). The latter two are associated with cell walls. Raffinose and stachyose have been implicated as contributing to flatulence (Wagner et al., 1977). MacArthur and D'Appolonia (1976) have shown a similar fractionation of sugars during air classification of wheat. Other components known to fractionate into the fines fraction include ash, crude fibre of cell wall origin, phosphorous, sulphur, potassium, zinc, calcium and phytate (Vose et al., 1976; Kon et al., 1977; Reichert, 1981; Elkowicz and Sosulski, 1982).

Isolates, proteinates and micellar proteins.

In the fabrication of processed foods frequent use is made of isolates. These isolates are of commercial interest because they have a bland appearance and taste, and improved nutritional and functional properties compared to flours and protein concentrates. They can be used as protein supplements in cereal based foods (eg. Thompson, 1977; Fleming and Sosulski, 1977), or as functional ingredients in processed foods. Compositional, nutritional, functional and quality aspects of soy isolates have been assessed by Mattil (1974).

Soy isolates generally have a protein content of greater than 90%. Vicia faba isolates and proteinates were produced with protein contents of 87 and 83% (N x 5.7) respectively. This is slightly lower than that of commercial soy isolates, but compares with values obtained for pea isolates (Sumner et al., 1981). The isolates were cream coloured, but formed darker beige products when they were neutralized. Fan and Sosulski (1974) and Fleming et al. (1975) produced tan coloured isolates from Vicia faba minor seeds. This was presumably due to the presence of pigments in the flours, and may be a problem in the utilization of these products in some fabricated foods. Sumner et al. (1981) suggested that coloured products were formed by oxidation of polyphenols during freeze drying. Murray et al. (1985) reported a 0.84% phenolic acid content in dehulled Vicia faba flour, and phenolic acids in flours and hulls and their involvement in producing coloured compounds was studied by Sosulski and Dabrowski (1984). Fleming et al. (1975) suggested that phenolic acids are oxidized to quinones, and complex with proteins under alkaline conditions as described by Sabir et al. (1974). This would explain the

development of colour after neutralization to form the proteinate.

Micellar proteins with 98% protein (N x 5.7) were produced from both Maris Bead and IVS-G flours. This compares with a value of 96% protein (N x 5.85) obtained by Murray et al. (1981). This is much higher than the protein contents of isolates and proteinates, and demonstrates the effectiveness of the method in excluding other compounds, eg. lipids and phytate. The two methods used for preparing micellar proteins yielded products with significantly different characteristics. The micellar protein formed without ultrafiltration precipitated very quickly when water was added. A thick gelatinous mass was formed at the bottom of the beaker, as previously observed by Murray et al. (1981). The freeze dried product was an off-white colour and more dense than the isolate and proteinate preparations. However, the micellar proteins formed after reduction of the supernatant by ultrafiltration did not precipitate so quickly or form a thick viscous layer in the bottom of the vessel. Precipitation was allowed to proceed over-night in a cold room, but in some cases a centrifugation step was necessary to collect the precipitated protein. The freeze-dried product resembled the isolate preparation in appearance and physical characteristics. It was considered to be more equivalent to a isolate.

Proteinates, isolates and micellar proteins were analyzed by SDS-PAGE and shown to be chiefly composed of the major storage proteins of Vicia faba, ie. legumin and vicilin (Figure 7). These proteins account for 80% of the total seed protein in V.faba, and are discussed in greater detail in the following section. Protein bands observed by SDS-PAGE of the flours, but absent from proteinates, isolates and micellar proteins probably correspond to proteins from the more soluble albumin fraction.

To test the homogeneity of the preparations they were subjected to gel filtration on Superose 6 (Figure 8). The micellar protein preparation was difficult to get into solution, so was not used. Insoluble material was removed by centrifuging all of the samples prior to testing. Therefore, only soluble aggregates were observed. The largest amount of high molecular weight material (Mr. approx. 3 million) was observed for the isolate. This probably reflects a greater extent of protein aggregation, possibly induced by acid precipitation. In these experiments severe but localized acid denaturation was avoided by adding dilute acid dropwise whilst stirring the sample. Arntfield and Murray (1981) demonstrated, using DSC analyses, that some denaturation does occur during isoelectric precipitation of Vicia faba proteins. Lillford and Wright (1981) observed that all of the soy protein precipitated at pH 4.5 could be recovered by adjusting the pH to 7.6, providing that the sample was freshly prepared. However, some of this protein may have been denatured but still retained its solubility. Alternatively, Vicia faba proteins may be more sensitive to isoelectric precipitation. The isolate also contained a large proportion of low molecular weight species. These could have been either dissociation products of the 7S or 11S components, low molecular weight proteins, eg. whey proteins, or proteolytic breakdown products formed during extraction. Whey proteins should remain in solution during isoelectric precipitation, so their involvement is unlikely. The former explanation is the most likely as SDS-PAGE revealed that most of the protein corresponded to subunits of the major storage proteins. However, some proteolytic breakdown products may also have been present, as some low molecular weight bands were observed by SDS-PAGE. Proteolytic breakdown products from vicilin subunits have been identified by Scholz et al. (1983).

The isolates produced by ultrafiltration had small amounts of low molecular weight material. The pore size in the membranes used provided a cut point of Mr. approx. 50 000. Low molecular weight proteins would be removed in the filtrate and only proteins in a relatively native state retained.

Although isolates, proteinates and micellar proteins have many superior qualities compared to protein concentrates, flours and grits there are also some disadvantages associated with their production.

Lipids tend to concentrate with the protein fraction during isolate and proteinate preparation, and may amount to 7.7 and 12% of isolates prepared from defatted or full-fat flours respectively (Wright and Bumstead, 1984). At these levels storage problems and effects on functionality become more pronounced. Storage problems emanate from the action of lipoxygenase on fatty acids to form hydroperoxides, which may react further to form a wide range of volatile compounds, giving rise to undesirable flavours and odours (Gardner, 1975; Rackis et al., 1979). Lipoxygenase has a strong affinity for linoleic acid which accounts for 55% of the component fatty acids in Vicia faba (Hinchcliffe et al., 1977). Faba bean lipoxygenases have been isolated by Eskin and Henderson (1974). However, as most faba bean fatty acids exist in esterified form as triacylglycerides and phospholipids the actions of lipases and phospholipases are also important. These enzymes have been isolated and characterized by Dundas et al. (1978) and Atwal et al. (1979). Henderson et al. (1981) proposed an overall mechanism for lipid degradation in faba beans. Lipids and their degradation products can bind to proteins and interfere with functional properties. In some cases it may be necessary to remove them using special solvent systems (Wolf, 1975). These lipid protein complexes have been studied by St.

Angelo and Ory (1975) and St. Angelo and Graves (1986).

Drying of isolates, proteinates and micellar proteins can be expensive. Also, the method of drying has been shown to affect the composition and appearance of pea isolates (Sumner et al., 1981). Drum-drying resulted in isolates with a toasted flavour and darker colouring. Freeze-drying produced isolates with a cerealy flavour, and was also implicated in the formation of coloured products. Spray-drying produced isolates with the most bland flavour and appearance. However, sodium proteinates generally had superior flavour characteristics compared to isolates, but were darker in colour.

Finally, the production of whey type liquids during isolate, proteinate and micellar protein production is a potential water pollution threat. This is also a perennial problem for the cheese industry, as 20% of milk proteins are contained in the whey fraction (Burgess and Kelly, 1979). However, whey proteins can be formed into useful by-products using ultrafiltration to concentrate the useful constituents (Lawhon et al., 1974). The resultant whey fraction can either be used directly in animal feeds, or separated to yield a soluble protein fraction, a soluble carbohydrate fraction and water for recycling (Vose, 1980). Whey proteins have been shown to have useful functional and nutritional properties (Burgess and Kelly, 1979; To et al., 1985).

Ultrafiltration with small pore size membranes has been used directly in the production of isolates from cotton seed, field pea and horse bean (Lawhon et al., 1977; 1978; Vose, 1980). These isolates will also contain whey proteins, sugars and ash, so would be expected to have different functional properties. The method also totally avoids the need for effluent disposal.

The effects of these processing methods on solubility and denaturation are discussed in subsequent sections.

Characterization of Purified Proteins.

The subunit structure of Maris Bead legumin has previously been studied by Maplestone et al. (1985). On non-reducing gels they observed a distinct band corresponding to a Mr. of 56 000, and fainter bands corresponding to Mr. values of 65 - 70 000 and 40 000. Similar results were obtained in this study but the 40 000 Mr. band was more distinct and the bands corresponding to Mr. values of 65 - 70 000 were either very faint or indiscernible (Figure 10). Under reducing conditions major bands were observed corresponding to Mr. values of 36 000, 23 000 and 21 000. This is in general agreement with previous studies (Wright and Boulter, 1974; Maplestone et al., 1985).

Typically purified vicilin does not migrate as a sharply defined band during electrophoresis under non-denaturing conditions (Ibuchi and Imahori, 1978; Gatehouse et al., 1981). Vicilin from V-faba in these experiments yielded a single broad peak during gel filtration and analytical scanning ultracentrifugation. Considerable heterogeneity of vicilin proteins has been demonstrated. Four major subunits with Mr. values of 66 000, 60 000, 55 000 and 38 000 were produced by SDS-PAGE. This is in agreement with the findings of Bailey and Boulter (1972). The subunit pattern was not altered by electrophoresis in the presence of 2-mercaptoethanol.

Legumin yielded more than one peak by gel filtration and during analytical scanning ultracentrifugation (Figures 11 and 12 respectively). Peaks 3 and 6 obtained by gel filtration were assigned a Mr. value of 600 000. This was considered to be a dimerized form of the 11S protein. When the protein was subjected to SDS-PAGE it formed

an identical band pattern to that of the 11S protein. A small peak with an Sobs value of 14-16S was observed during analytical ultracentrifugation. This was also attributed to dimerized 11S proteins. Some much larger aggregates were also observed during gel filtration of legumin preparations, particularly in those samples which were dialyzed against distilled water. This could be explained by the formation of disulphide linkages after precipitation during dialysis, or during lyophilization (Hoshi and Yamauchi, 1983). The addition of ammonium hydrogen carbonate rendered the protein more soluble. Sedimentation coefficients were similar to previously reported values (Derbyshire et al., 1976; Mori and Utsumi, 1979).

Solubility characteristics.

Dispersibility profiles of Maris Bead and IVS-G protein preparations were similar in character to those reported previously for peanut isolates (Rhee et al., 1973), soy isolates (Shen, 1976a; Hermansson, 1979), and mung bean, field pea, fababean, chick pea, lupin, lentil, pea bean and lima bean flours (Fan and Sosulski, 1974). Minimum nitrogen solubility occurred at pH values between 4 and 6. However, mung bean, field pea and soy bean had very narrow apparent isoelectric points, faba bean, chick pea and lupin had intermediate width ones, and lentil, pea bean and lima bean had a broad minimum solubility range. The results presented in this thesis are not directly comparable with those in the literature as different protein concentrations and methods were used. It was necessary to use 10% protein concentration as gel formation would not have occurred at lower levels. At higher protein concentrations minimum protein solubility is lower than at lower protein concentrations, and the apparent isoelectric point is broader (Figures 14 and 15). Shen (1976b) observed no effects on protein solubility using concentrations

up to 10%. However, different experimental procedures were employed. Smith et al. (1966) and Betschart (1974) did observe decreased extractability with increasing protein concentrations up to 10% for soy protein and leaf protein respectively. Blending speed, centrifugation time and intensity, equilibration time, temperature and effectiveness of mixing all affect the values obtained for solubility (Shen, 1976b).

Maximum nitrogen solubility was observed between pH values of 2 - 3.5 and >7 (Figures 19-22). Net charge on the protein molecules is highest at these pH values, and so intermolecular repulsive forces render the protein more soluble. Protein-protein interactions are favoured at intermediate pH values, resulting in some protein precipitation.

The proteinates were more soluble than the micellar proteins over the entire pH range, although the latter were expected to be in a more native state (Figures 19 and 21). The IVS-G micellar protein was prepared by ultrafiltration, and as discussed previously did not exhibit typical micellar protein characteristics. Extensive protein-protein interactions occur during micelle formation and these may be stabilized by subsequent disulphide bond formation, thus rendering the protein insoluble (Murray et al., 1981). Protein-protein interactions were less extensive in the IVS-G preparation as rapid protein precipitation did not occur. Isolates would be expected to have intermediate solubility characteristics compared to proteinates and micellar proteins. This is substantiated by protein contents of the 20% solutions at two pH values (Table 13). Lillford and Wright (1981) demonstrated that disulphide linkages are formed during storage of soy isolates rendering them less soluble. This could be reversed by the addition of reducing agents. Decreased solubility after

isoelectric precipitation of soy proteins has also been observed by Nash and Wolf (1967), Nash et al. (1971) and Anderson (1974). Improved solubility characteristics of proteinates, as compared to isolates, have also been observed for leaf proteins (Lu and Kinsella, 1972; Betschart, 1974). This can be attributed to a change in the surface distribution of charge on the protein molecules caused by adjusting the pH, and the higher salt content of the proteinate. Both of these factors increase solubility at the expense of protein-protein interactions.

The use of solubility profiles as a measure of protein denaturation is clearly not valid in comparing these samples. Furthermore, Hermansson (1979) reported that a completely denatured soy isolate could have a solubility profile similar to that of a native soy isolate. Differential scanning calorimetry (DSC) analysis is a more reliable indicator of denaturation, and has been used to monitor the effects of processing on soy isolates (Hermansson, 1979), field bean isolates and micellar proteins (Murray et al., 1981) and field pea, faba bean, soy and canola isolates (Murray et al., 1985). DSC analyses are discussed in the next section.

Turbidity values closely mirrored nitrogen solubility levels. However, high turbidity values were observed at relatively low protein concentrations for the Maris Bead micellar protein. Populations of vicilin micelles can undergo dynamic interactions. The most extensive network formations occur at pH 6 - 7, where extensive protein sheets are formed. As the pH increases these sheets may break up to form small micelles in aggregates or single micelles which can exist in solution (Ismond et al., 1986a). Similar populations are the likely cause of the high turbidity values observed (Figure 21). However, changes in turbidity may be attributed to changes in the number, size

or optical properties of particles.

Full-fat Maris Bead proteinate was more soluble than the defatted sample (Figures 19 and 20). However, a large proportion of the protein was in solution in a colloidal state. The apparent isoelectric point was also narrower than for the defatted sample. The interference of lipid-protein interactions with protein-protein interactions requires further investigation. St. Angelo and Graves (1986) observed increased solubility of full-fat peanut proteins compared to defatted samples. However it is possible that the defatting procedure was responsible for the loss of solubility.

The differences in sediment volume observed between proteinates and micellar proteins probably reflects the different structural characteristics of the samples. Micellar proteins may undergo extensive coalescence to form amorphous three-dimensional structures (Ismond et al., 1986a). These would be expected to pack very closely, resulting in low sediment volumes (Figures 13, 14 and 17).

The effects of salt on the dispersibility characteristics of a defatted Maris Bead proteinate were investigated at pH 7.7. In the absence of salt virtually all of the protein was extracted. Increasing the salt content to 2% caused a decrease in protein solubility (Figure 24). The effects of salt are pH dependent. Hermansson (1979) reported a sharp decrease in the protein solubility of a soy proteinate (pH 7) when the salt concentration was increased from 0 - 0.1M. Raising the salt content to 0.4M increased protein solubility. However, at pH 5 increasing the salt concentration increased solubility from < 10% with no salt to approximately 60% at 0.5M salt. Furthermore, isolates exhibited different behavioural characteristics. Small concentrations

of calcium chloride also profoundly affect solubility on the alkaline side of the isoelectric point (Hermansson, 1979). This is of particular significance in the fabrication of milk type products.

The effects of temperature.

The effects of temperature on food proteins have recently been reviewed by Kilara and Sharkasi (1986). The use of heat in the preparation of protein-rich products has been shown to have an adverse effect on the subsequent solubility of proteins from milk whey (Morr et al., 1973), soy beans (Wolf, 1970; Johnson, 1970), alfalfa leaves (Betschart, 1974), blood (Tybor et al., 1975), cottonseed (Lawhon and Cater, 1971) and great northern beans (Pilosof et al., 1981). However the use of heat is frequently necessary to destroy anti-nutritive factors and improve flavour characteristics.

Protein denaturation, caused either by heating or other processing methods, can be measured using DSC analysis. A large ΔH value indicates a relatively undenatured protein. Also the determination of denaturation temperatures for major bean components assists in predicting the behaviour of products in cooked foods by indicating the temperatures at which gelling or other major physical changes may occur.

The first major peak observed for the bean flour had a Tmax value of 337.7K, and was attributed to starch gelatinization (Table 20). The observed values of Tm and Tmax were in close agreement with previously reported values for Vicia faba (Biliaderis et al., 1980), but lower than those reported by Wright and Boulter (1980). This may be attributable to the different types of bean used; faba bean, horse bean and broad bean respectively. Starch gelatinization is important in a number of food processes, eg. the baking of bread and cakes,

extrusion, thickening and gelling. The observed starch gelatinization temperature is also similar to that reported for corn and potato starches (Biliaderis et al., 1980), suggesting that it may have potential applications in foods where corn and potato starches are conventionally used.

The transitions due to denaturation of vicilin and legumin from Maris Bead flour had similar values of Tmax to those reported previously by Wright and Boulter (1980). However, purified proteins had lower values of Tmax indicating that they are somehow stabilized against denaturation in the flours, possibly due to differences in the water environment. This phenomenon was previously reported by Wright and Boulter (1980). A broad peak was observed for vicilin, indicating heterogeneity in this protein.

The highest ΔH values were obtained for the micellar proteins suggesting that this processing method produces the least denaturation (Hermansson, 1979; Arntfield and Murray, 1981; Murray et al., 1985). The ultrafiltration product was also relatively undenatured. Isolates and proteinates were denatured to varying extents, possibly due to acid precipitation or protein aggregation.

Other characteristics which have been used to measure the extent of denaturation include a) optical properties, measured by optical rotatory dispersion (ORD) or circular dichroism (CD) (Kato et al., 1981; Matsuda et al., 1981), b) visible and u.v. spectra (Hermansson, 1979), and c) fluorescence and polarization properties (Yamagishi, 1982).

Gelling.

Many studies have been made on the factors involved in the gelling of soy proteins. Considering the structural similarities between legume proteins it is likely that similar mechanisms are involved in the gelling of V. faba proteins.

Heating a solution of soy proteins produces an irreversible viscous state (PROGEL). Cooling may result in gel formation, although this step may be reversed if heat is subsequently applied (Catsimpoolas and Meyer, 1970). The use of salts, reducing agents, denaturants and water miscible solvents has yielded information on the mechanisms involved in the soy protein gelation process (Circle et al., 1964; Catsimpoolas and Meyer, 1970; Hashizume et al., 1975; Shimada and Matsushita, 1980; Babajimopoulas et al., 1983; Utsumi and Kinsella, 1985a). These mechanisms have been shown to differ for gels formed from either a mixed globulin fraction or 7S or 11S fractions (Hashizume and Watanabe, 1979; Shimada and Matsushita, 1980; Utsumi and Kinsella, 1985a; b; Hermansson, 1985). These are summarized below.

The 11S protein undergoes a thermal transition at 90°C (Hashizume and Watanabe, 1979; German et al., 1982). In dilute solutions the 11S protein dissociates into subunits, and this is followed by aggregation and precipitation of the basic subunits, due primarily to hydrophobic interactions. This precipitation is enhanced in the presence of low concentrations of 2-mercaptoethanol (Catsimpoolas et al., 1967; Wolf and Tamura, 1969; Catsimpoolas et al., 1970; Catsimpoolas and Meyer, 1970; Mori et al., 1981a; German et al., 1982; Utsumi et al., 1984). At protein concentrations of approximately 14% gelling may occur. Two mechanisms for gel formation

have been proposed. Nakamura et al. (1984b; 1985a) and Mori et al. (1981a) suggested that heating causes only minor changes to the quaternary structure of the 11S oligomers. The 11S proteins polymerize to form strands which interact to form macroaggregates, which form the gel network. Hermansson (1985) suggested that dissociation occurred by the same mechanism as in dilute solutions, but there was no separation into acidic and basic subunits. Subunit pairs reassociated to form strands which were equivalent to the polymerized aggregates observed by Nakamura et al. (1984b) and Mori et al. (1981a). The strands were built up of hollow cylinders with an external diameter of 12-15 nm (Hermansson, 1985). The formation of strands as a result of aggregation, followed by interaction of the strands to form a gel matrix has been observed for other globular proteins (Tombs, 1974).

The contribution of individual acidic subunits to gel formation is not uniform. In particular acidic subunit III (ASIII) has been shown to promote gel hardness in reconstituted pseudoglycinins from a) isolated acidic subunits + total basic subunits (Mori et al., 1982), and b) from intermediary subunits (Nakamura et al., 1985b). Utsumi and Kinsella (1985b) demonstrated that ASIII was difficult to solubilize from gels. Similarly ASII has been shown to contribute to gel hardness in reconstituted pseudoglycinins from broad bean (Utsumi et al., 1983). Conversely, ASIV contributed marginally to gel formation. ASIV is the most acidic of the acidic subunits, and is not linked to its corresponding basic subunit by a disulphide bond (Mori et al., 1981a; 1982). However, its presence has been shown to reduce by half the time required for gelling to occur. This has been attributed to the more heat-labile nature of the non-covalent linkage between the acidic and basic subunits, resulting in conformational changes in glycinin which stimulate the formation of soluble

aggregates, and subsequent polymerization (Mori et al., 1981a). The release of ASIV from glycinin during heating has been reported (Mori et al., 1982; Nakamura et al., 1984a; 1985a). Furthermore it is the most readily solubilized acidic subunit from preformed gels (Utsumi and Kinsella, 1985b).

The gelling mechanism of the 7S protein has been less well studied. The 7S protein undergoes a thermal transition at a lower temperature than the 11S protein (Table 20). Gel formation will occur at a lower temperature (80°C) and protein concentration (approx. 11%) than for 11S proteins (Shimada and Matsushita, 1980). No dissociation products have been identified during gel formation of 7S proteins, and it is thought that aggregation occurs without prior dissociation (Hashizume et al., 1975). Heating causes a greater viscosity increase than for 11S proteins, however, viscosity does not increase as much during cooling (Babajimopoulas et al., 1983). Hydrogen bonds are believed to be the most important factor in the formation of hard and elastic gels from 7S proteins (Babajimopoulas et al., 1983; Utsumi and Kinsella, 1985a). All three of the 7S subunits appear to contribute equally to gel formation (Utsumi and Kinsella, 1985a; b). The network structure for 7S proteins has been reported to be denser and more cross-linked than that of 11S proteins (Hermansson, 1985). Many authors have reported differences in the gelling properties of 7S and 11S proteins (Saio and Watanabe, 1973; Saio et al., 1974; Hashizume et al., 1975; Shimada and Matsushita, 1980; Babajimopoulas et al., 1983; Utsumi and Kinsella, 1985a; b). However, these must be treated with caution. Many of the 7S preparations used have been only 70% pure and have contained large quantities of contaminating 11S protein. Furthermore, the presence of calcium ions in preparations has been shown to have a profound effect on gelling properties (Skurray et al.,

1980).

Gels formed from a 1:1 mixture of 11S and 7S proteins have superior gelling properties to either individually (Babajimopoulas et al., 1983). When dilute solutions of 7S and 11S proteins are heated there is no aggregation or precipitation of the 11S basic subunits (Hashizume and Watanabe, 1979; German et al., 1982). However, if salt is present aggregation and precipitation may occur (Hashizume and Watanabe, 1979). In the absence of salt soluble complexes are formed between the basic subunits of the 11S protein and conglycinin via electrostatic interactions (Damodaran and Kinsella, 1982). Disulphide bonds may also form between basic subunits (Utsumi et al., 1984; Yamagishi et al., 1984). The β subunit of conglycinin was involved in complex formation to a greater extent than the α or α_1 subunits (German et al., 1982; Utsumi et al., 1984; Utsumi and Kinsella, 1985b). However, in more concentrated solutions all of the conglycinin subunits appear to be equally involved in gel structure formation (Utsumi and Kinsella, 1985a). The major factors involved in gel formation were hydrogen bonds and hydrophobic interactions. Disulphide linkages were not necessary for gel formation, but may contribute to gel elasticity (Furakawa and Ohta, 1982; Babajimopoulas et al., 1983; Utsumi and Kinsella, 1985a). ASIII was again involved in gel formation and hardness (Utsumi and Kinsella, 1985a).

Gelling of V. faba proteins was favoured at pH values of 2.5 - 3.5 and 6 - 8, and was least favoured at pH values of <2.5, 4 - 5.5 and >10 (Figures 13-18). Close to the isoelectric point (pH 4 - 5.5) protein solubility is very low and so gel formation is limited by protein concentration. A similar decrease in gelling ability at extremes of pH has been observed by Catsimpoolas and Meyer (1970). Electrostatic interactions between the basic subunits of the 11S

protein and vicilin subunits are inhibited at extremes of pH, and the proteins tend to remain in solution.

Micellar proteins formed strong gels at pH 3.5 despite having a low protein solubility (Figure 17). Gels were also formed at pH values >8. All micellar protein gels had a highly aggregated structure. Murray et al. (1981) reported that micellar proteins tend to form fibres in aqueous environments, and that these fibres can be heat coagulated. The formation of fibres in solution could provide the basis for matrix formation.

The full-fat Maris Bead proteinate formed gels at a wider range of pH values than the defatted sample. Shimada and Matsushita (1981) observed that soy protein gels formed with added oils were always firmer than those produced without oil. The lipid content of the samples was not determined, but lipid contents of 12% have been reported for pea isolates (Wright and Bumstead, 1984). Catsimpoolas and Meyer (1971) reported that adding up to 40% mono-, di- or triglycerides increased gel strength. Generally viscosity was increased by a) decreasing the chain length of the fatty acids, b) decreasing the esterification of hydroxyl groups on the glycerol component, or c) increasing the degree of saturation. Phospholipids and cholesterol were also shown to enhance gelation. G values and rupture strength were also high for gels produced from full-fat samples (Table 12).

The effects of salt on gelling.

Gel strength increased with increasing salt content up to 1.25% (approx. 0.2M) for Maris Bead proteinate (Figure 23). Hermansson (1978) reported that thermal aggregates were formed during heating of soy proteins in 0.1M NaCl, and thermal aggregation was maximised at

0.2M NaCl. Raising the salt content further suppressed aggregation. Other studies have reported a decrease in viscosity by increasing salt content from 0 - 2M at pH 7 - 8. The observed decrease was attributed to the stabilization of the quaternary protein structure against dissociation, thus affording protection from thermal unfolding (Catsimpoolas and Meyer, 1970; Hermansson, 1978; Babajimopoulas et al., 1983). However, no measurements were recorded between 0 and 0.2M NaCl in these studies.

All of the gels formed were translucent, with the exception of those containing 1.5 and 1.75% NaCl. However, the gels were dark brown in colour. The pigments responsible for this colouration were not identified. However, it may prove necessary to remove them before the proteins can be used in food products.

SEM of gels.

Protein contents after centrifuging of the 20% protein solutions were all low, especially for the micellar protein (Table 13). This was partly attributed to difficulties in wetting the samples, and partly to poor solubility. All of the gels appeared to be composed of highly aggregated structures, particularly those of the proteinates and isolates in which spherical aggregates of approximately 12.5 μm diameter were observed. These are much larger than those reported by Hermansson (1985) in soy protein gels. She reported strands with a diameter of 12-15 nm. However, the relevance of the observed structures is doubtful, as using glutaraldehyde as a fixative has been implicated in the induction of aggregated structures in protein gels (Hermansson and Buchheim, 1981).

Rheological properties.

Rigidity of the gels was highest at pH 4 and 4.6 (Table 12). These gels were opaque and pasty. Closer examination revealed that they had little cohesion. The high rigidity values obtained were probably due to structural compaction during compression, and further emphasized by the increased cross sectional area produced by compression (Calzada and Peleg, 1978). Compression was accompanied by syneresis indicating that the protein matrix was coarse and had a low water holding capacity. The nature of the gel network is partly determined by the relative rates of the denaturation and aggregation steps. If aggregation is relatively slow a gel with a finer network of protein chains is formed. The resultant gel will tend to be translucent and elastic. However, if aggregation is relatively fast a coarse and inelastic gel is formed. At pH values close to the isoelectric point the latter is favoured (Hegg et al., 1979; Hegg, 1982). At pH 7 the protein is more soluble, and so the aggregation process is slower. Translucent and relatively elastic gels were formed at this pH. Generally soft gels were produced at all pH values, even at 20% sample concentration. Soy proteins have been shown to have superior gelling properties to bean proteins (Fleming et al., 1975; Utsumi et al., 1983). However, the increase in viscosity of heated broad bean globulin solutions was equivalent to that of soy proteins (Fleming et al., 1975). V. faba proteins may have a greater potential in mixtures of gelling agents designed to give a range of functional properties. The compatability of broad bean 11S protein with gelatin in gelling systems has been investigated by Andersson et al. (1985).

All polymer dispersions are viscoelastic by nature, ie. they exhibit solid-like elastic and fluid-like viscous behaviour

simultaneously (Tung, 1978; Mitchell, 1980). This is important where materials are exposed to constantly changing forces as in chewing, mashing, cutting, mixing, pouring, spreading or pumping. In a stress-relaxation experiment the sample is subjected to a constant strain and the residual stress is measured against time. The stress induced in the sample can relax by several methods: a) viscous flow of polymer units, b) breakage or formation of chemical bonds, and c) steric movement of polymer side chains. Relaxation times are significant to the consumer if they operate over time periods which are applicable to the act of mastication, ie. relaxation times of less than 1 second. These may alter the consumer's perception of firmness. The principal component of stress-relaxation is believed to be viscous flow (Bohlin, 1980). Three flow processes were identified in the protein gels (Table 14). However, it was not possible to identify them with specific processes. This has been successfully done for wheat gluten dough (Mita and Bohlin, 1983). Gluten has two flow processes with co-ordination numbers of 4 and 2. Electron microscopy has revealed that gluten is composed of non-aligned fibrillar structures ($z = 4$) arranged to form a lamellar superstructure ($z = 2$) (Bernardin, 1975). Co-ordination numbers were variable for protein gels, and the applicability of the theory to such gels is questionable as it is unlikely that a simple co-operative flow unit is involved (Table 15). SEM of soy protein gels suggests that the strands are extensively cross-linked (Hermansson, 1985). The intercepts of the F/F_0 axes of the rate - force plots gave positive values. This indicates that the system was not in a state of equilibrium prior to testing, and verifies the existence of cross-linkages and aggregates in the gels. The above factors partially arrest flow processes, and cause increases in relaxation times and co-ordination numbers (Mita and Bohlin, 1983).

Foaming

Two factors contribute to the foaming properties of a solution: a) foam expansion, and b) foam stability. These foaming properties depend on the ability of a surfactant to a) rapidly migrate to a new interface, b) to effectively lower interfacial tension at the interface, and c) to stabilize the interface. The first requirement is largely dependent on the diffusion coefficient of the protein, and generally smaller proteins will reach the interface fastest (MacRitchie, 1978). The interface is a highly denaturing environment for proteins. The reduction in interfacial tension is achieved by an increase in surface pressure due to the protein orientating and spreading itself at the interface. To facilitate this a significant proportion of exposed hydrophobic surface and an ability to rapidly unfold are desirable (Graham and Phillips, 1979a; b; Kato and Nakai, 1980; Kim and Kinsella, 1985). Once a monolayer has been formed subsequent association of proteins with the interface requires the ability to adsorb onto and insert into the pre-existing film without disrupting the film's integrity. Adsorption occurs continuously where either stretching of the film due to bubble rearrangements or gas diffusion, or removal of proteins due to coagulation occur. Coagulation commonly occurs at agitated air - water interfaces. Protein molecules are converted to insoluble particles which lose their foaming properties causing the foam to collapse. Coagulation is enhanced close to the isoelectric point. Ovalbumin is particularly susceptible to coagulation (Henson et al., 1970), and precipitated ovalbumin has been observed in whipped egg white (De Vilbiss et al., 1974). Adsorption on to the interface requires a tendency to bind to hydrophobic surfaces, and is enhanced close to the isoelectric point where protein - protein interactions dominate. For the same reason

foam strength is maximized close to the isoelectric point, or at the isoelectric point for proteins which remain soluble (Buckingham, 1970; MacRitchie, 1978; Halling, 1981; Kinsella, 1981; Kim and Kinsella, 1985). No attempt was made to optimize conditions for each protein in this study.

Samples like α-casein and sodium caseinate consist of small and soluble molecules which can rapidly migrate to the interface. On reaching the interface the readily available hydrophobic segments and the molecular flexibility of the protein facilitates orientation and spreading at the interface (Mercier et al., 1971; Ribadeau-dumas et al., 1972; Mercier et al., 1973). As judged by initial conductivity during sparging α-casein produced the greatest volume of foam (Table 16). Tornberg (1978a; b) has previously demonstrated the ability of α-casein to form large volumes of foam consisting of many small bubbles. Conventional measurements of foam volume do not account for bubble size. The advantage of the conductivity method is that it gives an estimate of the total volume of fluid held in the foam. BSA and hemoglobin also formed large volumes of foam. BSA also has a high hydrophobicity value and is relatively unstructured. The pH used for the foaming experiments was close to the isoelectric point of hemoglobin, myoglobin and γ-globulin. Hemoglobin and myoglobin both produced brittle foams with large bubbles, possibly caused by coagulation of proteins at the interface. The large foam volume produced by whipping the hemoglobin sample does not accurately reflect its foaming properties as bubble size is not considered. The measures of foaming power obtained by the sparging method probably more accurately reflect the foaming power of hemoglobin.

Proteins with a high degree of tertiary or quaternary structure tend to resist unfolding. A larger energy input is necessary

to form foams from these proteins, eg. lysozyme, ovalbumin and γ-globulin (Kato et al., 1983a; German et al., 1985). In this study all proteins were foamed with an equal energy input. It is assumed that this was insufficient for adequate film formation by lysozyme as only a small volume of foam composed of large bubbles was formed (Table 16). Soy proteins and V. faba proteins have compact quaternary structures. The relatively poor foaming properties of soy proteins have been attributed to the difficulties involved in the adsorption and unfolding of the protein at the air - solvent interface (Tornberg, 1978a). In the native state soy proteins have a low value of surface hydrophobicity (Kato and Nakai, 1980). Furthermore, unfolding is prevented by disulphide linkages. Treatment with a disulphide cleaving agent facilitates unfolding and also results in the exposure of hydrophobic residues, particularly on the basic subunits (German et al., 1982; 1985). A dramatic improvement in foam expansion was observed when soy proteins were treated with sulphite (German et al., 1985). Soy hydrolysates are widely used as foaming agents in the food industry (Eldridge et al., 1963). Greater foam expansion has been observed for hydrolysed soy proteins (Eldridge et al., 1963; Puski, 1975), egg white (Grunden et al., 1974) and whey proteins (Kuehler and Stine, 1974).

Foams tend to be highly unstable because of surface tension. For a film to be relatively stable the proteins must be a) able to form a cohesive network capable of retarding film coalescence and collapse of bubbles, b) capable of withstanding minor shocks due to bubbles bursting, and c) able to repel the approach of adjacent films (MacRitchie, 1978; Graham and Phillips, 1979a). A newly formed foam is composed of undistorted spherical bubbles. However, initial rapid drainage under the influence of gravity causes these to distort and

become polyhedral. Subsequent drainage may either occur from plateau borders at bubble intersections under the influence of gravity, or from the lamellar regions between bubbles into the plateau borders due predominantly to Laplace capillary pressure. The latter force is opposed by the negative pressure in the plateau borders and double layer repulsive forces between adjacent films. Drainage of liquid from the lamellar regions causes the interfacial films to become thin and fragile, and facilitates gas diffusion from small bubbles to larger ones resulting in the collapse of the bubbles (Halling, 1981; Kinsella, 1981). The film strength depends on rheological and adhesive properties, eg. surface topography, mechanical strength, viscoelasticity and water binding capacity (Graham and Phillips, 1979a). Generally proteins with a large molecular weight and a high degree of tertiary and quaternary structure form more stable foams. The bean and pea globulins produced the most stable foams, as measured by the sparging method. This reflects their compact quaternary structure. Isolates, proteinates and ultrafiltration products also produced relatively stable foams (Table 16). Micellar proteins only produced small quantities of foam, probably due to their poor solubility characteristics. Stable foams were also produced by sodium caseinate, BSA and soy whey protein. Sodium caseinate readily forms a viscous solution which enhances foam stability (Morr, 1981). However, BSA and whey proteins do not usually form stable foams (German et al., 1985). Under the conditions employed it would appear that insufficient adsorption and denaturation occurred at the interface for the formation of viscous films from the more structured proteins, eg. ovalbumin and γ-globulin.

No real differences in foaming properties were observed between 7S and 11S proteins from peas and beans. The observed

differences were probably due to relatively minor differences in processing conditions. Also, there was no preferential retention of 7S compared to 11S proteins, or vice versa, in foams produced from Maris Bead isolates (Figure 37). Poole et al. (1984) demonstrated that lysozyme was strongly involved in matrix formation in egg albumen foams. This was attributed to electrostatic interactions between lysozyme and other more acidic proteins.

Of the soy proteins tested the whey proteins had the best foaming properties (Figure 28). Whey proteins tend to be small, soluble and capable of producing large volumes of foam with a small energy input. However, they also tend to produce less stable foams than globulin proteins.

The effects of lipids on foaming properties are clearly demonstrated by comparing the results for the full-fat and defatted soy flours (Table 16). Eldridge et al. (1963) demonstrated that polar lipids were more detrimental to foam formation than neutral lipids, presumably due to their greater surface activity. Although lipid contents are much lower in peas and field beans compared to soy beans, isolates and proteinates may contain appreciable amounts of lipid. The effects of these are clearly demonstrated in both whipping and sparging experiments.

Foaming properties in defatted pea, bean and soy samples improved with increasing protein content. The higher protein contents of soy flours and isolates compared to those of pea and bean probably account for the superior foaming characteristics (Figures 30 and 32).

Values for foam expansion determined by the sparging and whipping methods were not strongly correlated (Table 17). As mentioned previously values for foam expansion determined by the whipping method

do not indicate the volume of liquid held in the foam. A relatively weak foam composed of large bubbles may have a greater volume than a dense foam composed of many small bubbles. The conductivity method is probably a better indicator of foam quality. Kato et al. (1983b) reported that foam volume and initial conductivity were correlated. However, his foam volume measurements were produced by sparging, and not whipping. In the experiments reported in this study only small differences in foam expansion after sparging were observed (Table 16). However, different operating procedures were used. Foam stability values produced by the two methods were more strongly correlated.

In conclusion, the sparging method was considered to be a better test of foaming properties for such comparative experiments.

Increasing the viscosity of the bulk solution should enhance foam stability, although it may also be detrimental to the rate of foam formation by decreasing the diffusion rate of the proteins. It has also been suggested that low concentrations of sucrose enhance foaming by creating an unfavourable environment for proteins, leading to protein deposition at the interface (Lee and Timasheff, 1981). However, adding up to 12% sucrose to a solution of Maris Bead proteinate slightly reduced both foam expansion and foam stability (Table 18). Presumably the energy input during foaming was insufficient to compensate for the increased viscosity of the solution. At concentrations greater than 12% the heavy consistency of the solution inhibited foam formation.

Partial denaturation of proteins has also been used to increase viscosity as a means of improving foam stability of egg proteins (De Vilbiss et al., 1974), whey proteins (Kuehler and Stine, 1974; Richert et al., 1974) and soy proteins (Eldridge et al., 1963).

Emulsifying properties.

The mechanics of emulsion formation are similar to those of foams, ie. a) diffusion of protein molecules to the interface where they are adsorbed in native form, b) surface denaturation of the adsorbed protein, and c) aggregation of the denatured protein into a coagulum devoid of surface activity which is forced out of the interface by the spreading pressure of native protein undergoing denaturation at the interface. If coagulation is extensive the coagulum may form a skin which will stabilize the emulsion (Acton and Saffle, 1970).

Emulsifying profiles have been correlated with solubility profiles for various proteins (Swift et al., 1961; Hegarty et al., 1963; Crenwelge et al., 1974; Sosulski et al., 1976b; Volkert and Klein, 1979). The latter authors observed that emulsifying capacity was not greatly affected when dispersible nitrogen was equalized over the whole pH range. In this study emulsion activity was as follows : proteinates > isolates > micellar proteins > protein concentrates > flours. Dispersible protein followed the same order. However, high values for nitrogen solubility index (NSI) are not necessarily indicative of good emulsifying properties (Smith et al., 1973; McWatters and Holmes, 1979a; b). Surface hydrophobicity together with solubility characteristics are considered the best indicator of emulsifying properties by Voutsinas et al. (1983a).

Flint and Johnson (1981) reported that soy proteins could form films at pH values below the isoelectric point and up to a value of 7.5. This range includes most food systems and all meat emulsions. In studies on soy beans, 7S proteins have been attributed with superior emulsifying properties compared to 11S proteins (Aoki et al., 1980;

Yamauchi et al., 1982). However, in this study 11S proteins performed slightly better than 7S proteins. Different methods of protein preparation, different species and different methods of determining emulsifying properties were used in all of the investigations.

The lowest values of EC and EA were obtained at the isoelectric pH for soy isolates and 7S proteins (Franzen and Kinsella, 1976; Kamat et al., 1978; Ramanatham et al., 1978; Volkert and Klein, 1979; Aoki et al., 1980). However, the 11S protein had the lowest values of EC and ES at pH 7. In this study emulsion activity was determined at one pH value only (pH 7.5), and the 11S protein from Maris Bead emulsified almost all of the available oil (Table 19).

Full-fat samples generally had improved emulsifying properties compared to equivalent defatted samples (Table 19). Lipids generally improve the emulsifying capacities of proteins (Wang and Kinsella, 1976; Sosulski et al., 1976b). Halling (1981) suggested that lipids may act co-operatively with proteins during emulsification. Many lipids contain small molecule surfactants, eg. mono-glycerides and free fatty acids.

Products with high protein contents generally had the best emulsifying properties (Tables 3, 9, 10 and 19), suggesting that under the conditions employed protein load was insufficient to test the surface activity of the proteins. Halling (1981) observed a sharp increase in emulsifying capacity with increasing protein content up to a critical value, after which only small increases in emulsifying capacity were observed. Differences between soluble proteins were considered to be small. However, emulsifying properties could not be explained by protein content alone. Fibre has been negatively correlated with emulsifying properties by Yasumatsu et al. (1972).

Coarse fractions generally had low values of emulsion activity, presumably due to the presence of hull material.

The bean proteins had much higher EA values than casein. The casein preparation used was prepared by rennet treatment and acid precipitation. The resulting product is highly insoluble and retains the original calcium content of the native casein micelle. It is non-functional as an emulsifier unless the calcium ion content is reduced. The emulsifying properties of α_{S1}-casein and its hydrolytic derivatives have been studied by Shimizu et al. (1983).

The soy isolate performed better than bean and pea isolates and proteinates. This possibly reflects its slightly higher protein content, although the method of production may also have been important. Aoki et al. (1980) and Ochiai et al. (1982) observed increased values of EC over the pH range 2 - 10 for hydrolysed soy proteins. This was attributed to either improved solubility characteristics, or exposure of more hydrophobic residues. Heat denaturation and lipophilization have also been reported to improve the emulsifying properties of soy proteins by Kamat et al. (1978) and Aoki et al. (1981) respectively. However, muscle proteins are considered the best emulsifying agents (Smith et al., 1973).

The difficulty of extrapolating from model systems to actual food systems with many interacting components is significant. Puski (1976) has suggested that three different methods for evaluating food additives as emulsifying agents should be used, depending on the type of product being developed, ie. a) comminuted type meat systems, b) low fat milk type systems, or c) high fat mayonnaise type systems. For inclusion in cooked meats temperature dependance, water binding and gelation are also important. Vaisey et al. (1975) used faba bean

and field pea protein concentrates as ground beef extenders in patties. It was necessary to texturize the protein to produce samples similar to the beef controls. 30% soy protein substitution is possible in ground beef and turkey loaves without causing adverse effects on fat content, water holding capacity, texture and flavour (Williams and Zabik, 1975). However a potential problem associated with adding soy proteins to meat systems is the development of rancid flavours due to the oxidation of lipids. This is particularly significant in poultry products which have a large unsaturated fat content. The use of non-meat meat extenders has recently been reviewed by Mittal and Usborne (1985).

Conclusion

In this study various processing methods were used to produce protein or starch enriched products which were then assessed for functionality in model tests. The processing methods used were defatting, air classification, and production of isolates, proteinates and micellar proteins. These ranged in protein content from 33 - 98%. A starch concentrate containing 60% starch was also produced.

The relatively high protein content of V. faba seeds make them an ideal material for producing large yields of high protein content products. Equivalent pea products tend to have lower protein contents, or can only be produced in low yields. However, the superior protein content and functionality of defatted soy flours compared to those of V. faba will make it difficult for the latter to compete in the same markets. Protein concentrates produced by air classification were more equivalent in protein content to defatted soy flours. However, they did not exhibit equivalent functionality in foaming tests. This was attributed to a high lipid content.

Air classification was used to produce fractions enriched in protein, starch and hull material. The technique has many advantages compared to conventional wet processing methods, including being able to avoid the use of potential protein denaturants, thus leaving the protein in its native state, and obviating the need to dispose of effluent. However, a major problem is the tendency of lipid to fractionate with the protein fraction. Defatting using solvents to extract the polar lipids may have a detrimental effect on protein functionality. Raffinose and stachyose also fractionate with the protein, and may affect the market potential of the product.

Impact milling efficiency was lower for IVS-G, possibly reflecting structural differences between the two seeds. Also, the levels of contaminating starch and residual protein were high in the protein and starch fractions respectively. The starch content of the fines fraction could have been attributable to damaged starch granules or a population of small starch granules. A different milling procedure may result in improved impact milling efficiency.

The use of hulls as a fibre additive may be limited due to tannins and other toxic constituents in the hull material. Tannin contents were not quantified in this study but Maris Bead probably had larger quantities than IVS-G as it has a darker coloured seed coat. The selection of white seeded varieties may increase the market potential of the hull.

Starch concentrates are of less commercial interest than protein concentrates. Starch functionality was not studied in this thesis. However, faba bean starch gelatinization was shown to occur at a similar temperature to potato and corn starches.

Isolates, proteinates and micellar proteins had protein contents ranging from 83-98%. Equivalent soy isolates and proteinates have slightly higher protein contents. The micellar proteins were shown to be the least denatured by DSC analyses. However the native state of the micellar protein was not reflected in its solubility profile. Low values of protein solubility were produced at all pH values. The decreased ΔH values obtained for isolates and proteinates were attributed to the effects of isoelectric precipitation or protein aggregation.

Protein functionality was assessed in a number of model tests. The difficulties in extrapolating from the results obtained to real food systems with many interacting components are significant. Micellar proteins did not perform well in any of the functional tests and this was attributed to lack of solubility. However, other studies have demonstrated that micellar proteins in aqueous environments form fibres which can be coagulated by heat. It was suggested that micellar proteins may exhibit desirable functional properties in dough type products. Proteinates were more soluble and generally exhibited greater functionality than isolates. Disulphide linkages formed during the storage of isolates were probably responsible for the loss of solubility. The presence of pigments in the products caused dark brown colours to appear at neutral and alkaline pH, or in the presence of salt. The pigments responsible were not isolated and characterized but reports in the literature have attributed the formation of coloured products to phenolic acids. This is likely to be a problem in many food products.

V. faba proteins did not form firm gels even at protein concentrations of 20%. Translucent and slightly elastic gels were formed at pH values > 7. Globular proteins are generally much less

effective as gelling agents than gelatin or commercially utilized carbohydrate products. However, at high concentrations V. faba globulins may contribute to matrix formation.

Gelling, foaming and emulsifying properties were largely dependent on protein concentration. However lipids and fibre did affect performances. Full-fat products had improved gelling and emulsifying, but inferior foaming properties compared to defatted samples. Isolates and proteinates generally had similar functional properties to equivalent soy products. Foaming and emulsifying properties could probably have been improved by treatment with a reducing agent. The conductivity method was considered to give a better measure of foam quality.

7S and 11S proteins did not differ significantly in their behaviour in foaming or emulsifying tests. The small observed differences were probably caused by minor differences in conditions during processing. However, the 7S protein does undergo a thermal transition at a lower temperature than the 11S protein, and they also exhibit different salt and calcium ion sensitivities. The development of cloning techniques to produce large quantities of homogeneous proteins, coupled with the development of micro-methods will facilitate a more comprehensive evaluation of these proteins. Significant genetic variation in the 11S : 7S protein ratio has been demonstrated for pea (Casey et al., 1982). Furthermore, different subunits have been shown to contribute to varying extents in gel formation. Eventually it may be possible to select specific varieties of legume to match specific functional requirements.

In conclusion, V. faba proteins and products had similar functional properties to soy products. This is not surprising

considering the similarities in structure. However, the higher protein content of the soy bean enables larger yields of protein rich products to be obtained. The ability of V. faba beans to compete with soy beans will depend on economic factors, and also the development of markets for the other major faba bean constituents, ie. starch and fibre.

Suggestions for further work.

A number of issues were raised in the course of this study which warrant further research. Differences were observed in impact milling efficiency of Maris Bead and IVS-G seeds. A quantitative isolation of cell wall material, and SEM of milled flours would help to elucidate the factors involved in limiting milling efficiency. SEM of milled flours would also give an estimate of the proportion of damaged starch granules, and a study of starch particle size distribution would indicate whether the contaminating starch in the protein fraction was due to a population of small starch granules or damaged starch. If it is due to damaged starch granules a change in the method of milling may improve the separation of protein and starch particles.

Calcium ions are known to enhance the formation of firm gels from soy proteins in the production of tofu. It is likely that firmer and more cohesive gels could be produced from Vicia faba proteins in the presence of calcium ions.

Finally, no attempt was made to investigate the effects of chemically or physically modifying the proteins on their functional properties. Modification by the addition of reducing agents, heat denaturation, hydrolysis, acetylation and succinylation have been shown to alter protein functionality by altering solubility, viscosity and hydrophobicity characteristics.

CHAPTER 6

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