Functional properties of enzymically hydrolysed fish waste

FUNCTIONAL PROPERTIES OF ENZYMICALLY HYDROLYSED FISH WASTE

by

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DEDICATION

To the spirit of those who died in the chemical weapon bombardment of Halabja and to my brother Mahmmode Hassan.

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by

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ABSTRACT

Keywords: Fish protein hydrolysate, functionality, trypsin, bromelain, taste panel.

Enzymic hydrolysis of cod fish waste was investigated using two enzymes (trypsin and bromelain). A fish protein hydrolysate (FPH) powder and frozen flake hydrolysate were produced using a spray drier and an ice flake machine.

Functional properties of the FPH were assessed with respect to the molecular weight (MW) spectrum. The characteristics of solubility and emulsification for the hydrolysate showed it to be suitable for use as a binder compared with egg albumin (EA) and soy bean isolate (ISB) for fish products.

Fishburgers with improved texture, succulence and reasonable cooking losses were made successfully from cod fish mince incorporating a vegetable oil/water emulsion stabilised by FPH. Taste panels were carried out and overall acceptability of the fishburgers made from the FPH emulsion was better than fishburgers containing EA and ISB emulsions.

Economic evaluation and specification of a pilot plant were done for both FPH powder and frozen flake hydrolysate production.

This work strongly emphasises that FPH should only be used in fish products which need a good binder and where the flavour/taint problems of use in other products e.g. beverages, pasta will not arise.

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CHAPTER 1

INTRODUCTION

Research in the field of fish processing and product development has resulted in many innovative methods and formulations using fish as an ingredient for human consumption. As the world population is increasing at such a rate that it will be 7-8000M by the year 2000 the need for protein will increase to 130-150 million tons/year. Clearly, to provide the necessary proteins for human consumption the volume of fish available will need to increase dramatically. It is generally recognised that a significant proportion of the extra demand must be satisfied by an improvement in fish utilisation and a reduction in the large amount of fish wastage i.e. that which is thrown away, or used for animal feeding and fertiliser and losses due to spoilage and infestation.

Fish waste (FW) can be from two sources: that remaining on the frames of fish after filleting and those caught but not processed because of 'non-white' colour, size, bony species, or physical damage. Fish processing industries are slowly moving into utilisation of the fish waste in ways designed to reduce wastage.

In the 1890's a product called 'fish flour' was produced on a commercial scale for human consumption which had both good storage properties and an agreeable taste. A German patent was also taken out

in 1934 for the manufacture of "albumen from fish". As a result of the above work the imports of egg proteins in Germany decreased while the production of fish proteins, called Wiking Eiweiss increased.

Recently most fish waste was utilised to produce fish silage, fish meal and fish protein concentrate (FPC). Most of these products do not have good functional properties because the production processes bring about denaturation of many of the proteins. New food protein products should ideally possess both functional properties such as solubility, emulsifying ability, water and oil binding capacity etc as well as nutritional value and no toxicity.

The functional and nutritional properties of fish proteins may be improved by the use of specific enzymes and choosing the proper set of hydrolysis parameters such as pH and temperature to partially hydrolyse the proteins. The work described in this thesis is concerned with their modification to produce an enzymically hydrolysed fish waste protein.

CHAPTER 2

LITERATURE SURVEY

2. UTILISATION OF FISH WASTE

2.1.1 Source

The source of FW mainly comes from by-products of the world fish catch and fish processing industries. About 30 percent of the world's annual fish catch at 70 million tons is available as by-products and includes a large amount of fish waste which is not directly suitable for human consumption.

By-products and FW are highly nutritious and this has resulted in its utilisation in the most effective way in several products such as protein rich residues, as fertiliser, fish meal and fish silage as animal feeds, fish oil and fish protein concentrate (FPC) for human consumption. [Wallerstein and Pariser, 1978; Windsor and Barlow, 1981; Disney, 1984].

However by-products and FW may more effectively be utilised nowadays to produce nutritional and functional proteins through enzyme hydrolysis which are valuable for direct application in food industries. Such processes have been tried out in several countries e.g. Chile, France, Norway and USA [Mohr, 1980].

2.1.2 Fish Products

2.1.2.1 Fish meal

The use of fish by-products in the form of fish meal for animal feeds for cattle, poultry, pigs etc is not a new idea. This use was originally mentioned in the Travels of Marco Polo at the beginning of the 14th Century in which his crew fed their animals dried fish [Windsor, 1971; Windsor and Barlow, 1981].

Fish meal can be defined as a solid product obtained by removing water and some oil from the fish waste or unwanted fish [Windsor, 1971]. The industrial production of fish meal and oil was started at the beginning of the 19th Century to utilise the high proportion of the world's supply of by-products [FAO, 1975; Windsor, 1981]. In recent years, the world catch of fish increased from 70 million tons in 1980 to 91 million tons in 1986 and 20 million tons (30%) and 40 million tons (42%) of this have been converted to fish meal, respectively (Bowman, 1982; FAO, 1987]. Fish meal is produced throughout the world in large amounts every year. The main countries which produce fish meal are the USA, UK, USSR, Denmark, Peru, Norway, Chile, Japan, South Africa, Canada, Thailand [Meade, 1971; Windsor and Barlow, 1981].

The high nutritional value makes fish meal a popular feeding stuff for all kinds of animals. It has an especially high level of essential amino acids (Methionine, Lysine) and B-complex vitamins (B_{12} 0.7-0.33 mg/lb), (Choline 1500 mg/lb), (Niacin 3000 mg/lb) and (Riboflavin 3 mg/lb).

The chemical composition and nutritional value of fish meal will vary with the raw materials and processing conditions (see Table 1) [Windsor and Barlow, 1981; Ockerman and Hansen, 1988].

2.1.2.2 Fish silage

The utilisation of fish by-products in the form of fish silage (either liquid or dried) as an animal feed (for pigs, poultry, cattle and fish) is also not a new idea. The methodology was developed originally 60 years ago by Virtanen in Finland [Cole and Barton, 1965; Tatterson and Windsor, 1974; Backhoff, 1976; Disney et al, 1978; Windsor and Barlow, 1981; Poulter and Disney, 1982; Raa and Gildberg, 1982].

Fish silage is the liquefied fish protein product obtained by the action of natural enzymes present in the whole fish or parts of fish or fish waste in the presence of an added acid to prevent microbial spoilage [Tatterson and Windsor, 1974; Windsor and Barlow, 1981; Raa et al, 1983].

The commercial production and development of silage has been carried out worldwide in recent years. The main countries involved are Denmark, Poland, Germany, USSR, Canada, UK, India, Thailand, Malaysia, Ghana, Australia and Malawi. Fish silage was also the subject of several patents in Poland in 1959 and Germany in 1970 [Tatterson and Windsor, 1974; Disney and Hoffman, 1976; Lan et al, 1979; Brown et al, 1984].

TABLE 1: CHEMICAL COMPOSITION OF FISH MEALS

Component %	Herring meals	Anchovy meals	Pilchard and Massbanker meals	Tuna (Mixed) species) offal meals	Menhaden meals	White fish meals
Crude protein	73.6	65.4	65.4	53.24	62.01	65.01
Moisture	6.93	8.1	9.0	6.20	8.25	8.49
Ash	-	-	-	-	-	20.92
Amino acids:						
Lysine	7.73	7.75	7.94	7.30	7.56	6.90
Methionine	2.86	2.95	2.71	2.75	2.82	2.60
Cystine	0.97	0.94	0.95	0.79	0.90	0.93
Tryptophan	1.15	1.20	1.02	1.05	1.07	0.94
Histidine	2.41	2.43	3.02	3.41	2.32	2.01
Arginine	5.84	5.82	5.95	6.43	6.04	6.37
Threonine	4.26	4.31	4.38	4.34	3.97	3.85
Valine	5.41	5.29	5.41	5.31	5.10	4.47
Isoleucine	4.49	4.68	4.48	4.46	4.40	3.70
Leucine	7.50	7.62	7.30	7.20	7.14	6.48
Phenylalanine	3.91	4.21	3.91	4.10	3.95	3.29
Tyrosine	3.13	3.40	3.23	3.28	3.22	2.60
Aspartic acid	9.10	9.49	9.37	0.30	9.07	8.54
Serine	3.82	3.84	4.27	4.18	3.61	4.75
Glutamic acid	12.77	12.96	12.92	11.93	12.70	12.79
Proline	4.15	4.17	4.52	5.43	4.58	5.34
Glycine	5.97	5.62	6.92	8.15	6.78	9.92
Alanine	6.25	6.31	6.17	6.76	5.94	6.31

Source: Windsor and Barlow (1981)

Fish silage products have considerable benefits to offer as a means of converting by-products into animal feed. Its nutritional value is adequate as a complete diet or as a food supplement. The nutritional value of fish silage has been reported as a good source of protein and has been tested as an animal feed [Disney et al, 1978; Trevino et al, 1982; Johnson et al, 1985].

Feed trials showed that fish silage can be used at a level of 5-10% of dry weight of foodstuff for pigs, 30% of total protein level for chickens and at the 12-23% level for broilers. The poor nutritional value of fish silage in some animal feed trials is possibly due to lipid oxidation and a high level of salt. The nutritional value and chemical composition of silage from different species of fish or parts of fish is fairly uniform and does not vary much with different raw materials (see Table 2) [Windsor and Barlow, 1981; Ockerman and Hanson, 1988].

2.1.2.3 Fish protein concentrate (FPC)

The idea of converting fishery by-products and industrial fish waste to make fish protein concentrate or to the earlier named 'fish flour' is not new [Halliday and Disney, 1977; Finch, 1970; Windsor, 1982]. The term FPC is new, but originally in ancient Roman times it was called 'liquamen' and was prepared from salted small fish [Halliday and Disney, 1977; Sikorski and Naczk, 1981].

TABLE 2: CHEMICAL COMPOSITION OF SILAGE

Component	White fish silage	Herring silage	
	White fish offal	Herring offal	Herring offal de-oiled
Crude protein	15.0	13.5	14.5
Oil	0.5	8.7	2.0
Moisture	78.9	75.4	80.8
Ash	4.2	2.6	2.8
Amino Acid			
Aspartic acid	6.6	3.7	
Serine	3.7	2.0	
Glutamic acid	9.7	5.7	
Glycine	6.1	3.1	
Alanine	5.4	3.0	
Valine	3.3	2.4	
Tyrosine	2.1	1.1	
Phenylalanine	2.3	2.4	
Histidine	1.8	1.2	
Arginine	5.1	3.7	
Leucine	4.5	3.7	
Isoleucine	2.8	1.9	
Threonine	3.1	2.2	
Cystine	0.5	0.3	
Methionine	1.8	0.8	
Lysine	4.7	4.2	

Source: Windsor and Barlow (1981)

Fish protein concentrate has been defined as any stable fish preparation, intended for human consumption, in which the protein is more concentrated than in the original fish by removing water and oil [Windsor, 1982; Windsor and Barlow, 1982].

There are three main categories or families of FPC products: Type A, a virtually odourless, tasteless powder with a maximum of 0.75% oil; Type B, a powder which has no specific limits of fish odour with a maximum of 3% oil; Type C, a normal fish meal produced hygienically [Finch, 1970; Finch, 1977; Windsor and Barlow, 1982].

The commercial scale production of FPC was started by Waage in the 1890's in Norway [H lliday and Disney, 1971]. In recent years FPC was widely publicised, especially during the late 1950's up the mid-1970's, and study projects were started in over 40 countries. The main countries involved were: Canada, USSR, Chile, USA, Morocco, Sweden, South Africa, UK, Peru and Brazil [Halliday and Disney, 1977; Crisan, 1970; Windsor, 1971; Wallerstein and Pariser, 1978].

The high nutritional value of FPC protein has been well established in recent years by so many feeding trials for direct human consumption as a protein supplement to foods. The products which contained FPC at different levels were bread, pasta, soups, rice dishes, breakfast cereals and biscuits etc. Nutritional studies showed beneficial effects for children (12g FPC/day was sufficient in diet) and nursing mothers [Finch, 1970; Stillings, 1974; Sidwell, 1974; Halliday and Disney, 1977; Windsor and Barlow, 1981].

The chemical composition and nutritional value of FPC obviously varies with different species of whole fish, fillets, head and eviscerated fish, and trimmings as the raw material and type of processing method (see Table 3) [Stillings, 1974; Sidwell et al, 1970]. It is important that FPC processing should be carried out in such a way that nutritional value will not be affected i.e. the solvents and temperatures used during processing do not result in loss of amino acids. However the action of many solvents and heat or drying on FPC is to denature the proteins and results in loss of functional properties.

FPC is completely unacceptable as a food in its own right therefore it is used as an additive. Unfortunately, production of FPC is expensive because of the method of production, it is only used as an additive and loss of nutritional value occurs in producing it and production of baked biscuits and bread (USA Type) [Finch, 1974; Stillings, 1974; Halliday and Disney, 1977].

2.1.2.4 Fish protein hydrolysate (FPH)

New proteins with functional properties from both conventional and novel sources will be needed [Kinsella, 1977] because of the increase in the population mentioned earlier. To increase the utilisation of these proteins in the human diet they must be capable of inclusion due to these functional properties not just nutritional properties.

TABLE 3: CHEMICAL COMPOSITION OF FPC

Components %	FPC			
	Red hake	Herring	Anchovy	Menhaden
Crude protein (N x 6.25)	80.9	87.5	80.0	78.5
Lipid	0.18	0.19	0.07	0.18
Ash	13.5	10.8	16.8	19.4
Amino Acid				
Lysine	8.28	8.53	8.06	7.89
Histidine	2.05	2.13	2.31	2.29
Arginine	6.47	6.12	6.25	6.44
Threonine	4.15	4.23	4.23	3.96
Valine	4.88	5.45	5.06	5.13
Methionine	3.24	3.19	3.05	2.96
Cystine	0.86	0.71	0.72	0.58
Isoleucine	4.33	4.37	4.34	4.12
Leucine	7.54	7.62	7.38	6.95
Phenylalanine	4.49	4.05	3.98	3.84
Tyrosine	3.21	3.16	3.05	3.00
Tryptophan	0.97	1.20	1.31	1.11

Source: Sidwell et al (1970)

The idea of protein hydrolysates was established long ago for the utilisation of protein-rich food waste and non-conventional food proteins (e.g. meat scraps and fish waste) [Fox, 1982]. The utilisation of fish by-products in the form of FPH was started 30 years ago [Mackie, 1982; Adler-Nissen, 1986].

Generally, FPH is considered as an liquified product made from whole fish (or FW) by the action of added proteolytic enzymes to accelerate hydrolysis, under conditions where pH and temperature are controlled, to achieve a range of final products [Windsor and Barlow, 1981; Mackie, 1982].

Since proteins play an important role in the properties of our food, growing interest in the fish hydrolysate started and aimed to use the product for animal feed, human consumption and non-dietary uses as several therapeutic preparations [Sen et al, 1962; Sripathy et al, 1964; Ostrander et al, 1977; Fox, 1982; Adler-Nissen, 1986].

The production and development of FPH by proteolytic enzymic digestion has been carried out worldwide. The main countries involved are Uruguay, Norway, USA, Japan, India, Denmark, France and UK [Mackie, 1982].

The nutritional value of FPH has been evaluated by several studies. It has been confirmed that the process does not cause any damage to essential amino acids and that the required functional properties are

achieved [Rutman and Heimlich, 1974; Labasidis et al, 1978; Mohr, 1980]. The chemical and nutritional value of FPH is generally summarised in Table 4 [Windsor and Barlow, 1981]. The FPH has been used for enrichment of food products, as a nutritional supplement in cereals, as milk replacers for animals (calves, pigs) and human consumption (especially young children) [Rutman and Heimlich, 1974; Yanez et al, 1976; Petchey et al, 1979; Windsor and Barlow, 1981; Mackie, 1982]. FPH has also been used as a substitute for egg albumen in food systems (ice-cream, meringues, cookies, gelled desserts etc) where good functional properties such as foam capacity, emulsion capacity, aeration and solubility are required [Miller and Groninger, 1976; Ostrander et al, 1977].

2.1.3 Fish Mince Products

The most efficient means of obtaining mince from fish has been achieved over the last 40 years by using mechanical deboners and has enabled the fish processing industries to convert some fish by-products into good quality mince [Keay et al, 1982; Field, 1988].

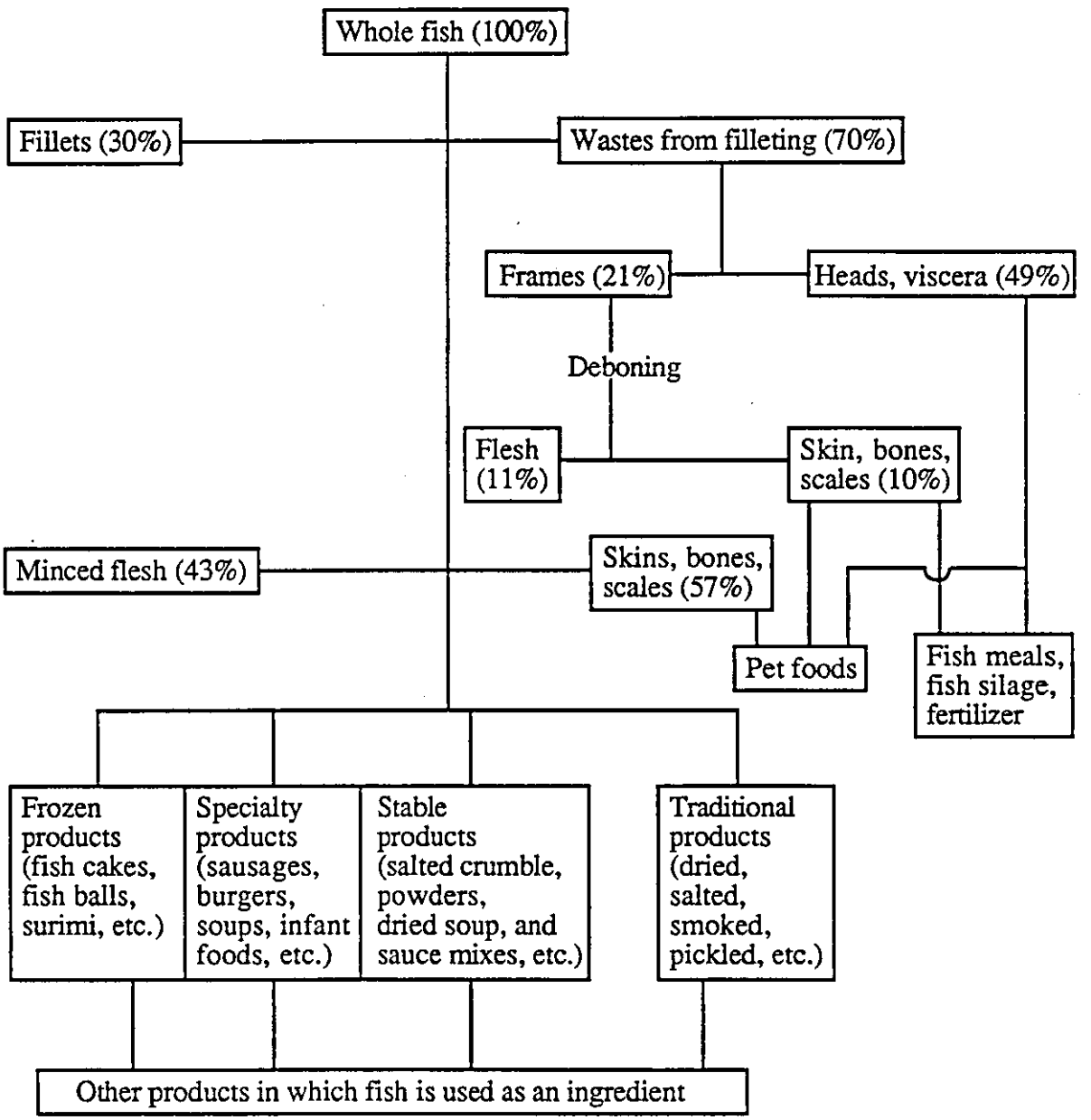
Fish mince is the flesh recovered from filleted fish (beheaded, gutted, cleaned) by a mechanical flesh bone separator which simultaneously removes unwanted parts (skin and bones). Generally, it is this technological breakthrough which can provide the additional seafood products from under-utilised species of whole fish which are wasted because of size, bony species, physical damage and other waste materials during fish processing e.g. filleting (see Figure 1) [Bligh

TABLE 4: COMPOSITION OF FISH PROTEIN HYDROLYSATE

Component	%
Moisture content	3 - 8
Crude protein	70 - 87
Crude fat	1 - 23
Crude ash	1 - 9
Phosphorus	0.4 - 0.8
Calcium	0.1 - 0.8
Chlorides	2 - 3
Iron	40 - 50 ppm
Amino Acid	
Lysine	6.9 - 8.9
Methionine	2.5 - 3.0
Cystine	0.7 - 1.0
Tryptophan	0.6 - 1.2
Arginine	6.4 - 7.1
Threonine	3.5 - 3.9
Isoleucine	3.7 - 4.3
Leucine	6.0 - 7.1
Valine	4.3 - 4.9
Histidine	1.8 - 2.1
Phenylalanine	2.4 - 3.7
Tyrosine	2.5 - 3.3
Glycine	2.5 -12.1
Glutamic acid	12.5 -14.0
Aspartic acid	8.5 - 8.8
Proline	4.3 - 6.5
Serine	4.0 - 4.9
Alanine	6.2 - 7.3

Source: Windsor and Barlow (1981)

Fig. 1. Approximate recoveries in processing of by-catch fish.



Source :- Allsopp (1982)

and Regier, 1976; Pigott, 1976; Allsopp, 1982; Hansen, 1982; Keay et al, 1982; Misuishi, 1982].

Fish mince-based products can be found throughout the world especially in European countries. Some of the 46 fish species and shellfish which are currently used for minced fish products are Atlantic and Pacific cod, mackerel, Alaska and Pacific pollock, haddock, hake, herring, trout, rock fish, saithe, salmon and lobster [Martin, 1976].

The most common products and also new, experimental products utilise mechanicallly recovered fish mince, they include: fish cakes, frozen minced fish (surimi), kamaboko (fish cake), salted-dried minced fish cakes, fish sausages, fish pastes, fish balls (smoked fish bits), fish sticks, fish mince blocks (for fish fingers and portions), cook-freeze dishes, traditional fish pie, crackers, biscuits, spun fish protein fibres, soups and savoury mince [Del Valle et al, 1973; Herborg, 1976; Newman, 1976; Pannell, 1976; Keay, 1980; Suzuki, 1981; Keay et al, 1982; Mackie and Thomson, 1982; Poulter, 1982; Lee, 1984; Field, 1988; Ockerman and Hansen, 1988; Yu and Tan, 1990].

The quality of fish mince and the setting of standards are complex matters, especially in new product development. The types of mince used as raw materials for foods include mince from fillet trimmings (without skin), minced fish from selected portions of frames (backbone, skin and blacknape removed), minced fish from headed and gutted fish (not fillets) with or without backbone removed, minced fish from invertebrates without viscera, minced fish from very small

fish and minced fish for preparing stable and salted products [Martin, 1976].

It also seems clear that minced fish material, from single species or mixed species, shows properties which are much wider ranging than other 'flesh' foods. This is due to species differences, unique seasonal and biological variations and chemical composition [Keay, 1980; Weinberg, 1983].

The mincing process and the different parts of fish used (as mentioned earlier) have an effect on the physical and chemical nature of the minced fish - mainly texture, flavour and colour. It is well known that the quality of the comminuted fish products is related to the functional properties of the raw, fish mince [Howgate, 1976; Keay, 1980; Sidwell, 1980; Regenstein, 1986]. For example, the texture of fish mince from cod fillets is succulent, juicy, fibrous and slightly soft to firm. However, the mince prepared from other fractions of cod fish is different in texture from cod fillet mince. Most parts of cod give mince which is dry, fibrous, crumbly and has a chewy residue but the mince from the backbone is sloppy, mushy and contains gelatin which contributes greatly to texture. However the minced fish offers great flexibility of processing to improve texture in the production of conventional fish minced blocks [Miyauchi et al, 1975; Howgate, 1976].

The use of additives is necessary to any industry intent on using minced fish for fish products which require moulding or shaping. The improvement in texture and functional properties including solubility, emulsion, water binding and gelling capacity has been well established by using common additives such as salt, polyphosphates, egg albumen, sugar, textured vegetable protein, starches, alginate and food grade gums [Hing et al, 1972; Miyauchi et al, 1975; Martin, 1976; Ravichander and Keay, 1976; Sorensen, 1976; Weinberg, 1983].

2.1.3.1 Formed fish products

Fish mince can be formed into various shapes and sizes depending on which type of fish product is required.

Fish cakes are commonly a mixture of minced fish (cod, haddock, saithe, salmon, hake and coley), potato and seasoning which is then coated with batter and breadcrumbs [Pannell, 1976; Keay et al, 1982].

Long ago, preservation techniques and the development of fish products were achieved in the form of a salted-dried minced fish cake which represents a high quality protein food for human consumption [Del Valle and Gonzalez-Inigo, 1968; Del Valle and Nickeson, 1968; Del Valle et al, 1973; Del Valle, 1974; Del Valle et al, 1976; Bligh and Duclos, 1982; Young, 1982; Poulter and Poulter, 1984; Akande et al, 1988].

A variety of uniform shapes and sizes have also been produced from frozen fish blocks (80% fillet and 15% minced fish) such as fish fingers, fish portions (fish sticks) which are subsequently coated with batter and breadcrumbs [Cole and Keay, 1976; Sorensen, 1976; Keay et al, 1982; Martin, 1986].

Fish pastes, spreads, pates, sausages and fish balls are good examples of fish products utilising fish mince made in food industries of today and the past. The species commonly used to produce these products are mackerel, cod, haddock, salmon, pilchard, shrimp, crab, smoked kipper, bloater and lobster. These products are generally a mixture of fish mince with salt, spices, flavours, colouring, starch, vegetable oil, sodium glutamate and cereal fillers, except in the case of fish balls where the minced fish is mixed with milk powder, water and flour to produce a smooth emulsion [Hing et al, 1972; Herborg, 1976; Keay et al, 1982; Peterson, 1981; Poulter and Trevino, 1983].

2.1.3.2 Surimi/Kamaboko

Modern fish processing industries developed surimi in the 1960's from the traditional methods of fish preparation which originated in Japan over 1500 years ago.

Traditionally, surimi is a Japanese term used for mechanically deboned, freshly washed, refined fish mince. It can be cold stored or frozen and the necessary functional properties still retained by incorporating additives such as sucrose (4%), sorbitol (4%),

polyphosphate(0-3%) and salt (0-2.5%) which act as cryoprotectants. Surimi is an intermediate gel product which is mainly used as a material for neriseihi (various kinds of kamaboko, fish sausage, fish ham), and other foods such as hamburgers, fish sticks [Suzuki, 1981; Lee, 1984; Hastings, 1989; Johnston, 1989].

At present there are two main types of surimi gel products being made: one, frozen surimi which can be salted (ka-en-surimi) and unsalted (mu-en-surimi): a second type of raw surimi (nama surimi) has a higher water holding capacity than the frozen surimi but it has to be manufactured into final products on the same day. The fish species most widely used as a material for surimi are Alaska pollock (Theragra chalcogramma), Atka mackerel (Pleurogrammus azonus), horse mackerel (Trchurus japonicus) and lizard fish (Saurida undosquamis) [Suzuki, 1981].

Since frozen surimi is a material for kamoboko products, it should be made from fresh fish. However, the quality of surimi will be influenced by many factors such as fish species, seasonal variation and size of material. Therefore, careful selection of the raw material, handling and storage conditions of surumi is necessary to achieve a good strength of "ashi" character (i.e. resilient texture) in production of kamaboko. The fish species in particular has a great influence on the degree of "suwari" or setting of the fish paste (i.e. plasticity of meat paste resulting in strong gel causing shaping to be difficult). The essential factor in the forming of the 'ashi'

TABLE 5: COMPONENTS OF VARIOUS KINDS OF KAMABOKO

Components %	Steamed kamaboko	Broiled kamaboko	Fried kamaboko	Chikuwa	Hampen
Moisture	74.4	72.0	66.2	69.1	75.7
Protein	12.0	16.2	12.3	12.2	9.9
Lipids	0.9	0.8	4.5	2.1	0.3
Carbohydrates	9.7	7.4	13.9	13.5	11.4
Ash	3.0	3.6	3.1	3.1	2.7
Calcium (mg)	25	25	60	15	15
Sodium (mg)	1000	1200	1000	1000	800
Phosphorus (mg)	60	60	70	110	110
Iron (mg)	1.0	1.0	1.5	2.0	1.0
Vitamin A (g) (retinol)	0	0	0	0	0
B1 (mg)	0	0	0.05	0.05	0
B2 (mg)	0.01	0.01	0.01	0.08	0.01
Niacin	0.5	1.5	0.5	0.7	0.7
C (mg)	0	0	0	0	0
Sodium (g) chloride	2.5	2.9	2.4	2.4	1.9

Source: Suzuki (1981)

character of kamaboko is actomyosin which should be protected from denaturation in surimi production [Suzuki, 1981; Lee, 1984; Lanier, 1986; Lee, 1986; Roussel and Cheffel, 1988].

Kamaboko is a heat-gel fish mince protein product which is shaped and cooked in different ways to give various products. Kamaboko cooked by broiling includes hatsuki, chikawa (a tubular shaped product) and hampen. Fried kamaboko products include satsumage (ball, bar and square shaped) and Tempura.

Generally the ingredients for kamaboko are starch, vegetables (green beans, yam, burdock), seaweed, egg yolk, boiled quails eggs, cheese, ham, shrimp, squid etc) [Suzuki 1981; Johnston, 1989; Hamann et al, 1990]. Chemical composition of various kamaboko is given in Table 5.

There are various fish species used commercially for production of kamaboko, those as for surumi, and also croakers (Argyrosomus argentatus, Nibea mistsukurii), sharp-toothed eel (Muraenesox anereus), cutlass fish (Trichiurus lepturus), shark and flounders. Many deep sea fish and fresh water fish species have also been investigated for the production of kamaboko, since the interest in fish and fish products for human consumption, in particular kamaboko, has increased [Suzuki, 1981; Min et al, 1982; Johnston, 1989; Hastings et al, 1990; Roussel and Cheffel, 1988].

Kamaboko gel formation is a complex process of various chemical and physicochemical changes of fish flesh proteins. The gel-forming ability is influenced by freshness, handling, and proper washing of the materials. The actomyosin as a main component of myofibrillar protein is solubilised with salt solution in the meat paste to form actomyosin sol. This will turn into a viscous gel at the end of processing (see Figure 2). Some experiments have shown that myosin also has the same ability to form a myosin sol in forming kamaboko gel [Suzuki, 1981].

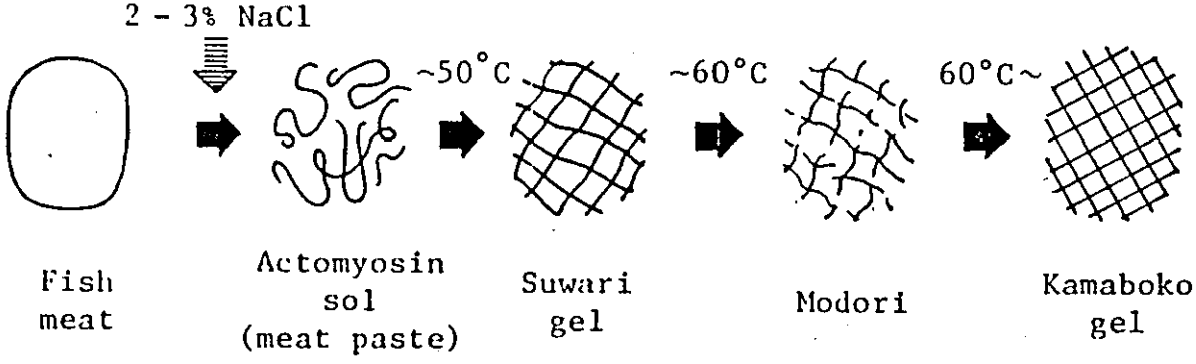
2.2 FUNCTIONAL PROPERTIES

Functionality is defined as "any property of a food or food ingredient, except its nutritional ones, that influences its utilisation [Pour-El, 1979]. Therefore, it it necessary for FPH to have functional properties in its food systems i.e. give desirable characteristics to the food system for its utilisation.

Functionality is an important property of a protein. The terms 'functionality' and 'functional properties' are related and the area has various terms associated with protein functionality which depend on different researchers interests [Sathe et al, 1984; Adler-Nissen, 1986].

Functional properties can be defined as the overall physicochemical behaviour or performance of proteins in food systems during

FIGURE 2: MECHANISM OF KAMABOKO GEL FORMATION



Source: Suzuki (1981)

processing, storage, preparation, consumption. It reflects the complex interactions that are influenced by protein composition, its structural conformation and intermolecular association(s) of protein with other food ingredients (such as water, carbohydrate and lipid) and the nature of the environment in which these properties are measured. These characteristics influence the quality and organoloptic attributes of food systems [Kinsella, 1976; Kinsella, 1982].

The functional properties which proteins give to the final product are the most important factors improving the quality of the food system. This particularly applies to comminuted meat products and fish products. The addition of proteins with several functional properties as ingredients will improve the quality of the final products as follows:

1. Improve uniform emulsion formulation and stabilisation.
2. Improve firmness (gelation), pliability and texture.
3. Facilitate cleaner, smoother slicing.
4. Reduce cooking shrinkage and drip by entrapping binding fats and water.
5. Prevent fat separation.
6. Enhance binding of meat particles without stickiness.
7. Improve moisture-holding and mouth feel.
8. May give antioxidant effects [Kinsella, 1982].

2.2.1 Classification of Functional Properties

The functional properties of proteins are generally classified, in terms of their application in food systems (see Table 6) [Kinsella, 1976; Kinsella, 1982].

Generally, the classes of functional proteins are utilised for their unique functional properties or for several additive functional activities. These must be determined and manipulated to enhance their use in food applications [Anglemier and Montgomery, 1976; Richardson, 1982].

As far as the food systems are concerned, it is the protein that provides the functional properties. However proteins with a wide range of functional properties are required to provide the demand for the food industry. Table 7 shows the type of food systems and related functional properties [Briskey, 1970; Richardson, 1977; Kinsella, 1982].

2.2.2 Protein Structure and Functional Properties

Most of our food is an extremely complex mixture of many chemical units, such as carbohydrate, lipid, protein and water.

Generally proteins consist of only about 20 structural units, the amino acids. They consist of different amino acid classes and their disposition in the polypeptide chain affects functional properties (a high proportion of apolar residues affects interpeptide interaction,

TABLE 6: CLASSES OF FUNCTIONAL PROPERTIES OF PROTEINS IN FOOD APPLICATIONS

Property	Functional Criteria or Specific Functional Properties*
Organoleptic	Colour, flavour, odour
Kinesthetic	Texture, mouthfeel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling, syneresis, viscosity
Surface	Emulsification, foaming (aeration, whipping), film formation
Binding	Lipid-binding, flavour-binding
Structural	Elasticity, cohesiveness, chewiness, adhesion, network cross-binding, aggregation, dough formation, texturisability, fibre formation, extrudability
Rheological	Viscosity, gelation
Enzymatic	Coagulation (rennet), tenderisation (papain), mellowing ('proteinases')
'Blendability'	Complementarity (wheat-soy, gluten-casein)
Antioxidant	Off-flavour prevention (fluid emulsions)

* Varies (1) pH (2) Temperature (3) Protein concentration (4) Protein species (5) Ionic strength (6) Dielectric constant of the medium (7) Macromolecules (carbohydrate, lipids) in the medium (8) Processing treatment (i.e. modification

Source: Kinsella (1976); Kinsella (1982)

TABLE 7: TYPICAL FUNCTIONAL PROPERTIES PREFORMED BY PROTEINS IN FOOD SYSTEMS

Functional Property	Mode of Action	Food System
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water; Entrapment of water (no drip)	Meat, sausages, breads, cakes
Viscosity	Thickening; Water binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds cheese
Cohesion-adhesion	Protein acts as adhesive material	Meat, sausages, baked goods, pasta products
Elasticity	Hydrophobic bonding in gluten: Disulphide links in gels	Meats, bakery
Emulsification	Formation and stabilisation of fat emulsions	Sausages, bologna soup, cakes
Fat absorption	Binding of free fat	Meats, sausages, doughnuts
Flavour-binding	Adsorption, entrapment, release	Simulated meats, bakery, etc
Foaming	Form stable films to entrap gas	Whipped toppings, Chiffon desserts, Angel cakes

Source: Kinsella (1982)

solubility and surface activity). The hydrophobic regions also directly influence surfactant properties e.g. emulsifying, foaming.

The charged amino acids may be involved in stabilising globular proteins and in water binding which is an important factor for hydration, solubility, gelation and surface activity [Kinsella, 1982]. The structure of proteins is usually classified into four levels. The primary structure (amino acid sequence in the polypeptide) is linked by two reactive groups (α-carboxyl and α-amino). The secondary structure (folding of primary chains) is stabilised by hydrogen bonding (e.g. α-helix or β-pleated sheet and random coil). The tertiary structure (folding of secondary structure to give a compact form i.e. three-dimensional structure) is formed by hydrogen, hydrophobic force, Van der Waals and disulphide bonds). The quaternary structure (association of secondary or tertiary structures to form an oligomeric unit i.e. arrangement of polypeptide chains of protein) is stabilised by similar forces as in the tertiary structure.

However, the bonds and forces in protein structures play the role of mediators affecting changes in size and conformation. They play a major part in the original protein structure as well as modifications leading to altered functionality [Berk, 1976; Pour-El, 1981; Kinsella, 1982].

The combination of secondary, tertiary and quaternary structures is referred to as the conformation of a globular protein. The solution of

globular protein has its non-polar sections (hydrophobic i.e. having no affinity for water) inside the globular structure and its charged polar groups (hydrophilic i.e. having an affinity for water) on the outside in contact with water. The uncharged groups (polar i.e. carbonyl, hydroxyl, amide, carboxyl and sulphydryl) can also be inside and out. There is a correlation between the hydrophobicity and ability to perform as a functional protein [Bigelow, 1967]. The nature of hydrophobicity and its relationship to functional properties of protein and interface has been investigated [MacRitchie, 1978; Graham and Philips, 1979].

The important factor relating to functional properties in food systems is the conformation of a globular protein in which the polar amino acids are in contact with the aqueous phase which favours solubility. This predisposition of molecules is caused by protein conformation and is also related to emulsifying or foaming agents, while unfolding elongated conformation of proteins is related to gel, stabilised foams and fibre spinning [Franks, 1975].

The water binding of food proteins also correlates strongly with the polar residues (carboxyls and basic groups). Polar amino acids (ionised) will bind to a large amount of water while the non-ionised acids bind to an intermediate amount and the hydrophobic groups bind to little or no water [Kinsella, 1982].

There has been research into the underlying properties of functional proteins in foods through the physicochemical phenomena i.e. inter-relationships between composition, structure and functional properties to explain enzyme mechanisms, protein-protein association and ligand-protein binding. There have been further studies to predict the changes in protein conformation through the polypeptide chain of a certain amino acid sequence [Buckingham, 1970; Hermansson et al, 1971; Graham and Phillips, 1979; Hermansson and Akesson, 1975; Franks, 1975].

2.2.3 Measurement of Functional Properties

Functional properties are affected by several factors which include protein source, method of preparation, concentration, modification (enzymatic, alkaline, or acid hydrolysis, chemical e.g. crosslinking agents), drying/dehydration and others including temperature, pH, and ionic strength.

However, the determination of functional properties, for any protein to be evaluated, is presently complicated because of the multitude of different methods that are employed. This variety reflects the fact that functional proteins are assessed for specific purposes in specific food systems so the equipment and conditions are dedicated to that application.

There is a need for universal standardised methods for each measurement of a specific property to allow comparison of data of

functional properties of different proteins. The measurement of some functional properties of proteins will be discussed below.

2.2.3.1 Solubility

Solubility is a very important functional character of food proteins which is related to protein-water interactions in food systems e.g. solubility, viscosity, gelation, foaming and emulsification. Several parameters are known to affect protein solubility such as pH, temperature, processing conditions, ionic strength, and presence of materials capable of binding with proteins, the universal solvent is water. Ideally, solubility provides a good index of native structure, the optimisation of processing and determining the effects of treatment which might affect potential applications [Hermansson, 1973; Kinsella, 1976; Kinsella, 1982; Sathe et al, 1984].

The pH-solubility range is being used as a guide to relate directly to many important functional properties, e.g. beverages, emulsifications, foaming capacity and gelation. Solubility profiles as a function of pH have been studied by many workers [Lawhon and Cater, 1971; Morr et al, 1973; Tybor et al, 1975; Betschart, 1974; Wu and Inglett, 1974; Kinsella, 1979].

Solubility has been the topic of investigation of many researchers to examine different materials such as soybean isolate (SB), whey protein concentrate (WDC), FPC, FPH, alfalfa leaf protein, bovine plasma,

animal plasma and globin isolate and peanut flour. Different conditions of stirring, temperature and centrifuging time were used but almost always the Kjeldahl method was used to determine soluble nitrogen which was converted to a protein content [Hermansson, 1973; Hermansson et al, 1971; Spinelli et al, 1972; Chen et al, 1975; Tybor et al, 1975; Hevia et al, 1976; Miller and Groninger, 1976; Wang and Kinsella, 1976; Hevia and Olcott, 1977; Kinsella, 1979; McWatters and Holmes, 1979; Hindi, 1979; Jasim, 1983; Hill, 1986].

Several descriptions are used to represent solubility including water-soluble nitrogen (WSN), water soluble protein (WSP), protein-solubility index (PSI), water-dispersible protein (WDP), nitrogen solubility index (NSI) and protein dispersibility index (PDI) [AOAC, 1980].

Generally, the determination of protein solubility involves dispersing the protein in water (the amount of protein varies up to 2%), mixing at a certain temperature (perhaps with alteration of pH), and centrifuging for a certain time (varies). The solubility will be expressed as total extractable nitrogen by the Kjeldahl procedure and solubility will be described by one of the above terms [Hermansson et al, 1971; Hill, 1986].

2.2.3.2 Oil Absorption Capacity

Binding is an important functional property in relation to lipid, water, flavours and other food components. The ability of proteins to

bind fat or oils is very important for its application in food systems as meat replacers and extenders. This means improvement of the quality of a wide variety of food products including meats, sausages, and frankfurters in mouth feel and flavour in simulated foods [Kinsella, 1976].

Oil adsorption and binding properties are related to physical entrapment of oil and is normally attributed to the proteins [Kinsella, 1976; Hutton and Compbell, 1977]. The major driving forces involved in oil adsorption are the non-covalent bonds of primary protein structure such as hydrophobic, electrostatic and hydrogen bonds [Cornwell and Horrocks, 1964]. Generally oil adsorption of proteins is affected by protein sources, protein composition, processing, particle size and temperature [Kinsella, 1976]. However, a few researchers have investigated oil adsorption in relation to the amount of soluble protein/total protein, bulk density and particle size [Lin et al, 1974; Wang and Kinsella, 1976; Hutton and Compbell, 1977; Jasim, 1983; Hill, 1986].

Oil adsorption and binding capacity are usually determined by mixing liquid oil with a protein sample or food ingredient, holding the mixture for a certain time and centrifuging. The volume of free oil was measured and the oil retained in the sample was expressed as oil adsorbed in ml of oil or gram oil/g of sample. The methods for the measurement of fat or oil adsorption have varied among investigators [Lin et al, 1974].

2.2.3.3 Water Uptake Capacity

Protein-water interaction is the most important criterion in the functional application of food proteins. The water uptake by the protein matrix generally involves interaction of water with proteins and as a result of this water remains with the protein, and loosening of polypeptides occurs (as in swelling of proteins). This ability of the protein structure to prevent water and other components from being released is of importance in food applications industrially [Labuza, 1968; Kinsella, 1982; Hermansson, 1986].

Various terms are used to describe water uptake according to the method employed including: water absorption capacity (WAC), water binding capacity (WBC), water holding capacity (WHC), water hydration and swelling capacity [Kinsella, 1976].

The term water binding is defined as the water retained (bound and entrapped) by the protein after centrifugation, and water sorption defines water absorbed by dry protein after equilibration against water vapour of a known relative humidity [Kinsella, 1979].

Generally, methods for measuring water uptake involve adding water to proteins, mixing and using external force such as pressure or centrifuging. The amount of water retained within a protein matrix after centrifuging is expressed as water adsorption capacity (WHC) which is the amount of water bound per gram of protein [Hutton and Campbell, 191; Kinsella, 1982; Hermansson, 1986]. Swelling capacity

is another way of measuring water absorption and refers to the spontaneous expansion on uptake of water. Both swelling and water adsorption capacity are directly correlated and they are a very good index of protein functionality in food applications such as sausages, custards and doughs [Hermansson, et al, 1971; Kinsella, 1976]. The degree of swelling is influenced by the intermolecular force or bonding (hydrogen bond, electrostatic interaction) between adjacent polypeptides and the facility with which water can disrupt these and replace protein-protein with protein-water bonding.

Swelling and water absorption are generally influenced by protein source, protein concentration, particle size, pH, ionic strength, temperature, mixing time, denaturation of proteins and surface topography [Kinsella, 1976; Kinsella, 1982; Hermansson, 1986].

Proteins from different sources have been investigated by many workers for water uptake using different methods and conditions. The major materials examined include: acylated fish protein, FPC, fish plastein, FPH, whey protein, soy protein, bovine plasma, blood, alfalfa leaf protein and bean protein [Hermansson, 1971; Miller and Groninger, 1976; Wang and Kinsella, 1976; Hindi, 1979; Sathe et al, 198.; Jasim, 1983; Hill, 1986].

2.2.3.4 Emulsion Properties

The emulsifying properties of protein have been a subject of great interest for many researchers as one of the major aspects of functional requirements in many food systems.

Emulsions are defined as dispersed immiscible droplets (the dispersed phase) within another liquid (the continuous phase) stabilised by interphasic compounds. In emulsions stabilised by proteins they act as an adsorbed surfactant active component (emulsifier) and they promote the formation of an emulsion by lowering the surface tension of the system which contains water (as dispersion or continuous phase) and oil (as dispersed or globular phase). Generally the surfactant should contain hydrophilic and hydrophobic (lipophilic) parts in which they will be adsorbed at the globular/continuous interface [MacRitchie, 1978; Pour-El, 1981].

The interface between the two phases (i.e. water and oil) is very important to the stability of the whole system. The stability of emulsion droplets is obtained through the formation of layers around oil globules causing mutual repulsion and/or by formation of a film membrane around the droplets by proteins which cause a decrease in interfacial energy and prevents the separation of oil and water phases e.g. coalescence (a process by which the small individual droplets are destroyed and bigger drops are formed). Emulsions of oil-in-water (o/w) and water-in-oil (w/o) are thermodynamically unstable because of the positive free energy causing interfacial tension [Sathe et al, 1984].

The factors which influence the emulsion stability are interfacial tension (oil and water phases), properties of adsorbed film, electrical charge on the globule, oil: water ratio (o:w) viscosity of

the dispersion phase, dispersed and dispersion ratio (w/v) and size and surface/volume ratio of globules [McRitchie, 1978; Graham and Phillips, 1979].

Emulsion capacity (EC) is a characteristic of the protein solution or suspension necessary to emulsify oil. It has been defined as the volume of oil (ml) that can be emulsified by protein (gm) before phase inversion or collapse of emulsion occurs i.e. maximum amount of oil which can be emulsified without losing its emulsion properties [Kinsella, 1976; Pour-El, 1981]. Many workers have investigated the inversion or collapse of emulsions based on the visual change in the appearance, sudden drop in viscosity and increase in electrical resistance [Swift et al, 1961; Webb et al, 1970; Satterlee et al, 1973; Crenwelge et al, 1974; Haque and Kinsella, 1989].

Generally, the determination of emulsion capacity involves the addition of oil to the aqueous solution or suspension containing a protein (e.g. 2%) which is stirred strongly until the emulsion is formed. The maximum amount of oil which can be added to the protein solution/suspension before phase inversion under specific conditions such as amount of protein, type of protein, stirring time, temperature, pH is called the emulsion capacity (g-oil/g-protein) [Swift et al, 1961; Hill, 1986].

Emulsion stability refers to the ability of the protein to form an emulsion that remains unchanged for a certain duration of time under specific conditions [Swift et al, 1961; Kinsella, 1976].

Proteins from different sources have been studied by many researchers for their emulsifying properties using different techniques and conditions. The main materials which have been examined including fish protein, FPC, FPH, whey protein, soya protein, blood protein, bovine plasma, meat protein, alfalfa leaf protein and ground nut protein [Swift et al, 1961; Ivey et al, 1970; Spinelli et al, 1972; Groninger, 1973; Hermansson, 1973; Keuhler and Stine, 1974; Chen et al, 1975; Koury et al, 1975; Miller and Groninger, 1976; Wang and Kinsella, 1978; Hutton and Campbell, 1977; Ramanatham et al, 1978; Cante et al, 1979; Hindi, 1979; Kinsella, 1979; Yamauchi et al, 1980; Hill, 1986].

2.2.3.5 **Foaming properties**

One of the functional properties of protein which has received great attention is foaming. Foaming is important in several products e.g. cakes (angel, sponge), fudges, souffles, whipped toppings and meringues [Kinsella, 1976].

Food foams usually consist of gas (air bubbles) dispersed in and well-enclosed by a liquid containing a soluble surfactant. The ability of proteins to stabilise a foam is well related to adsorbed layers of the air-water (A/W) interface. However, in protein foams the surfactant protein will perform two essential functions. It will permit film formation by reducing surface tension and interfacial tension of the dispersed phase and form a structurally strong spontaneous cohesive layer around the gas (air bubbles) molecules [Kinsella, 1976;

Stainsby, 1986; Clark et al, 1987]. The film strength depends upon the magnitude of protein-protein interaction and protein-water interaction [Hermansson et al, 1971].

Foam capacity is a measure of the ability of the protein to form a gas-filled cellular system through incorporation of gas (air bubbles) by whipping, agitation or under specified conditions. In practice, agitation is also called foaming which involves solution and air entrapment. In whippability measurements, the volume of foam is usually expressed as the percentage volume increase. The volume of the foam i.e. foam expansion indicates the extent of volume increase.

Foam stability is a measure of the capability of foam to be sustained and to resist collapse over a period of time. It can be expressed by the rate of leakage of fluid from the foam [Kinsella, 1976; Pour-El, 1981; Stainsby, 1986].

Soluble proteins differ widely in their ability to stabilise foams. Increased hydrophobicity of proteins leads to increased foam stability whereas more hydrophilic derivatives showed reduced stability, drainage and collapse. The insolubility of proteins and more coagulation will lead to stability of the foam (Graham and Phillips, 1979; Stainsby, 1986]. However, complete denaturation in foam formation is undesirable because the stability and elasticity of protein films around the gas (air) droplets depends on the degree of denaturation. The greater the degree of denaturation the more fragile

the foam and collapse will occur, see Table 8 for the sequence of events occurring during foam formation and coalescence [Kinsella, 1976; Cherry and McWatters, 1981].

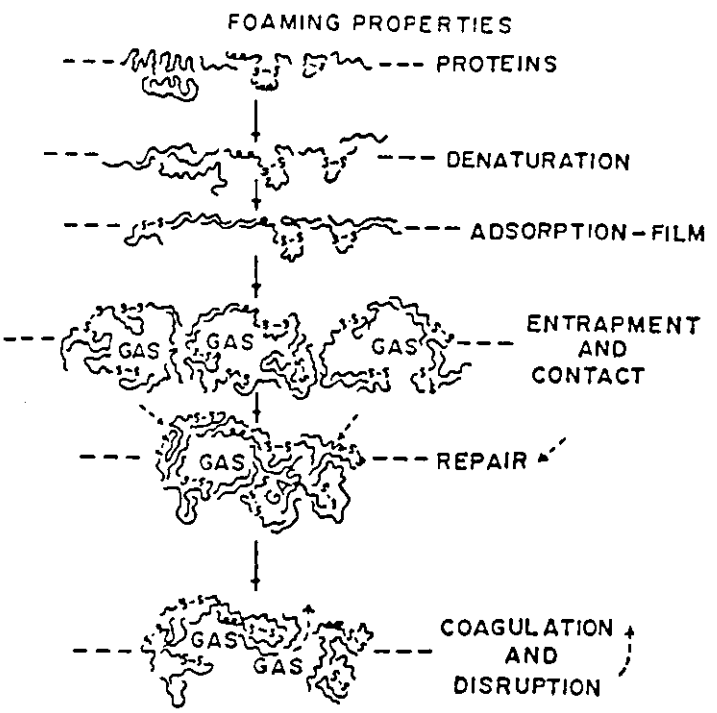
Several factors influence the foaming properties of proteins such as protein source, composition, concentration, solubility, method of preparation, pH, temperature, duration of heating, presence of salt, sugars, lipid and method of measurement.

Methods used for determining foaming properties vary greatly. The most fundamental essential factors involve measuring the volume of the foam which is obtained from a known volume and concentration of protein dispersion, following whipping/beating, agitation and shaking or bubbling/sparging gas (air) through the solution. Other researchers also studied the dynamic lifetime of the foam volume, foaming power which is equal to gas (air) volume/liquid volume, loss of light on transmission through a layer of foam, foam strength, surface tension of the foam and the viscosity of the protein solution [Halling, 1981; Kitabatake and Doi, 1982; Clark et al, 1987].

Different proteins have been investigated for foaming properties by many workers using different techniques and conditions. The main proteins examined include fish proteins; fish protein concentrate; acylated fish proteins; succinylated fish protein; fish plastein; soy bean protein; egg albumen; bovine plasma; whey protein concentrate; sunflower protein and peanut protein (MacDonnell et al,

TABLE 8: SEQUENCE OF EVENTS OCCURRING DURING FOAM FORMATION AND COALESCENCE

1.	Denaturation:	uncoiling of protein polypeptides
2.	Adsorption:	formation of a monolayer or film of denatured protein at the surface of the colloidal solution
3.	Entrapment:	surrounding of gas at the interface by the film and formation of bubbles.
4.	Repair:	continued adsorption or formation of a second monolayer around the bubbles to replace coagulated regions of the film.
5.	Contact:	protein films of adjacent bubbles come in contact and prevent flow of the liquid.
6.	Coagulation:	interacting forces between polypeptides increase causing protein aggregation and weakening of the surface film followed by bursting of the bubble; weakening of the film also occurs when the Repair step ceases because of a deficiency of denatured protein.



Source: Cherry and McWatters (1981)

1955; Hermansson et al, 1971; Lawhan and Carter, 1971; Baldwin and Sinthalavais, 1974; Richert et al, 1974; Groninger and Miller, 1975; Miller and Groninger, 1976; Hindi, 1979; Jasim, 1983; Hill, 1986].

2.2.3.6 Viscosity

Viscosity (η) is defined as the ratio of the shear stress (τ) to shear rate or the slope of the flow curve (i.e. viscosity is the resistance of the liquid to flow).

The flow properties and viscosity of protein dispersions are important in relation to processing and process design such as feed rate, spray drying and to evaluate the thickening power of proteins and property of fluid foods (soups, beverages, batters and comminuted meats and fish products). They give information about the physicochemical interaction between proteins which is a good index of structural changes in proteins and of the hydrodynamic/rheological properties of modified food proteins [Sone, 1972; Kinsella, 1976; Kinsella, 1979; Acton et al, 1983].

Several parameters govern the flow properties including molecular size, shape, surface charge, solubility and swelling capacity of protein molecules which are influenced by the environmental conditions (temperature, pH, concentration, ionicity and processing history of proteins) [Kinsella, 1976; Kinsella, 1982].

Information and studies about the viscosity/rheological properties of proteins are important because they help to explain the conformation (shape) and interaction (hydration, aggregation) of molecules in solution, and they allow reduction of functional properties to physical properties and they also provide a good tool for process monitoring [Rha and Pradipasena, 1986]. Several instruments have been used for measuring viscosity and rheological properties in relation to flow on dispersion, slurries and pastes. These types are rotational viscometers (Brookfield viscometer with Helipath stand and various spindles, Haake viscometer), standard capillary viscometer (Ostwald, Canon-Fenske and Ubbelohde viscometers) and the falling sphere ball viscometer [Sone, 1982].

Many authors have studied the viscosity and flow properties of proteins and products using different types of instruments to suit different needs. A glass capillary tube viscometer (Canon-Fenske type) was used to measure the viscosity of eggs and egg products in which a linear relationship between shear stress and shear rate was obtained and the viscosity data were reported as centipoise (Cps) [Scalzo et al, 1970]. A Brookfield LVI viscometer with Helipath stand was also used to measure the viscosity of soy bean dispersion and the data were reported as apparent viscosity [Catsimpoolas and Meyer, 1970]. A Haake rotaviscometer and Ostwald type were used to measure the viscosity of the following materials: whey protein, fish plastein, bean protein, bovine plasma and the data was reported also as Cps [Hermansson, 1972; Sathe et al, 1984; Jasim, 1983; Hill, 1986].

2.2.3.7 Gel formation

The ability of a protein to form a gel through intermolecular bond interactions provides a structural matrix as semi-solid for holding materials such as water, flavours, sugars and other food ingredients. This provides also a useful additional dimension to protein functionality in products including: baked goods, processed meats, sausages, confectionery and many oriental textured foods e.g. tofu [Kinsella, 1976; Kinsella, 1979; Kinsella, 1982].

Gelation can be defined as a protein aggregation phenomenon in which polymer-solvent and polymer-polymer interactions as well as attractive and repulsive forces are balanced such that a well-ordered tertiary network or matrix is formed [Schmidt, 1981]. Gossett et al (1984) has discussed the terminology which is associated with the gelation phenomenon such as denaturation, aggregation, coagulation and gelation. Food materials which provide a good gel matrix employed by the food industry include starch, pectin, gelatin, alginate, agar, carrageenans and plant gums.

Gelation is characterised by a relatively high viscosity, plasticity, elasticity and yield values. However, coagulation and gelling will be brought about by many factors including heat, salts, pH, alcohol, pressure, urea which in turn unfold the protein in gelation systems. Various factors influence gelation such as electrostatic charge, protein (composition and concentration) and formation of disulphide bonds [Gossett et al, 1984]. The bonds involved in the structure of a

protein gel matrix include hydrogen bonding, disulphide binding and hydrophobic attraction which play major roles in crosslinking (by covalent and non-covalent bonds) and stabilising the gel [Catsimpoolas and Meyer, 1970; Howell and Lawrie, 1985].

Many different terms are used to describe protein gels (see Table 9). Some of the terms have been interpreted as a force/distance curve to measure the texture profile using the Instron machine (see Figure 3) [Szczesniak, 1963]. Measurement of gel properties can be done using different techniques including resistance to penetration, gel strength, breaking strength, ability to hold water, viscosity of gel, gravimetric analysis, structural changes of the gel, electrophoresis of protein bonds, solubility and formation of disulphide linkages [Gossett et al, 1984]. For example the penetration test has been used to measure the depth of penetration of a punch into a food under constant force for a given time and also can be used to define the compression coefficient and shear coefficient [Hill, 1986].

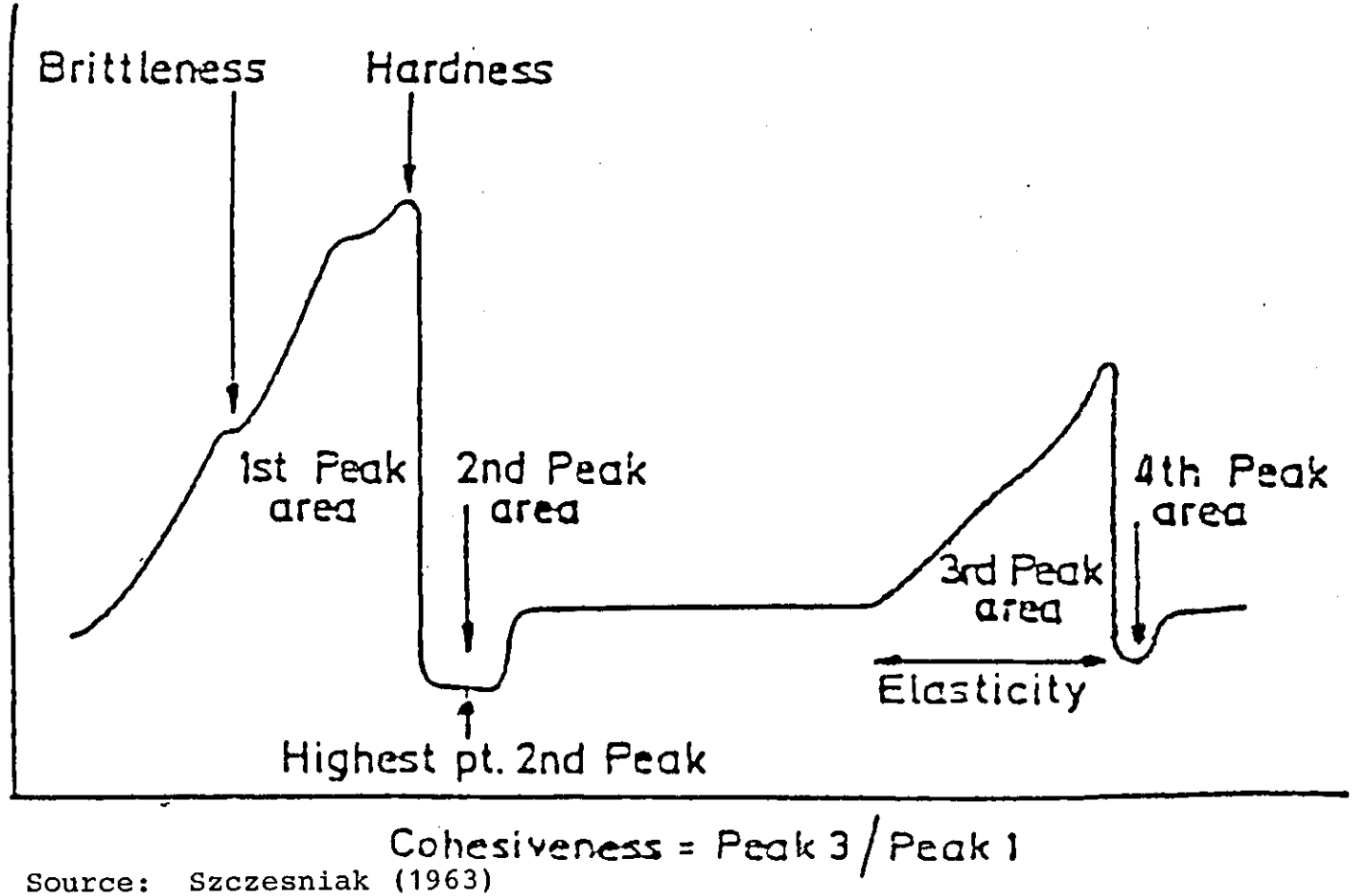
Many food proteins have been investigated for gelling capacity by many workers using different techniques and conditions. The main materials examined include acylated fish protein, fish protein concentrate, fish plastein, bovine plasma, soya bean protein, egg albumen, milk protein, whey protein and muscle protein [Catsimpoolas and Meyer, 1970; Tsai et al, 1972; Miller and Groninger, 1976; Hindi, 1979; Jasim, 1983; Gossett et al, 1984; Schmidt and Morris, 1984; Ziegler and Acton, 1984; Howell and Lawrie, 1985; Hill, 1986].

TABLE 9: TEXTURAL PARAMETERS AND POPULAR NOMENCLATURE

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

Source: Szczeniak (1963)

FIGURE 3: TEXTURE PROFILE CURVE



Source: Szczesniak (1963)

2.3 ENZYME PROCESSING

2.3.1 Enzyme Science and Food Technology

Enzymes are proteins with the capability to catalyse specific biochemical reactions [Berk, 1976]. Enzymes are a specific group of naturally occurring proteins of relatively high molecular weight that are synthesised by living cells. Generally they are colloidal, thermolabile, exhibit a high degree of stereo-chemical substrate specificity and can be isolated from living cells. Many thousands of the biochemical reactions that constitute the metabolism of a cell will be catalysed by enzymes [Kay, 1968; Bickerstaff, 1987]. Enzymes can be classified into six major groups according to reaction specificity, see Table 10. Generally enzymes have an active site which interacts with the substrate by binding to it to form an enzyme-substrate complex. Enzymes are usually very much larger than their substrates, and may consist of a single polypeptide chain with only one active site but some enzymes with quaternary structure may have several active sites [Leadlay, 1978].

Immobilised enzyme technology has overcome the problems associated with soluble enzymes and cost considerations. They can be used for prolonged periods in continuous processing, and can be recovered and re-used a number of times. The contact time between enzyme and substrate and product can be more easily controlled. The term immobilisation implies the artificial confinement of the enzyme protein so that it remains accessible to its substrates, but is

TABLE 10: ENZYME CLASSIFICATION

Enzymes	Specific Action
Oxidoreductase	Oxidation-reduction reactions (e.g. dehydrogenases, reductases, oxidases, oxygenases, hydroxylases)
Transferase	Transfer atoms or groups of various chemicals (e.g. ethyl, acetyl, aldehyde, ketone, amine, phosphate residues etc) from one substrate to another
Hydrolase	Responsible for the hydrolytic cleavage of bonds e.g. lipase, proteinase, pectinesterase, amylase, maltase
Lyases	Responsible to remove groups from their substrate and leaving double bonds e.g. carboxy-lyases, aldolases, hydrolases
Isomerases	Transfer their substrates from one isomeric form to another such as racemases and epimerases
Ligase	This name is now given to a group of enzymes which catalyse a certain type of synthesis

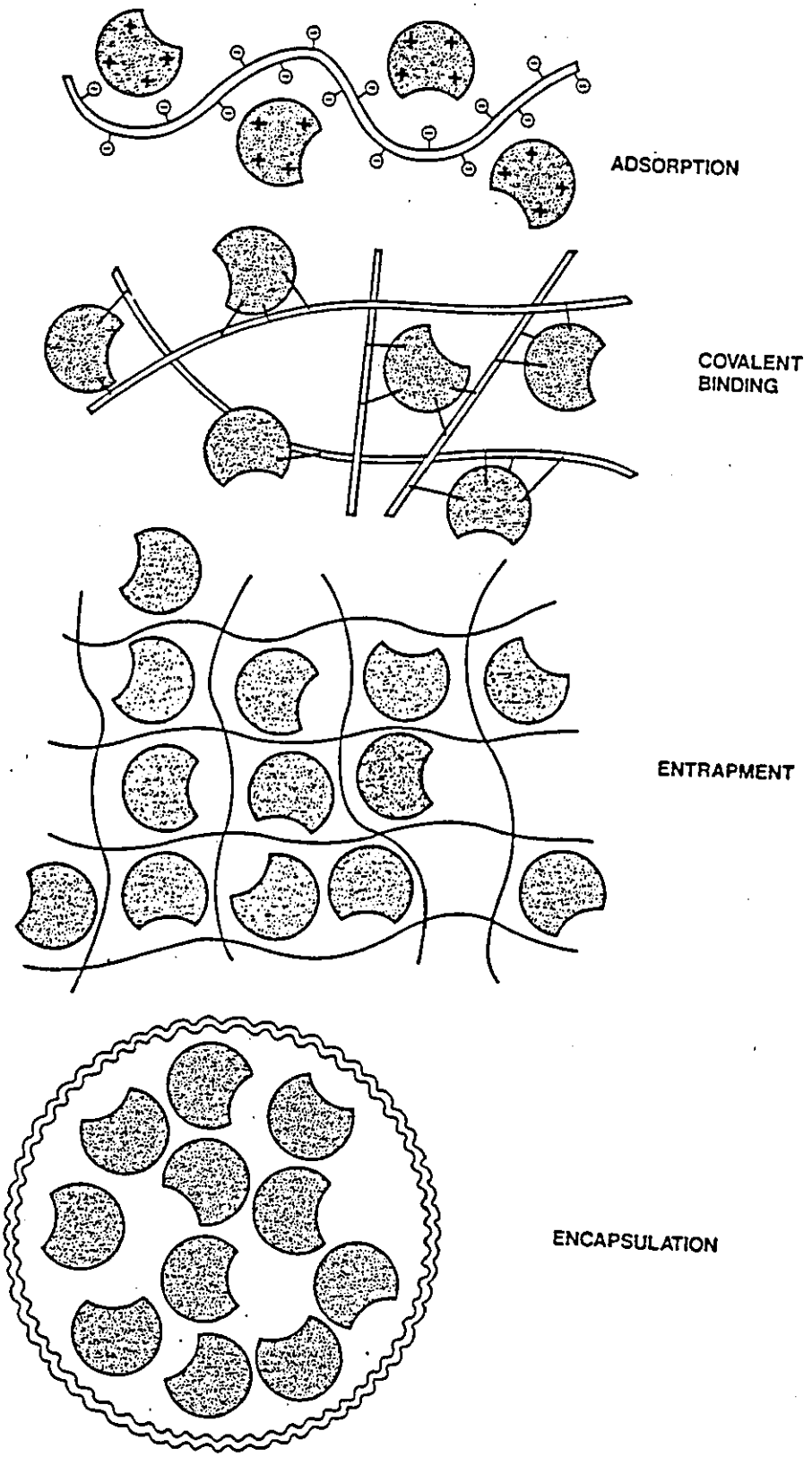
Source: Berk (1976)

readily separable from them. There are four approaches to achieve enzyme immobilisation: adsorption, covalent binding, entrapment and encapsulation (see Figure 4). Immobilised-proteases have been applied successfully for food hydrolysis for example pepsin, trypsin and α-chymotrypsin [Olson and Richardson, 1974; Barker, 1978; Leadlay, 1978; Halling and Dunnill, 1979; Bickerstaff, 1987].

The use of enzymes to facilitate a huge industrial manufacturing process has many attractions compared to chemical reactions i.e. modification of properties of proteins by chemicals [Feeney, 1977; Whitaker, 1977]. Industry has a long history of manufacture and use of enzymes in food and medicine. A large proportion of current enzymes are used in the food and drink industries [Leadlay, 1978; Bickerstaff, 1987; Righelato and Rodgers, 1987; West, 1988; Wong, 1989].

Proteolytic enzymes are used to modify proteins through hydrolysis of peptide bonds which lead to changes in functional properties. They are the principal hydrolases that act on protein modification (see Table 11) through cleavage of peptide bonds [Whitaker, 1977; Peterson, 1981]. Proteolytic enzymes include the serine proteinases such as chymotrypsin, trypsin, elastase and thrombin; the cysteine proteinases such as papain, ficin and bromelein; the metal peptidases such as amino peptidase and carboxypeptidase; and peptidases such as pepsin and rennin.

FIGURE 4: ENZYME IMMOBILIZATION



Source: Bickerstaff (1987)

TABLE 11: PROTEOLYTIC ENZYMES IN PROTEIN MODIFICATION

Food	Purpose or Action
Baked goods	Softening action in doughs. Cut mixing time, increase extensibility of doughs. Improvement in texture, grain and loaf volume. Liberate α-amylase.
Brewing	Body, flavour, and nutrient development during fermentation. Aid in filtration and clarification. Chillproofing.
Cereals	Modify proteins to increase drying rate, improve product handling characteristics. Production of miso and tofu.
Cheese	Casein coagulation. Characteristic flavour development during ageing.
Chocolate-cocoa	Action on beans during fermentation.
Egg, egg products	Improve drying properties.
Feeds	Waste product conversion to feeds. Digestive aids, particularly for pigs.
Fish	Solubilization of fish protein concentrate. Recovery of oil and proteins from inedible parts.
Legumes	Hydrolysed protein products. Removal of flavour. Plastein formation.
Meats	Tenderisation. Recovery of protein from bones.
Milk	Coagulation in rennet puddings. Preparation of soybean milk.
Protein hydrolysates	Condiments such as soy sauce and tamar sauce. Bouillon. Dehydrated soups. Gravy powders. Processed meats. Special diets.
Antinutrient factor removal	Specific protein inhibitors of proteolytic enzymes and amylases. Phytate[a]. Gossypol[a]. Nucleic acid[a].
Wines	Clarification[a]
In vivo processing[b]	Conversion of zymogens to enzymes. Fibrinogen to fibrin. Collagen biosynthesis. Proinsulin to insulin. Macromolecular assembly.

a In large part caused by other than proteolytic enzymes
b Representative examples given

Source: Whitaker (1977)

Various enzymes catalyse the hydrolysis of peptides, amides and esters. Endopeptidase enzymes such as trypsin, α-chymotrypsin, pepsin, papain, bromelein, ficin, and pronase act on the peptide bonds within the protein chain. Exopeptidases such as carboxypeptidase and aminopeptidase act on the peptide bond from the N or C terminal ends of protein chains [Richardson, 1977; Whitaker, 1977; Peterson, 1981; Adler-Nissen, 1986].

The major recent advance in enzymatic hydrolysis of proteins is with proteolytic enzymes in the food area. The most useful property of proteolytic enzymes is their specificity through their ability to hydrolyse peptide bonds formed by specific amino acids compared with acid hydrolysis which does not distinguish between bonds. Alkaline hydrolysis can destroy some amino acids such as tryptophan and cause racemisation of most amino acids [Waley and Watson, 1954; Blau and Waley, 1954; Sela and Kalchalski, 1959; Adler-Nissen, 1986].

2.3.2 Application of Enzymes in the Fish Field

Enzymes traditionally are used for various food processing applications, for example cheese, baking production, and meat tenderisation. However, the use of enzymes within the field of fish processing is minimal, because emphasis has been put on mechanisation of all stages of processing. Recently the fish industry has realised the importance of enzymes in particular areas of seafood processing e.g. for the removal of skins from herring, squid, skate and thin membranes from fish and other seafoods [Stefansson, 1988].

Much work has been done on the application of enzymes including bromelain, papain and ficin in the production of soluble fish protein for the manufacture of fermented fishery products such as fish sauce, pastes and silage [Mackie, 1974; Beddows and Ardeshir, 1979; Owens and Mendoza, 1985]. The use of enzymes in traditional methods for making enzyme hydrolysed products represents a successful solution to the problems of how to control enzyme activity in order to obtain the desired degree of textural change and flavour development without bacterial spoilage or toxin formation [Owens and Mendoza, 1985].

Extensive work on the application of enzymes has been done to improve fish protein functionality without losing their nutritional value. Generally enzymes have been used to solubilise fish proteins to improve the yield of usable proteins from food processing residues [Beck and Scott, 1974]. Various projects have been carried out on the application of different enzymes and their effect on protein functionality e.g. solubility of fish proteins and fish products such as FPC and FPH [Hale, 1969; Cheftel et al, 1971; Spinelli et al, 1972; Tarky et al, 1973; Mohr [illegible], 1978; Hindi, 1979; Mackie, 1982; Jasim, 1983].

2.4 PRODUCT AND ECONOMIC EVALUATION

The development of foods to feed human and animal populations using fish as a protein source has recently received increasing industrial interest. It is necessary to design plant and equipment for each

process so that profitability may be achieved [Backhurst and Harker, 1973].

Economic studies of fish products such as fish silage and meal, FPC and FPH have been carried out and emphasised the importance of availability of raw material and the amount of processing to which each product is subjected [Sripathy et al, 1964; Rutman and Heimlich, 1974; Chakraborty and James, 1976; Nicholson, 1976; Pigott et al, 1978; Aagaard et al, 1980; Merritt, 1982]. Detailed information on the cost of the plant and equipment for production of fish protein hydrolysate is not available, but a rough assessment and comparison with fish meal has been done (see Table 12) for a detailed process, investment cost (£), annual production cost (£) and total cost of production [Merritt, 1982].

2.5 AIM OF THE PRESENT WORK

The idea of processing fish proteins for human consumption is ancient. It was put forward in order to help meet the demand for food for both nutritional and functional purposes.

The processing of fish causes a large amount of FW and other by-products. The waste has, up to now, been mainly utilised to feed animals. However, FW can be more efficiently utilised to produce a nutritional and functional protein product which is valuable for direct application in food industries. One such potential product is

TABLE 12: PROCESS AND ECONOMIC EVALUATION OF FISH MEAL AND FPH

The Process

Stage	Input	Output
1 Comminute	Fish (100 kg)	
2 Digest	Water (100 kg)	
	Enzyme (0.050 kg)	
	Acid/alkali	
3 Sieve		Bones etc (3 kg)
4 Decant		Sludge/liquors
5 Centrifuge	Liquors	Oil
6 Evaporate	Water solubles	
7 Spray dry		Product (14 kg)

Investment cost (£)

	Fish meal	Hydrolysate
Plant, machinery	700 000	1 400 000
Freight, etc	80 000	160 000
Plant buildings @ £200/m^2	40 000	40 000
Office & workshops at £400/m^2	40 000	40 000
Storage for 1200 tonnes of product	60 000	60 000
Miscellaneous	80 000	100 000
TOTAL:	1 000 000	1 800 000

Annual production cost (£)

	Fish meal	Hydrolysate
Annual fixed costs		
Amortization (factor 0.20)	200 000	360 000
Insurance (2%)	20 000	36 000
Maintenance and repairs (6%)	60 000	108 000
Interest, lease of land, etc	25 000	35 000
TOTAL:	365 000	611 000
Variable costs, per tonne of raw materials		
Raw materials	25	25
Fuel	8	10
Electricity	1	1
Bags	1	1
Labour	1	6
Water, chemicals etc	1	1
Enzyme (0.5 kg)	-	18
TOTAL:	40	62

/Continued

Table 12 (continued)

Total cost of production (£)

Cost	Amount of raw material per year			
	7500 tonnes		15000 tonnes	
	Fish Meal	Hydrolysate	Fish Meal	Hydrolysate
Annual fixed	365 000	611 000	365 000	611 000
Variable	300 000	465 000	600 000	930 000
Annual total	665 000	1 076 000	965 000	1 541 000
Per tonne of product	493	1 025	357	734

Source: Merritt (1982)

enzymically-hydrolysed fish protein called fish protein hydrolysate (FPH). The aim of this work was to study the production of FPH as a powder or frozen flake, in reproducible quality for human consumption and to be used in fish products.

The functional properties of FPH powder would be evaluated to establish the variability in product quality.

The suitability and functionality of FPH for food systems was determined by producing fishburgers from cod mince which was stabilised by an FPH/vegetable oil/water emulsion. Panel tests were done to compare reference fishburgers stabilised by egg albumen (EA) and soya bean isolate (ISB) emulsions with those stabilised by FPH.

Thus the project involves the establishment of methods to give a functional protein fit for human consumption and to improve the utilisation of minced fish in fish products and to evaluate the reproducibility of the final product. Process specification and costing of FPH were also outlined.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Raw Materials

The fish waste (fw) was the residue from filleting cod (Gadus morhua) supplied by Humberside College, Grimsby between December 1985 and April 1988.

The fw was chopped immediately into batches of 5 kg with a bowl chopper (Hobart Model A120) at speed 2 for two minutes. The chopped fw was stored in amounts of 8 kg in polyethylene bags at -30°C until required.

Four bathes of fw (Batches A-D) were used to produce fish protein hydrolysate (FPH) in this investigation. Batch A was divided into 2 x 8 kg batches which were hydrolysed as in Table 13 and the liquid FPH was combined and spray dried. This is sample T60/1. Batch B was divided into 4 x 8 kg batches which were hydrolysed as in Table 13 and the liquid was combined and spray dried. This is sample T60/2. Batch C was divided into 2 x 50 kg batches which were hydrolysed as in Table 13 and the liquid FPH was combined and spray dried. This is sample T60/3. Batch D was divided into 9 x 8 kg batches which were hydrolysed as in Table 13 and each sample was spray dried

TABLE 13: DETAILED EXPERIMENTAL DESIGN

Batches	Cod fillet waste used (kg)	Enzyme used	Time of hydrolysis	Sample	'Average' sample*
A	16	Trypsin	60 mins	T60/1	T60
B	32			T60/2	
C	100			T60/3	
			20 mins	T20/1	T20
				T20/2	
				T20/3	
D	72	Bromelain	60 mins	B60/1	B60
				B60/2	
				B60/3	
			20 mins	B20/1	B20
				B20/2	
				B20/3	
Control egg albumen					(EA)
Control soya bean isolate					(ISB)

* T and B stand for Trypsin and bromelain, 20 and 60 stand for time (minutes) of hydrolysis

individually. These are samples T20/1, T20/2, T20/3, B60/1, B60/2, B60/3, B20/1, B20/2, B20/3.

The above batches were hydrolysed in a small vessel, capacity 20 kg, using an electric heater (see Figure 5) except batch C which was hydrolysed in a large steam vessel of capacity 200 kg (see Figure 5). The mean results of the analysis and functional tests for each batch under the same conditions of enzyme hydrolysis were called 'average' samples as in Table 13. This was done to indicate the reproducibility of FPH from different batches of fw and the batches of the same condition of enzyme hydrolysis.

Egg albumen (EA) and soya bean isolate (ISB) were used as controls. The detailed experimental design for all the batches are summarised in Table 13.

3.1.2 Enzymes

Commercial enzymes were supplied by Sigma Chemical Co Ltd., Fancy Road, Poole, Dorset and BDH Ltd, Broom Road, Poole, Dorset respectively.

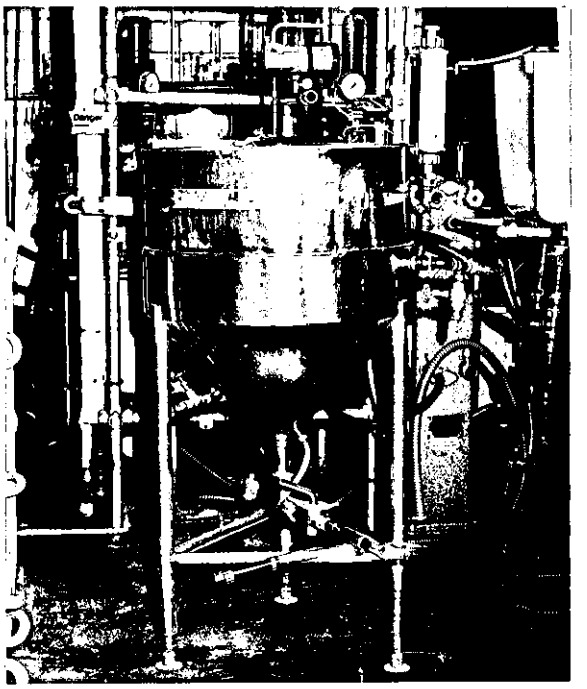
Bromelain enzyme (EC 3.4.22.4).

Trypsin enzyme (3.4.21.4).

3.1.3 Commercial Proteins

Commercial proteins were supplied by BDH Chemicals Ltd, Poole, Dorset, as follows: egg albumen powder and soya bean isolate.

FIGURE 5: REACTION VESSELS FOR ENZYMIC HYDROLYSIS



Large capacity vessel (200 kg)

Small scale vessel (20 kg)

3.2 ENZYME PROCESSING

The fw was thawed at room temperature and blended with water in the proportion 1:1. The pH was adjusted from 6.8 to 8 with 1N sodium hydroxide (NaOH). Enzyme hydrolysis was done at 55°C using steam or electrical heating and continuous mixing with a stirrer at a speed of 40 rpm. Trypsin or bromelain enzyme suspension was added at 1% of the total protein content in the fw. Two fixed hydrolysis times (20 and 60 minutes) were used. The pH was continuously checked during hydrolysis and adjusted to pH 8. The fw was heated for 20 minutes at 70°C in order to inactivate the enzyme and sieved (250 mm) to remove the bones and undigested materials. The FPH was spray dried at 200°C inlet temperature, 87°C outlet temperature and was packed in a glass or plastic container and stored at room temperature. A sample of FPH was flaked using an ice flake maker for alternative uses.

3.3 FUNCTIONAL TESTS

3.3.1 Solubility

Solubility was measured by the method of Hindi (1979) modified as below. Solutions of 1% FPH in distilled water were made and adjusted to the required pH (2, 4, 6, 8) by 1N HCl and 1N NaOH. The suspensions were agitated by means of a magnetic stirrer for 45 minutes in a water bath at 30°C. Solutions were centrifuged at 1470 x g for 10 minutes [Burgess and Kelly, 1979]. The total nitrogen content of the FPH (PX) and the total nitrogen in the supernatant (PY) were determined by the

Kjeldahl method [AOAC, 1979]. The solubility was calculated according to the following equation:

$$\text{Solubility } \% = \frac{PY}{PX} \times 100$$

3.3.2 Oil Absorption Capacity

The method of Hindi (1979) was used. Samples of FPH (0.5g) were weighed into a 15 ml conical centrifuge tube and 3 ml of corn oil was added. The tubes were shaken for 1 minute to disperse the FPH in the oil and then held at room temperature for 30 minutes. The tubes were centrifuged at 1000 x g for 25 minutes. The volume of oil absorbed as ml/g FPH was determined by subtracting the volume of free oil from the total volume added.

3.3.3 Water Absorption Capacity

The method of Jasim (1983) was used. A sample of FPH (0.5g) was weighed into a 15 ml centrifuge tube and 10 ml of distilled water was added. The tubes were shaken for 2 minutes to disperse the FPH and then held at room temperature for 5 minutes. The tubes were centrifuged at 1000 x g for 15 minutes. The volume of supernatant was subtracted from the original 10 ml water and the water absorbed was expressed as ml/g of FPH.

3.3.4 Swelling Capacity

The apparatus used in this test was a modification of that used by Hermansson (1972). It consisted of a sintered glass filter funnel No.

1 connected to a horizontal (0.2 ml) graduated capillary pipette through a 90° bend. The apparatus was filled with distilled water (total 0.2 ml) and clamped in a retort stand in order to remove any air bubbles. Then filter paper (Whatman No. 4) was placed in the funnel and individual samples of FPH (15, 25, 45, 55, 75, 100 mg) were carefully placed as evenly as possible on the filter paper. The volume of water absorbed after 30 minutes at room temperature was measured by movement of water along the pipette and expressed as (ml/mg FPH).

3.3.5 Emulsion Capacity

Emulsion capacity was measured by the method of Hill (1986). A 2% (w/v) solution of FPH was prepared and the specific gravity of the solution noted. To an aliquot of this solution (25 ml) corn oil (60 ml) was added and mixed for 2 minutes using a Silverson homogeniser at 8000 rpm speed. The emulsion (25g) was placed in a 100 ml beaker and corn oil added by means of a peristaltic pump at a rate of 5 ml/min. The Silverson homogeniser was used to mix the added oil into the emulsion until the emulsion was broken as seen by a change in viscosity or visually. Emulsion capacity was expressed as g oil added by pump/g protein.

3.3.6 Viscosity

Solutions of 5, 10 and 15% (w/v) of FPH were prepared and mixed for 2 minutes. The solutions were then left for about 30 minutes at room temperature to allow the samples to swell. The dispersion was mixed again for 2 minutes. A portion of dispersion samples was used to fill

the measuring head of the viscometer. Viscosities of the samples were determined using a Haake Rotovisc RV2 (at different temperatures of 20, 30 and 40°C) with a NIVI measuring system with sensor factor 50 DMK (G(Cp/scale grade-min)). The slope of a graph of rpm versus scale reading (shear stress) was used to calculate the viscosity (Appendix 3) as follows:

$$\text{Viscosity (Cp)} = \frac{\text{G x shear stress}}{\text{rpm}}$$

G = 30.9 (sensor factor at 50 DMK).

3.3.7 Gel Formation

The method of Hill (1986) was used. A 10% w/v of FPH solution in distilled water and 2% or 3% NaCl was prepared and 40 ml of solution was poured into glass universal bottles, and firmly capped. Then the contents were placed on the rack in the water bath for 30 minutes at 80°C. The samples were removed and left for 26 hours at room temperature. The gel formation was examined visually and by placing each bottle containing gel under a penetometer so that the cone and plunger could enter the gel without fouling the bottle. The height of the cone was precisely adjusted too. The cone and plunger were released for 8 seconds. The results were recorded as depth in mm, if the cone had penetrated, or as follows: if the cone reached the bottom of the gel the reading was recorded as 400. If no gel formed the arbitrary figure of 600 was recorded to the product. If movement occurred on inversion, the figure 500 was used to define it.

3.4 PRODUCT DEVELOPMENT METHOD

3.4.1 Method of Fishburger Production

Fishburgers were made from skinless minced cod fillet fish and an emulsion stabilised by FPH and the controls ISB and EA (0.5g of FPH or ISB or EA + 25 ml water + 70 ml of corn oil). The amounts of emulsion added to fish mince by weight were as follows: 10%, 15%, 20% and 25%. The mixture was stirred for 2 minutes and shaped by a beefburger machine and fishburgers were deep fried in vegetable oil for 2 minutes at 180°C.

3.4.2 Method of Cooking Loss Estimation

A simple method of cooking loss was used to measure the weight loss of fishburgers. Ready made fishburgers were weighed before cooking (S). After cooking for 4 minutes by deep frying the fishburgers were left to cool down for 1 minute on paper tissue to remove the excess oil and weighed again (W). The percentage of cooking loss was calculated as follows:

$$\text{Cooking loss } \% = \frac{S-W}{S} \times 100$$

3.4.3 Taste Panel Assessment

Test panel assessment of fishburgers was carried out by a method based on Land and Shepherd (1984) by 8 panellists. Samples of fishburgers (containing FPH, or ISB or EA emulsions) were numbered and score values of (5-1) were given for quality parameters such as colour,

odour, taste, texture and overall preference. A score of 5 represented the best quality and number 1 represented the lowest quality (see Table 14).

Score values of each individual quality parameter were plotted against the amount of emulsion added (10%, 15%, 20% and 25%).

TABLE 14: TEST PANEL SCORE SHEETS

PRODUCT RATING

Please indicate your assessment of each of the four quality parameters for the samples as listed below by ticking ( ) in the appropriate box:

1. COLOUR

	Sample No Scores
5. Golden Brown	5
4. Yellowish Brown	4
3. Light Brown	3
2. Dark Brown	2
1. Grey White-Brown	1

2. ODOUR

	Sample No Scores
5. Very strong fish odour	5
4. Strong fish odour	4
3. Slight fish odour	3
3. Weak fish odour	2
1. No fish odour	1

3. TASTE

	Sample No Scores
5. Very strong fish taste	5
4. Strong fish taste	4
3. Slight fish taste	3
2. Weak fish taste	2
1. No fish taste	1

4. TEXTURE

	Sample No Scores
5. Juicy and tender	5
4. Slightly tender/ juicy	4
3. Neither tough/dry nor tender or juicy	3
2. Slightly tough/dry	2
1. Tough and dry	1

Please indicate your own opinion of its overall acceptability.

5. OVERALL PREFERENCE

	Sample No Scores
5. Like very much	5
4. Like slightly	4
3. Neither like nor dislike	3
2. Dislike slightly	2
1. Dislike very much	1

Additional comments (if any)

PRINT NAME ..... DATE .....

Signature of the Panelist

3.5 ANALYTICAL METHODS

3.5.1 Degree of Hydrolysis

The method of Yamashita et al (1970) was used. A sample of hydrolysate (4g) was weighed and mixed with 2 ml of 10% TCA (Trichloroacetic acid). The mixture was filtered and total nitrogen of the filtrate was determined by the Kjeldahl procedure (AOAC, 1970]. Total nitrogen was also determined for the hydrolysate before adding the 10% TCA. The degree of hydrolysis was calculated according to the following equation:

$$DH\ \% = \frac{\text{Total nitrogen in the filtrate (after TCA)}}{\text{Total nitrogen in the hydrolysate}} \times 100$$

3.5.2 Yield of Fish Protein Hydrolysate

The yield was calculated according to the equation as follows:

$$\text{Yield} = \frac{\text{Total protein in FPH}}{\text{Total protein in FW}} \times 100$$

3.5.3 Total Solids

The hydrolysate samples (26g) were analysed in large flat bottomed dishes. The samples were evaporated to dryness on a water bath and dried at 70°C in a vacuum oven at 26 Hg for 2 hours. The total solids was determined as follows:

$$\text{Total solids} = \frac{\text{Dry weight of hydrolysate}}{\text{Weight of sample (26g)}} \times 100$$

3.5.4 Protein Content

Protein determinations were carried out by the Kjeldahl method of Pearson (1973). A sample of FPH (0.2g) was weighed into a digestion flask. N-free sulphuric acid (2-3 ml) and 1.1g of potassium sulphate (pentahydrate) and 100 mg of $CUSO_4$ as catalyst were added. The digested flask content mixture was initially warmed carefully to minimise forthing, then the heat was increased and the sample boiled until the solution became green in colour. Then the samples were left to cool and 5 ml of distilled water were added. The digestion flask was then placed in the distilling unit and 40% NaOH (15 ml) was added. The protein nitrogen was collected in a 250 ml conical flask containing 25 ml of 2% solution of boric acid. The final determination of nitrogen was done by titrating the above solution against a 0./N HCL using screened methyl red as indicator. Blank titration was carried out also and did not exceed 0.05 ml of acid titre. In the titration of HCl against the boric-nitrogen solution, Pearson (1973) indicated that 1 ml of 0.1N HCl is equivalent to 0.0014g of nitrogen and the factor used to convert grams of nitrogen to protein was x 6.25.

3.5.5 Lipid Determination

Lipid content was determined by the method of Lees (1975). The samples of FPH (5g) were weighed directly into an extraction thimble and then the end was plugged with cotton wool. Then the thimble was placed in the syphon portion of Soxhlet apparatus. Previously the 250 ml conical joint flask was weighed and 150 ml of chloroform was added.

The flask was then connected to the Soxhlet unit. The reflux was done for 6 hours. Then the chloroform was distilled off using a rotary evaporator and then the conical flask placed in an oven at 103°C until the chloroform was completely evaporated from the extracted oil. The conical flask was cooled down in a des. cator and weighed. The lipid content was determined from the weight of oil held in the receiver flask as a percentage of the FPH sample weight.

3.5.6 Moisture Content

The moisture was determined by the method of Pearson (1981). A sample of FPH (5.0g) was weighed into a previously weighed stainless steel dish. The dish was placed in an oven at 103 ± 2°C for 24 hr and the samples cooled in a desiccator and weighed. The drying procedure was repeated for a further 30 minutes in order to get constant weight.

The moisture content was calculated as follows:

$$\text{Moisture } \% = \frac{S-(W-w)}{S} \times 100$$

where W = weight of sample FPH after 24 hr with stainless steel dish

w = weight of stainless steel dish

S = weight of sample FPH

3.5.7 Ash Content

The ash content was determined by the method of Lees (1975). The sample of FPH (2g) was weighed into a known porcelain crucible and it was ignited using a bunsen burner. The samples were ashed at 550-600°C

until a white ash was obtained. The samples were allowed to cool down in a desiccator and weighed. The ash content (%) was calculated from the difference between constant weight of ash and known porcelain crucible weight divided by the original FPH sample weight.

3.5.8 Amino Acid Determination

The samples of FPH (20 mg) were hydrolysed with 6N HCl (5 ml) in an evacuated tube at 110°C for 48 hours. The hydrolysate was dried under vacuum and analysed using an amino acid analyser (Chromakon Kontron 500).

3.5.9 Molecular Weight Determination

Molecular weight distribution was carried out by Fast Protein Chromatography (Pharmacia, Milton Keynes, England) using Sephadex G15 and G25 in columns (1.5 x 110 cm), eluent TRIS buffer (pH 8) and detection at 280 nm. A sample of FPH (2% protein) was made up in TRIS buffer and filtered through 0.2 µm filter paper. The standards, 2% solutions of Blue dextran, Glucagon, Bacitracin, Vitamin B12, Tyrosine, were used to calibrate the columns in the molecular weight range below 5000 Daltons. Molecular weight (MW) of FPH, ISB and EA were determined from log MW and elution volume plots (Appendix 1).

3.5.10 Particle Size Determination

Particle size was determined using Malvern Instrument Easy M6.02. A representative homogenised sample of FPH was suspended in Propalyn solvent and placed in the Malvern Instrument. The results of particle size were obtained through a computer print out of cumulative percentage against particle size (µm) (Appendix 2).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 HYDROLYSIS AND FPH PRODUCTION

Initial experiments were designed to assess the reproducibility of FPH produced by enzyme hydrolysis from different batches of fw. Table 15 shows the proximate chemical composition of fw (wet/wt). It can be seen that the composition was not affected greatly by the source of raw material and filleting process. A knowledge of fw composition was obviously important to find the protein content in order to calculate the amount of enzyme to be added. It can also be seen that the fat contents (from 0.93-1.9%) were very low and not affected by seasonal variations as happens for fatty fish species. Mackerel for example, throughout the year has a moisture content of 56-74%, and accordingly the fat content will be in a typical range of 23-16% (i.e. the higher the moisture content, the lower the fat content will be). The protein contents ranged from 14.1-16.4% and again were unaffected by seasonal variation as happens for white fish such as herring which has a range of protein content of 16-19% (Stroud, 1970). However McLay (1985) found no significant difference in the protein and moisture content of different edible individual parts of whole fish such as cod fillet, trimmings from the fillet, and tissue recovered from the skeleton after filleting over a 12 month period. This means that it could be possible to obtain fw from the same fish species after filleting with only a slight variation in composition.

TABLE 15: CHEMICAL COMPOSITION (WET WT) OF COD FILLET WASTE BATCHES (A-D) USED FOR FPH PRODUCTION

Batch	Moisture %	Protein %	Lipid %	Ash %
A	73.10 (0.20)	16.40 (0.14)	1.70 (0.20)	7.50 (0.20)
B	76.30 (0.27)	15.76 (0.16)	1.90 (0.20)	5.70 (0.2)
C	75.70 (0.53)	15.42 (0.10)	1.90 (0.3)	5.8 (0.5)
D	79.80 (0.61)	14.14 (0.51)	1.60 (0.35)	4.44 (0.30)
E	82.40 (1.21)	15.00 (0.2)	0.93 (0.2)	1.10 (0.2)

* The results given in the table are the averages of five readings; standard deviations are given in parentheses.

E = Cod fillet used for fishburger production.

Generally any seasonal variation in fish species could be reflected in the composition of fw and this could be explained by variation in size, nutritional state, maturity of the fish, fish species and where the fish was caught (Murray and Burt, 1969). Therefore any seasonal variation of fw should be taken into account in production of FPH because it could be reflected in the composition of the FPH e.g. a high fat content in fw may cause the FPH to have a shorter storage life.

Table 16 shows the production conditions for FPH in detail. It can be seen that fish protein hydrolysis was carried out by two enzymes (trypsin and bromelain) for fixed times (60 and 20 minutes) and at a ratio of fish waste to water of 1:1 and ratio of enzyme to substrate of 1:100. It was found that the degree of hydrolysis (DH) of different batches of fw by trypsin and bromelain for 60 or 20 minutes was not significantly different for each enzyme. This was possibly because of careful control of enzyme hydrolysis conditions such as temperature, pH and time etc. However, the DH for 'average' samples T60 and B60 was found to be higher than for 'average' samples T20 and B20. This could be explained by the greater number of peptide bonds cleaved during 60 minutes hydrolysis which resulted in the presence of more soluble protein when measured by TCA solubility index (i.e. the percentage of nitrogen which is soluble in TCA under certain conditions). The TCA solubility index is a crude measure of the molecular weight distribution of protein hydrolysate, as it precipitates all large protein molecules and a considerable fraction of peptides (Adler-Nissen, 1986).

TABLE 16: PRODUCTION CONDITIONS OF FPH

Enzyme	Trypsin								Bromelain							
Time of Hydrolysis	60 minutes				20 minutes				60 minutes				20 minutes			
Batches*	A	B	C						D							
Samples	T60/1	T60/2	T60/3	'Average' Sample T60	T20/1	T20/2	T20/3	'Average' Sample T20	B60/1	B60/2	B60/3	'Average' Sample B60	B20/1	B20/2	B20/3	'Average' Sample B20
Degree of hydrolysis	59.7	56.9	58.3	58.3 (1.4)	45.9	43.9	47.1	45.6 (1.6)	65.0	66.1	63.7	64.9 (1.2)	50.1	52.6	53.8	52.2 (1.9)
pH of hydrolysate	7.8	7.8	8.0		8.0	7.9	7.8		8.0	8.0	7.9		8.0	7.7	8.0	
Total solids (% w/w)	8.5	9.3	8.7		8.9	8.9	8.6		8.6	8.5	8.6		8.6	8.6	8.5	
Yield (% w/w)	43.2	51.9	53.6	49.6 (5.6)	43.6	45.5	47.8	45.6 (2.1)	56.8	54.2	52.2	54.4 (2.3)	54.3	48.8	52.6	51.9 (2.8)

* Ratio of waste to water (1:1)
Ratio of enzyme to substrate (1:100)

It was found also that the DH of 'average' sample B60 was higher than 'average' sample T60. A similar pattern was also found for 'average' sample B20 and 'average' sample T20. This can be explained by the specificity and activity of the enzymes used. It is known that trypsin hydrolysis of peptides and proteins involves cleavage at only two peptide bonds involving the carboxyl group of lysine and arginine residues. In bromelain (sulfydryl group active side) hydrolysis of proteins involves cleavage at four peptide bonds marked by lysine, arginine, phenylalanine or tyrosine residues. This should result in a higher DH (by TCA method) after bromelain hydrolysis (i.e. more peptide bonds cleaved) than after trypsin hydrolysis (i.e. less peptide bonds cleaved) (Barker, 1978; Adler-Nissen, 1986). The amino acid analysis of the FPH shows that lysine, arginine, phenylalanine and tyrosine were all present in the fw (Table 18).

It was found also that the yield (% w/w) of trypsin-treated 'average' samples was lower than the bromelain-treated 'average' samples and 60 minutes hydrolysis of both enzymes resulted in a higher yield than 20 minutes hydrolysis. This could be explained by the low DH for trypsin-treated 'average' sample compared to the bromelain treated 'average' samples. The yield is related to the recovery of nitrogen for fw and therefore also includes a variable proportion of suspended protein nitrogen (not measured by TCA solubility). It was obvious after 60 minutes hydrolysis that the residue (bone) was free of undigested materials compared with the 20 minutes hydrolysate.

The proximate composition of FPH and controls are shown in Table 17. It can be seen that the protein content for samples after 60 and 20 minutes hydrolysis by the same enzyme are in the same range. Bromelain-treated samples have higher protein content even on a dry weight basis than the trypsin-treated 'average' samples. This could be explained by the fact that bromelain hydrolysis (with high DH) resulted in the recovery of more protein from fw than trypsin hydrolysis. It can be seen the fat content of the FPH is still low. Generally a high fat content might reduce the storage time of FPH and cause rancidity problems. These results are in agreement with the general composition of FPH and FPC (Windsor and Barlow, 1981).

Generally all the FPH samples had a slight fish odour, were slightly bitter in taste and creamy white to yellowish in colour. The bitterness of FPH might be associated with oligopeptides containing particular hydrophobic amino acids produced by enzyme hydrolysis. These amino acids usually are found within the structure of native proteins and therefore prevented from contact with the taste buds. Enzymic hydrolysis produces smaller peptides which expose these interior amino acids to the taste buds and an increasingly bitter taste may result. For example, gelatin which contains comparatively few hydrophobic acids will produce hydrolysates with little bitterness, whereas casein containing many hydrophobic amino acids, will tend to give bitterer hydrolysates than gelatin (Fujimaki et al, 1977; Peterson et al, 1984; Adler-Nissen, 1986).

TABLE 17: CHEMICAL COMPOSITION OF FPH AND CONTROLS

Batches	A	B	C	D													
Samples	T60/1	T60/2	T60/3	'Average' Sample T60	T20/1	T20/2	T20/3	'Average' Sample T20	B60/1	B60/2	B60/3	'Average' Sample B60	B20/1	B20/2	B20/3	'Average' Sample B20	EA	ISB
Moisture	9.20 (0.30)	8.13 (0.21)	8.30 (0.26)	7.88 (1.60)	8.9 (0.10)	8.80 (0.2)	9.00 (0.10)	8.90 (0.10)	7.90 (0.21)	7.8 (0.20)	7.90 (0.2)	7.80 (0.10)	7.60 (0.10)	8.10 (0.10)	8.1 (0.20)	7.90 (0.3)	6.00 (0.10)	5.80 (0.20)
Protein	80.0 (1.60)	82.40 (0.90)	80.20 (0.60)	81.0 (1.36)	81.0 (0.56)	82.10 (0.10)	80.20)0.10)	81.10 (0.10)	84.94 (0.91)	84.28 (0.62)	84.61 (0.97)	84.60 (0.30)	85.49 (1.00)	84.39 (0.83)	83.84 (0.94)	84.60 (0.80)	93.2 (0.30)	78.63 (0.50)
Lipid	3.60 (0.32)	3.94 (0.27)	4.00 (0.29)	3.85 (0.22)	2.60 (0.40)	2.33 (0.28)	2.70 (0.40)	2.54 (0.20)	2.40 (0.20)	2.60 (0.30)	2.60 (0.30)	2.33 (0.31)	2.00 (0.30)	2.20 (0.30)	2.40 (0.20)	2.20 (0.20)	- -	- -
Ash	6.40 (0.27)	6.20 (0.21)	6.28 (0.30)	6.29 (0.10)	6.00 (0.20)	6.50 (0.50)	6.10 (0.30)	6.20 (0.26)	5.90 (0.2)	5.33 (0.38)	6.0 (0.3)	5.74 (0.36)	6.21 (0.2)	5.60 (0.40)	6.40 (0.30)	6.10 (0.42)	- -	- -

Results are the mean of triplicate readings, and standard deviations are given in parentheses

Table 18 shows the amino acid profile of FPH and controls together with the model essential amino acid composition (FAO, 1957). It can be seen that there is little difference in the amino acid composition of FPH products. FPH products had a higher content of lysine and threonine than the reference protein and the controls EA and ISB. However, the FPH had lower contents of the other essential amino acids, valine, methionine, isoleucine, leucine and phenylalanine. These results are in agreement with the amino acid composition of FPH and FPC given by Windsor and Barlow (1981). The FPH products are highly nutritious as they are rich in essential amino acids.

The molecular weight (MW) distribution of the samples were determined by gel chromatography on Sephadex G15 and G25 and Appendix 1 shows the standard curve of protein standards which were used to calibrate the columns.

Table 19 shows the MW distribution for FPH 'average' samples and controls. Samples after hydrolysis by the same enzyme and for the same time are not very different in the proportion of peptides in the molecular weight ranges determined. It can also be seen that 'average' samples T60 and B60 had a lower proportion of peptides in the molecular weight range above 5000 Daltons than 'average' samples T20 and B20. It follows that 60 minutes hydrolysis resulted in a greater proportion of small peptides than after 20 minutes hydrolysis. Sample T20 gave no peptides less than 5000 Daltons but B20 gave 10% of

TABLE 18: AMINO ACID COMPOSITION (g/100g) FOR FPH 'AVERAGE' SAMPLES AND CONTROLS COMPARED WITH AN ESSENTIAL AMINO ACID REFERENCE

Samples / Amino Acids (%)	'Average' Samples ISB	EA	T60	T200	B60	B20	Essential* amino acid
Aspartic acid	9.93 (0.1)	9.12 (0.2)	9.41 (0.8)	8.98 (0.41)	8.32 (0.20)	7.53 0.14)	
Threonine	2.72 (0.1)	1.58 (0.1)	4.19 (0.23)	3.73 (0.17)	3.59 (0.01)	3.61 (0.25)	2.80
Serine	4.8 (0.1)	7.55 (0.1)	5.18 (0.2)	4.28 (0.20)	4.39 (0.2)	4.55 (0.38)	
Glutamic acid	12.93 (0.30)	11.99 (0.1)	12.22 (0.2)	11.45 (0.53)	12.76 (0.38)	12.83 (0.21)	
Glycine	3.73 (0.1)	3.12 (0.1)	7.40 (0.43)	6.24 (0.28)	9.33 (0.39)	8.60 (0.40)	
Alanine	4.1 (0.2)	5.11 (0.2)	7.57 (0.42)	6.29 (0.40)	6.60 (0.42)	5.91 (0.11)	
Cysteine	0.4 (0.03)	0.4 (0.1)	0.36 (0.11)	0.20 (0.01)	0.2 (0.1)	0.30 (0.11)	
Valine	3.75 (0.1)	5.67 (0.1)	2.86 (0.65)	3.71 (0.23)	3.44 (0.40)	3.74 (0.37)	4.20
Methionine	2.45 (0.1)	2.55 (0.1)	1.39 (0.10)	1.77 (0.16)	1.96 (0.26)	2.80 (0.10)	2.20
Isoleucine	3.98 (0.1)	4.68 (0.1)	2.39 (0.20)	2.52 (0.2)	2.47 (0.15)	3.71 (0.15)	4.20
Leucine	6.82 (0.1)	6.92 (0.1)	5.54 (0.20)	4.67 (0.30)	4.29 (0.58)	4.8 (0.22)	4.80
Tyrosine	3.25 (0.1)	2.36 (0.1)	2.91 (0.57)	1.73 (0.31)	1.95 (0.11)	2.69 (0.40)	
Phenylalanine	4.83 (0.1)	7.95 (0.1)	2.57 (0.11)	2.11 (0.27)	2.88 (0.27)	3.20 (0.30)	2.80
Ammonia	6.25 (0.2)	6.44 (0.1)	3.35 (0.20)	3.29 (0.19)	4.36 (0.37)	4.59 (0.40)	
Lysine	5.5 (0.2)	6.68 (0.1)	8.74 (0.44)	7.44 (0.23)	7.27 (0.7)	7.44 (0.30)	4.20
Histidine	2.45 (0.1)	2.1 (0.1)	2.36 (0.51)	2.24 (0.20)	1.77 (0.15)	1.92 (0.12)	
Arginine	6.51 (0.1)	4.99 (0.1)	7.17 (0.56)	6.33 (0.29)	7.52 (0.11)	6.16 (0.32)	
Proline	-	-	-	-	-	-	
Tryptophan	-	-	-	-	-	-	1.9

* FAO, 1957

TABLE 19: MOLECULAR WEIGHT (DALTONS) DISTRIBUTION FOR FPH SAMPLES WITH 'AVERAGE' SAMPLES AND CONTROLS (PROPORTION OF AREA OF EACH PEAK TO TOTAL AREA)

Samples / MW Daltons	Proportion			'Average' Sample T60	Proportion			'Average' Sample T20	Proportion			'Average' Sample B60	Proportion			'Average' Sample B20	EA	ISB
	T60/1	T60/2	T60/3		T20/1	T20/2	T20/3		B60/1	B60/2	B60/3		B20/1	B20/2	B20/3			
>5000	0.36	0.45	0.36	0.39 (0.05)	1.0	1.0	1.0	1.0	0.72	0.62	0.69	0.68 (0.05)	1.0	1.0	0.90	0.97 (0.06)	1.0	1.0
1000-5000	0.27	0.24	0.36	0.28 (0.06)					0.28	0.38	0.31	0.32 (0.05)						
<1000	0.38	0.34	0.30	0.34 (0.05)														

peptides in the range 1000-5000 Daltons. This could be explained by the longer time of hydrolysis, by either enzyme, allowing more cleavage of protein/peptides. The higher the DH the greater the number of peptide bonds broken, giving small peptides in the hydrolysate. Trypsin hydrolysis gave more small peptides after 60 minutes hydrolysis than bromelain. This did not correlate with the higher DH of bromelain hydrolysates (measured by TCA solubility) which should reflect a larger proportion of small peptides than for the trypsin hydrolysate. TCA solubility is a measure of the percentage of protein which is soluble in TCA under certain conditions as TCA precipitates all large protein molecules and a considerable fraction of peptides (Adler-Nissen, 1986). However TCA solubility is not a good measure of soluble protein molecular weight distribution because the MW at which precipitation occurs is variable for different proteins (Adler-Nissen, 1986; Arai et al, 1970).

It could be argued that protein precipitation is due to a change in pH, which occurs on the addition of TCA solution, which would be different for different proteins so that TCA solubility is a function of the protein charge (amino acid composition) not molecular weight. In this particular case the addition of TCA solution to FPH gives a pH change from 8 to 1.3 and at this low pH the FPH could have low solubility (see Table 22). The DH may be better represented for instance by measuring the number of peptide bonds broken as suggested by Adler-Nissen (1986) as there is a relationship between DH and peptide chain length (PCL).

Table 20 shows the particle size distribution for spray-dried FPH using a Malvern particle size laser diffraction instrument. The results were calculated from a graph of cumulative weight (%) against the size in microns plotted on a logarithmic scale. The values used were cumulative weight 50% and cumulative weight 90%. A cumulative weight of 50% means that 50% by weight of the whole sample has a particle size less than a given value and 50% higher than that value. A cumulative weight 90% means 90% by weight of the whole sample has a particle size less than a given value and 10% higher than the given value. For example, for EA the 50% cumulative weight is at a particle size of 34 μm and the 90% cumulative weight is at 61 μm. FPH were tested for particle size distribution to find out the uniformity of FPH particle size during processing and spray drying. It can be seen from Table 20 and Figure 6 (see Appendix 2 for individual graphs) that samples from the same enzyme hydrolysis (i.e. trypsin and bromelain for 60 and 20 minutes were not found to be significantly different in size ($P > 5\%$) at cumulative weight 50% and also for the cumulative weight 90% ($P \geq 5\%$). The particle size of FPH after 60 minutes hydrolysis with either enzyme is slightly smaller than after 20 minutes hydrolysis. This variation in particle size distribution could be due to enzyme hydrolysis where greater hydrolysis (high DH) would be expected to give smaller particles. Aggregation of protein particles during spray drying might occur which would increase particle size. This aggregation of protein particles might be dependent on the particle size after hydrolysis. Therefore the particle size distribution of the final product would ultimately

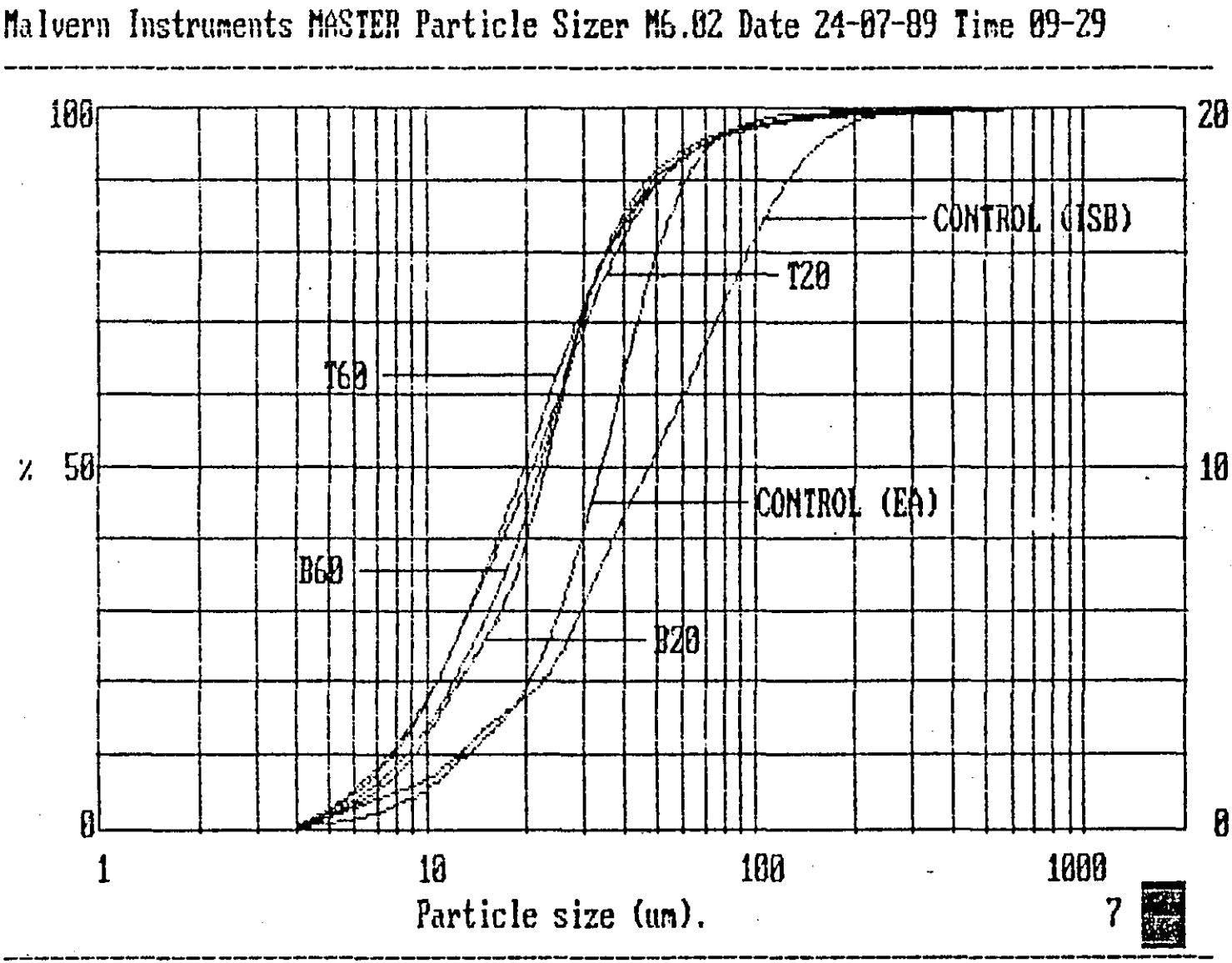
TABLE 20: PARTICLE SIZE DISTRIBUTION FOR SPRAY-DRIED FPH SAMPLES

Particle Size µm %	Sample†			'Average' Sample	Sample†			'Average' Sample	Sample†			'Average' Sample	Sample†			'Average' Sample
	T60/1	T60/2	T60/3	T60	T20/1	T20/2	T20/3	T20	B60/1	B60/2	B60/3	B60	B20/1	B20/2	B20/3	
50	20.8 (0.1)	18.6 (0.1)	20.2 (0.1)	19.9 (1.1)	20.0 (0.1)	25.3 (0.4)	18.6 (0.1)	21.3 (3.5)	20.5 (0.4)	22.8 (0.1)	23.0 (0.1)	22.1 (1.4)	21.2 (0.2)	23.8 (0.1)	23.1 (0.2)	22.7 (1.3)
90	59.1 (1.7)	43.5 (0.5)	50.1 (0.4)	50.9 (7.8)	56.2 (1.9)	50.8 (0.8)	46.3 (1.5)	51.1 (5.0)	47.1 (0.7)	41.1 (0.7)	52.5 (1.0)	46.9 (5.7)	56.2 (2.2)	51.2 (0.5)	42.3 (1.0)	49.9 (7.0)

† Results are the means of six readings of Malvern printout; standard deviations are given in parentheses.

† Samples were not found to be significant at level P < 5% for cumulative weight 50% and P < 1% for cumulative weight 90%.

FIGURE 6: PARTICLE SIZE DISTRIBUTION OF THE 'AVERAGE' SAMPLE AND CONTROLS (MALVERN PRINT OUT)



depend on the enzymic hydrolysis. Rutman and Heimlick (1974) showed that enzymic hydrolysis of FPH by bromelain for a short-time, 15 min, provided the right particle and molecular size distribution in the final product and this provided good functional properties and flavour. The uniformity of the final FPH product is an important factor for the quality of the product in the food industry and could have an effect on the functional properties in general.

Table 21 shows the particle size distribution of spray-dried FPH 'average' samples and controls. It was found that there was no significant difference between 'average' samples T60, T20, B60 and B20 at cumulative weights 50% and 90%. This indicates that it could be possible to produce FPH with a reproducible quality. It was also found that all 'average' samples T60, T20, B60 and B20 had a significantly lower particle size distribution than control EA ($P < 5\%$) and ISB ($P < 1\%$) at cumulative weights 50% and 90%. This indicates that FPH had a finer particle size distribution than the controls.

Soy isolates are prepared from heat-treated soy flour by dissolving the protein in dilute alkali (pH 8.0), removing the insoluble materials by centrifugation (or filtration), and precipitation of the protein at pH 4.5. Then it is neutralised with sodium hydroxide and spray dried, (Kinsella, 1979). This process could give rise to large particle sizes.

TABLE 21: PARTICLE SIZE DISTRIBUTION FOR SPRAY-DRIED FPH 'AVERAGE' SAMPLES AND CONTROLS

Particle Size µm %	Controls		'Average' Sample†				Significance Level††													
	ISB	EA	T60	T20	B60	B20	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to EA	T20 to EA	B60 to EA	B20 to EA	EA to ISB
50	47.2 (0.2)	33.8 (0.1)	19.9 (1.1)	21.3 (3.5)	22.1 (1.4)	22.7 (1.5)	(-)	(-)	(-)	(-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+)	(+)	(+)	(+++)
90	123.5 (0.4)	60.7 (0.3)	50.9 (7.8)	51.1 (5.0)	46.9 (6.7)	49.9 (7.0)	(-)	(-)	(-)	(-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+)	(+)	(+)	(+++)

† Results are the mean of sample means except for the controls, standard deviations are given in parentheses.

†† Significant level is indicated as follows: not significant (-), significant (+), highly significant (++), very highly significant (+++), corresponding to P > 5%, P < 5%, P < 1% and P < 0.1% respectively.

4.2 FUNCTIONAL PROPERTIES OF FPH

4.2.1 Solubility

Solubility behaviour provides a good indication of the extent of denaturation and potential application of proteins. Useful information on the optimisation of processing procedures and for assessing the effect of various treatments on functional properties of proteins can also be gained.

Solubility results are given in Table 22 as the percentage of the soluble protein of spray-dried FPH and controls as measured by Kjeldahl estimation after centrifugation at various pH. It was found that samples from the same enzyme and time of hydrolysis, at a particular pH were not significantly different ($P > 5\%$) in solubility. It was also found that the highest protein solubility of FPH was at the natural (or iso-ionic) pH (6.8-7.0) and minimum solubility at around pH 4.0 in all the samples. This agrees with the general solubility profile of proteins in having lower solubility around their iso-electric point. Proteins at a pH other than the iso-electric point possess net changes and they repel each other, thus allowing protein molecules to disperse easily in solution and this will increase their solubility [Anglemier and Montgomery, 1976].

Miller and Groninger [1976] in their discussion of protein solubility when measured by the Kjeldahl and Lowry methods, of acylated and succinylated fish protein described the highest solubility around pH 7-9 and the minimum solubility around pH 3-6.

TABLE 22: PROTEIN SOLUBILITY (%) OF FPH SAMPLES AT VARIOUS pH

pH	Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'
	T60/1	T60/2	T60/2		T20/1	T20/1	T20/1		B60/1	B60/2	B60/2		B20/1	B20/2	B20/3	
2	76.7 (1.2)	74.8 (1.0)	78.6 (0.3)	76.7 (1.9)	74.6 (1.8)	71.3 (1.6)	72.7 (1.8)	72.1 (1.8)	82.5 (1.7)	83.1 (1.7)	81.8 (1.2)	82.5 (0.7)	67.9 (0.9)	68.5 (1.2)	71.5 (1.0)	69.3 (1.9)
4	74.3 (1.4)	73.8 (1.0)	75.9 (2.1)	74.7 (1.1)	69.8 (1.3)	68.6 (0.5)	69.8 (1.9)	69.4 (0.7)	80.2 (0.5)	82.9 (1.0)	79.7 (1.2)	80.9 (1.7)	70.1 (1.3)	71.6 (1.7)	72.5 (1.2)	71.4 (1.2)
6	82.1 (1.8)	80.2 (2.1)	81.4 (1.1)	81.2 (1.0)	79.8 1.4)	75.0 (0.5)	78.9 (0.6)	77.9 (2.6)	86.2 (0.8)	84.4 (1.6)	83.6 (1.2)	84.7 (0.3)	75.6 (1.6)	75.7 (1.7)	74.7 (1.1)	75.3 (0.6)
8	83.2 (1.3)	82.5 (2.2)	80.8 (1.3)	82.2 (1.2)	79.2 (0.6)	77.7 (0.9)	79.7 (1.3)	78.9 (1.0)	86.6 (0)	85.7 (1.1)	85.9 (1.2)	86.1 (0.5)	77.5 (1.2)	77.8 (0)	76.3 (1.2)	77.2 (0.8)
Natural 6.8-7	85.4 (2.9)	84.8 (1.0)	86.8 (1.2)	85.7 (1.0)	81.1 (2.1)	79.2 (0.2)	82.3 (2.1)	80.9 (1.6)	87.7 (1.2)	87.3 (1.7)	86.7 (1.3)	87.2 (0.5)	77.9 (1.2)	78.9 (1.2)	80.5 (1.2)	77.1 (1.3)

† No significant difference was found for the FPH samples from the same enzyme hydrolysis
Results are the mean of four readings. Standard deviations are given in parentheses.

Hermansson et al [1971] studied the effect of pH also on solubility of FPC. Low solubility values (5%) were found at pH 1-6 and a relatively high solubility value (35%) was noted at pH 12. An increase in ionic strength also lowered the solubility of FPC. Hindi [1979] also showed a decrease in solubility of FPC at the iso-electric point, but showed an increase in solubility as a function of ionic strength from 42% (obtained with distilled water) to 51% using 0.51M salt solution. An increase in ionic strength above 0.51M salt solution once again gave a decline in solubility. Wang and Kinsella [1976] also presented values for the solubility of alfalfa leaf protein where all protein preparations showed minimum solubility between pH 3 and 4. Meinke et al [1972] also found minimum values for fish protein solubility between pH 5.5-6 for three fish proteins (carp, mullet and golden croaker).

Table 23 shows the significance level of protein solubility of 'average' samples of FPH and controls at various pH. It was found that all 'average' samples T60, T20, B60 and B20 had significantly lower protein solubility ($P < 1\%$) than EA at pH 2-8. It was also found that all 'average' samples T60, T20, B60 and B20 had significantly higher protein solubility ($P < 0.1\%$) than ISB. This indicates that FPH is an intermediate product between EA and ISB in terms of solubility.

FPH 'average' sample T60 had a significantly higher protein solubility ($P < 5\%$) at pH 2-8 than T20, and B60 also had a significantly higher

TABLE 23: PROTEIN SOLUBILITY (%) OF FPH 'AVERAGE' SAMPLE AND CONTROLS AT VARIOUS pH

pHs	Control		'Average' Samples				Significance Level †												
	EA	ISB	T60	T20	B60	B20	T60 to EA	T20 to EA	B60 to EA	B20 to EA	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20	EA to ISB
2	94.6 (1.6)	17.2 (1.0)	76.7 (1.9)	72.7 (1.7)	82.5 (0.7)	69.3 (1.4)	(++)	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+)	(++)	(++)	(++)	(-)	(+++)	(+++)
4	94.1 (1.9)	6.2 (1.1)	74.7 (1.1)	69.4 (0.7)	80.9 (1.7)	71.4 (1.2)	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+)	(++)	(++)	(++)	(-)	(+++)	(+++)
6	96.1 (1.0)	14.5 (1.3)	81.2 (1.0)	77.9 (2.6)	84.7 (1.3)	75.3 (0.6	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+)	(++)	(++)	(++)	(-)	(+++)	(+++)
8	96.6 (1.7)	17.3 (1.1)	82.2 (1.2)	78.9 (1.0)	86.1 (0.5)	77.2 (0.8)	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+)	(++)	(++)	(++)	(-)	(+++)	(+++)
Natural 6.8-7	100.6	19.9	85.7	80.9	87.2	79.1	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+)	(-)	(++)	(++)	(-)	(+++)	(+++)

† Significance level is indicated as follows: not significant (-), significant (+), highly significnat (++), very highly significant (+++)

protein solubility ($P < 0.1\%$) at pH 2-8 than B20. This should be associated with the higher degree of hydrolysis (i.e. TCA solubility) of 'average' samples with the longer time of hydrolysis which resulted from more cleavage of peptide bonds.

It was found that 'average' sample T60 had a significantly lower solubility ($P < 1\%$) than 'average' sample B60 and no significant difference ($P > 5\%$) was found in solubility for 'average' samples T20 and B20. This could again be explained by the specificity of the action of two different enzymes by which trypsin resulted in less cleavage of peptide bonds (i.e. lower degree of hydrolysis) and bromelain resulted in more cleavage of peptide bonds (i.e. higher degree of hydrolysis).

4.2.2 Oil Absorption Capacity

The ability of a protein powder to bind oil is very important in food applications principally because it enhances flavour retention and improves mouth feel. It is attributed mostly to physical entrapment of oil and the activity of hydrophilic/hydrophobic amino acids.

Table 24 shows oil absorption capacity (g/g) and (ml/g) of FPH samples at natural pH. All samples of FPH were shown to bind oil. The samples of the same enzyme and time of hydrolysis showed no significant difference in oil binding capacity ($P > 5\%$). The 'average' sample T60 was found to have a higher oil binding capacity than the other 'average' samples T20, B60 and B20. This could be explained by the

TABLE 24: OIL ABSORPTION CAPACITY (g/g) AND (ml/g) OF FPH SAMPLES AT NATURAL pH

		Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'
		T60/1	T60/2	T60/3		T20/1	T20/2	T20/3		B60/1	B60/2	B60/2		B20/1	B20/2	B20/3	
Oil Absorption Capacity	g/g	2.2 (0.14)	2.03 (0.20)	2.15 (0.24)	2.13 (0.10)	1.98 (0.15)	1.96 (0.12)	1.90 (0.08)	1.95 (0.04)	1.86 (0.08)	1.83 (0.18)	1.72 (0.10)	1.80 (0.07)	1.68 (0.23)	1.61 (0.20)	1.64 (0.20)	1.64 (0.04)
	ml/g	2.38 (0.15)	2.24 (0.23)	2.34 (0.25)	2.34 (0.10)	2.16 (0.17)	2.14 (0.13)	2.08 (0.08)	2.13 (0.04)	2.04 (0.10)	1.98 (0.17)	1.88 (0.11)	1.97 (0.10)	1.84 (0.25)	1.76 (0.22)	1.70 (0.76)	1.77 (0.10)

† No significant difference was found for the FPH samples from the same enzyme hydrolysis

Results are the mean of triplicate readings. Standard deviations are given in parentheses.

ability of the protein mass to bind oil which depends on the nature of the particle size distribution, the conformation of the surface of the particle and pores within the particle and hydrophobic surface forces.

The particle size distribution for the 'average' samples (Table 21) shows that of T60 to be the least thus giving a large surface area for oil binding compared with the other 'average' samples.

Table 25 shows oil absorption capacity (g/g) and (ml/g) of FPH 'average' samples and controls at natural pH (6.8-7.0). It can be seen that all 'average' samples of FPH (T60, T20, B60 and B20) showed significantly higher oil binding capacity than EA and ISB ($P < 1\%$). No significant difference was found among average samples except that T60 showed significantly higher oil binding capacity than B20 ($P < 1\%$).

Jasim [1983] presented values of oil absorption capacity of fish plasteins, egg albumen and soya bean isolate of 1.42, 1.52 and 1.83 (g/g) respectively. He also noted that there is a clear relationship between average particle size and oil binding capacity, the smaller the particle size distribution the more the oil binding per unit volume there will be.

The variability in oil binding capacity will be dependent on the source of protein and the conditions under which materials are dried. Wang and Kinsella [1976] presented some functional properties of

TABLE 25: OIL ABSORPTION CAPACITY (g/g) AND (ml/g) OF FPH 'AVERAGE' SAMPLE AND CONTROLS AT NATURAL pH

	Control		'Average' Sample				Significance Level†													
	EA	ISB	T60	T20	B60	B20	T60 to EA	T20 to EA	B60 to EA	B20 to EA	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20	EA to ISB
Oil Absorption Capacity (g/g)	1.04 (0.15)	1.23 (0.15)	2.13 (0.10)	1.95 (0.04)	1.80 (0.10)	1.64 (0.04)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(-)	(-)	(++)	(-)	(-)	(-)	(-)
Oil Absorption Capacity (ml/g)	1.14 (0.17)	1.34 (0.16)	2.34 (0.10(	2.13 (0.04)	1.97 (0.10)	1.77 (0.10)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(-)	(-)	(++)	(-)	(-)	(-)	(-)

† Significance level is indicated as follows: not significant (-), significant (+), highly significant (++), very highly significant (+++)

several alfalfa leaf protein concentrate samples in which oil absorption capacity was in the range 4.30-1.75 ml oil/g and for promosoy 100 and promine D (these are soya products) 1.10 and 1.70 ml oil/g respectively. Sathe and Salunkhe [1981] also reported values for oil binding capacity of blood albumen and globulin as 1.0 and 3.25 g/g respectively.

These figures suggest that FPH samples have higher oil binding capacity values which would enable the product to be comparable with, if not superior to, some other protein sources.

4.2.3 Water Absorption Capacity

The water absorption capacity is an important function of protein in different types of products particularly viscous foods such as soup, meat rolls, comminuted meats, processed cheeses and doughs etc. The water absorption capacity of different proteins must be measured to facilitate adjustment in food formulations when new protein sources are used. Some proteins with a high water absorption capacity, when added to food formulae, may bind a disproportionate amount of water and dehydrate other components. Therefore some adjustment in the amount of added water may be necessary to obtain the required viscosity in the products, thus the ability of a protein to bind water is an important property to measure.

Table 26 shows water absorption capacity (ml/g) of FPH samples at various pH. All samples of the FPH were able to absorb water under

TABLE 26: WATER ABSORTION CAPACITY (ml /g) OF FPH SAMPLES AT VARIOUS pH

pH*	Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'	Sample †			'Average Sample'
	T60/1	T60/2	T60/3		T20/1	T20/2	T20/3		B60/1	B60/2	B60/3		B20/1	B20/2	B20.3	
2	1.00 (0.10)	0.95 (0.10)	1.05 (0.10)	1.00 (0.10)	0.60 (0.10)	0.66 (0.20)	0.60 (0.12)	0.62 (0.10)	0.44 (0.06)	0.45 (0.05)	0.47 (0.06)	0.45 (0.08)	0.38 (0.08)	0.34 (0.09)	0.36 (0.10)	0.36 (0.06)
4	0.90 (0.20)	0.90 (0.23)	0.93 (0.19)	0.91 (0.10)	0.56 (0.11)	0.60 (0.10)	0.60 (0.13)	0.58 (0.08)	0.40 (0.06)	0.40 (0)	0.42 (0.05)	0.40 (0.06)	0.34 (0.09)	0.32 (0.10)	0.34 (0.10)	.0.33 (0.08)
6	0.85 (0.10)	0.75 (0.10)	0.90 (0.12)	0.83 (0.08)	0.55 (0.15)	0.60 (0.16)	0.50 (0.15)	0.55 (0.05)	0.55 (0.07)	0.53 (0.05)	0.45 (0.06)	0.51 (0.06)	0.38 (0.10)	0.33 (0.10)	0.43 (0.10)	0.38
8	0.80 (0.16)	0.85 (0.10)	0.70 (0.12)	0.78 (0.10)	0.62 (0.10)	0.55 (0.10)	0.62 (0.10)	0.59 (0.04)	0.53 (0.06)	0.46 (0.10)	0.43 (0.05)	0.47 (0.05)	0.35 (0.10)	0.30 (0.08)	0.40 (0.08)	0.35 (0.05)
Natural 6.8-7	1.12 (0.20)	1.08 (0.19)	1.0 (0.16)	1.07 (0.10)	0.64 (0.11)	0.73 (0.11)	0.65 (0.10)	0.67 (0.05)	0.68 (0.05)	0.57 (0.04)	0.50 (0.10)	0.55 (0.04)	0.39 (0.05)	0.37 (0.08)	0.44 (0.05)	0.40 (0.04)

† No significant difference was found for the FPH sample from the same enzyme hydrolysis

* pH relates to that of the water absorbed

Results are the mean of triplicate readings. Standard deviations are given in parentheses.

all test conditions. It was found that there were no significant differences ($P > 5\%$) for samples produced by the same enzyme and hydrolysis time at each pH ranging from 2-natural. However, there is a change in water absorption capacity with pH for FPH from both enzymes and hydrolysis times. This change is the inverse of the pH-solubility relationship because water absorption capacity is a function of the insoluble part of the FPH. Lin et al [1974] observed for sunflower meal that decreased solubility resulted in increased water binding capacity. Jasim [1983] also found for fish plastein that less water was absorbed at pH 4, 6, 8 and 10 and more at pH natural (unchanged).

It was also found that trypsin-treated samples had higher water absorption capacity than the bromelain-treated samples. This could be explained by the fact that each enzyme has its own characteristic mode of action in hydrolysis. It would not be expected that the same water absorption capacity would result from different enzymes giving a different degree of hydrolysis. In this case the trypsin-treated samples with a lower degree of hydrolysis and hence lower solubility and a higher water absorption capacity than bromelain-treated samples with a higher degree of hydrolysis.

Table 27 shows water absorption capacity (ml/g) of FPH 'average' sample and controls at various pH. It was found that 'average' sample T60 had a significantly higher water absorption capacity ($P < 1\%$) than the controls as did T20 ($P < 5\%$) at natural pH only. The 'average' samples B60 and B20 were not found to be significantly different ($P >$

TABLE 27: WATER ABSORPTION CAPACITY (ml/g) OF FPH 'AVERAGE' SAMPLES AND CONTROLS AT VARIOUS pH

pH	Control		'Average' Sample				Significance Level†											
	EA	ISB	T60	T20	B60	B20	T60 to EA	T20 to EA	B60 to EA	B20 to EA	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20	EA to ISB
2	0.55 (0.10)	4.5 (0.10)	1.00 (0.10)	0.62 (0.10)	0.45 (0.08)	0.35 (0.06)	(++)	(-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)	(+++)	(+)	(+)	(+)	(+++)
4	0.60 (0)	4.9 (0.10)	0.91 (0.10)	0.58 (0.08)	0.40 (0.06)	0.33 (0.03)	(++)	(-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)	(+++)	(-)	(+)	(+)	(+++)
6	0.56 (0.10)	4.3 (0.10)	0.83 (0.08)	0.55 (0.05)	0.51 (0.06)	0.38 (0.10)	(++)	)-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+)	(++)	(+)	(+)	(+)	(+++)
8	0.51 (0.10)	4.7 (0.2)	0.78 (0.10)	0.62 (0.08)	0.47 (0.05)	0.35 (0.05)	(++)	(-)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(++)	(++)	(+)	(+)	(+)	(+++)
Natural 6.8-7	0.48 (0.18)	5.1 (0.24)	1.07 (0.10)	0.67 (0.05)	0.55 (0.04)	0.40 (0.04)	(++)	(+)	(-)	(-)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)	(+++)	(+)	(+)	(+)	(+++)

† Significance level is indicated as follows: not significant (-), significant (+), highly significant (++), very highly significant (+++)

5%) to the control EA. This will indicate that FPH had more insoluble material than EA (note EA had a much more higher solubility than FPH samples). It was also found that all 'average' samples T60, T20, B60 and B20 had significantly lower absorption capacity ($P < 1\%$) than the control ISB at all pHs. This also would be due to the conformational characteristics, hydrophilic/hydrophobic balance of amino acids in the protein molecules. There is a good linear relationship between content of hydrophilic groups (hydroxyl, carboxyl, basic moieties), less amides and water binding capacity for protein of vegetable origin. When carbohydrate is associated with proteins (FPH does not contain carbohydrate but soya bean does) the carbohydrate is a hydrophilic constituent which could bind more water than the protein alone. This result is in agreement with those of Hutton and Campbell [1977]; Kinsella [1979] and Wang and Kinsella [1976] which also showed high water absorption capacity for soya bean isolate (ISB).

Jasim [1983] showed maximum values of water absorption capacity for fish plastein samples, in the range of 1.8-2.6 (g/g), at natural pH and at other pH (ranging from 4, 6, 8, 10) showed decreased water absorption capacity. Miller and Groninger [1976] also showed high water absorption (ml/g protein) for enzyme-modified acylated fish protein derivatives using succinic and acetic anhydrides and bromelain (1:800) hydrolysis. He obtained 25 and 50 ml/g water absorption capacity for acetylated and succinylated fish protein respectively without bromelain hydrolysis (controls). With acetylation and succinylation plus bromelain (1:800) hydrolysis fish protein showed decreased water absorption capacity to 15 and 25 ml/g respectively.

4.2.4 Swelling Capacity

Swelling refers to the expansion of protein by spontaneous uptake of solvent. Protein swelling by adsorption of water is an important functional property in food, such as sausages, custards and doughs. In these products, proteins hold water but do not dissolve because there is insufficient water present. Therefore they swell and impart characteristics such as body, thickening and viscosity [Kinsella, 1976; Kinsella, 1979].

Table 28 shows swelling capacity (ml/mg) of FPH samples and controls at natural pH 6.8-7.0. The ability of FPH and controls (EA and ISB) to adsorb water was investigated using varying sample weights of 15, 25, 45, 55, 75 and 100 mg in order to obtain a representative sample weight and information on the behaviour of protein modification. The results of Table 29 have been calculated from the slope of a curve of sample weight vs swelling capacity determined from linear-regression to ensure that the results obtained were independent of sample size.

It can be seen that all the samples tested were able to adsorb water. However, the extent of water uptake varies among the samples of trypsin and bromelain hydrolysis (Table 28). From Table 29 it was found that FPH 'average' sample T20 had higher swelling capacity than T60, and B20 also had higher swelling capacity than B60. This could be explained by the fact that 'average' samples T20 and B20 had a lower protein solubility than T60 and B60.

TABLE 28: SWELLING CAPACITY (ml /mg) OF FPH SAMPLES AND CONTROLS AT NATURAL pH†

Sample Weight (mg)	Sample			'Average' Sample T60	Sample			'Average' Sample T20	Sample			'Average' Sample B60	Sample			'Average' Sample B20	EA	ISB
	T60/1	T60/2	T60/3		T20/2	T20/2	T20/3		B60/1	B60/2	B60/3		B20/2	B20/2	B20/3			
15	0.010 (0.001)	0.009 (0.002)	0.009 (0.001)	0.009 (0.001)	0.008 (0.003)	0.009 (0.001)	0.007 (0.002)	0.008 (0.001)	0.007 (0.001)	0.007 (0.002)	0.006 (0.001)	0.007 (0.001)	0.008 (0.001)	0.008 (0.001)	0.009 (0.002)	0.008 (0.002)	0.006 (0.001)	0.059 (0.002)
25	0.016 (0.003)	0.015 (0.003)	0.014 (0.002)	0.015 (0.001)	0,016 (0.002)	0.018 (0.002)	0.017 (0.001)	0.017 (0.001)	0.014 (0.002)	0.013 (0.001)	0.012 (0.001)	0.013 (0.001)	0.015 (0.002)	0.016 (0.001)	0.015 (0.002)	0.015 (0.001)	0.010 (0.002)	0.113 (0.006)
45	0.030 (0.003)	0.026 (0.004)	0.032 (0.003)	0.029 (0.003)	0.030 (0.002)	0.031 (0.001)	0.030 (0.002)	0.030 (0.002)	0.020 (0.003)	0.017 (0.002)	0.019 (0.002)	0.019 (0.002)	0.016 (0.003)	0.018 (0.004)	0.017 (0.003)	0.017 (0.001)	0.020 (0.002)	0.130 (0.009)
55	0.035 (0.003)	0.033 (0.003)	0.035 (0.002)	0.034 (0.002)	0.034 (0.002)	0.033 (0.001)	0.032 (0.003)	0.033 (0.001)	0.026 (0.004)	0.028 (0.003)	0.025 (0.001)	0.026 (0.002)	0.029 (0.002)	0.026 (0.004)	0.030 (0.003)	0.028 (0.002)	0.022 (0.001)	0.190 (0.010)
75	0.051 (0.003)	0.044 (0.004)	0.052 (0.002)	0.049 (0.004)	0.047 (0.001)	0.045 (0.002)	0.046 (0.002)	0.046 (0.001)	0.036 (0.003)	0.033 (0.002)	0.033 (0.001)	0.035 (0.002)	0.040 (0.002)	0.042 (0.002)	0.044 (0.003)	0.042 (0.002)	0.034 (0.001)	0.207 (0.008)
100	0.063 (0.002)	0.060 (0.002)	0.064 (0.002)	0.062 (0.002)	0.060 (0.001)	0.060 (0.002)	0.056 (0.002)	0.058 (0.002)	0.048 (0.001)	0.045 (0.002)	0.044 (0.002)	0.046 (0.002)	0.050 (0.001)	0.051 (0.002)	0.053 (0.002)	0.051 (0.002)	0.044 (0.002)	0.227 (0.006)
r	0.998	0.998	0.971		0.997	0.995	0.991		0.997	0.988	0.999		0.978	0.980	0.977		0.997	0.950

Results are the mean of triplicate readings, standard deviations are given in parentheses

† Natural pH 6.8-7

TABLE 29: SWELLING CAPACITY (ml/g) OF FPH SAMPLES, 'AVERAGE' SAMPLES AND CONTROLS AT NATURAL pH

Swelling Capacity	Sample			'Average' Sample T60	Sample			'Average' Sample T20	Sample			'Average' Sample B60	Sample			'Average' Sample B20	EA	ISB
	T60/1	T60/2	T60/3		T20/1	T20/2	T20/3		B60/1	B60/2	B60/3		B20/1	B20/2	B20/2			
ml/g	0.60	0.55	0.59	0.58 (0.03)	0.62	0.67	0.66	0.65 (0.03)	0.52	0.42	0.40	0.45 (0.06)	0.50	0.46	0.48	0.48 (0.02)	0.46	4.02

Almost all the 'average' samples T60, T20, B60 and B20 had greater swelling capacity than control (EA) and lower than control (ISB).

Hermansson et al [1971] reported high swelling capacity for hot alkali-treated FPC with a value of 38 ml/g in pure water and 35 ml/g in 0.5M NaCl solution. Jasim [1983] presented swelling capacity for fish plastein samples in a range of 1.18-1.49 ml/g in pure water and 0.93-1.20 ml/g in 0.2M NaCl solution and for a fw hydrolysate 1.13 ml/g. Hermansson [1973] also presented values for swelling capacity of some protein sources. It was found that each 20 mg of soy bean isolate, sodium caseinate and whey protein concentrate had swollen to entrain 6, 3 and 2 ml H_2O/g protein respectively. Hindi [1979] also reported the swelling capacity of FPC as 0.8 ml/g.

It is difficult to make any comparisons between results obtained from different sources using investigations by empirical tests, in which great variances may be inherent in the tested materials, equipment and techniques. In comparing different experimental results caution must be taken and the functionality measurement of different proteins must be determined in a simple system to facilitate adjustments in food formulation when using or testing new protein sources [Kinsella, 1976].

4.2.5 Emulsion Capacity

The function of emulsifiers such as egg protein, whey protein, casein, soya bean protein and muscle protein decreases surface tension at the interface and interacts with each phase to form the network structures. Generally an emulsifier possesses a chemical structure containing hydrophilic and lipophilic groups similar to surface active agents. The use of an emulsifier-stabilised emulsion is found in various branches of the food industry such as meat emulsions (e.g. sausage), salad dressing, mayonnaise and ice-cream etc [Ivey et al, 1970; Tornberg and Hermansson, 1977; Yamuchi et al, 1980; Haque and Kinsella, 1989]. See also Section 2.2.3.4 and method of measuring in Section 3.3.2.

Table 30 shows the results of emulsion capacity (g oil/g protein) for FPH samples at various pH. It was found that individual samples from the same enzyme and time of hydrolysis were not significantly different ($P > 5\%$) at each pH. The results for the pH-emulsifying capacity profile of FPH samples and controls indicated a maximum at the natural pH (6.8-7.0) and lower values at pH 2, 4, 6 and 8. This corresponds to the pH-solubility profile where the protein solubility is least at pH other than the natural (see Section 4.2.1). It has been suggested that pH influences emulsion capacity in an indirect manner by affecting the solubility of the protein. Crenwelge et al [1974] reported a good correlation between the pH-emulsifying capacity and pH-solubility profiles of four proteins (soya protein, cottonseed, bovine haemoglobin and non-fat dry milk) from pH 3 to 10. Both

TABLE 30: EMULSION CAPACITY (g oil/g protein) FOR FPH SAMPLE AT VARIOUS pH

pH	Sample†			'Average' Sample T60	Sample†			'Average' Sample T20	Sample†			'Average' Sample B60	Sample†			'Average' Sample B20
	T60/1	T60/2	T60/3		T20/1	T20/2	T20.3		B60/1	B60/2	B60/3		B20/1	B20/2	B20/3	
2	258.6 (3.5)	255.2 (5.2)	256.7 (4.1)	256.8 (1.7)	238.1 (3.8)	236.8 (2.1)	235.8 (1.7)	236.9 (1.2)	210.1 (6.0)	207.1 (1.7)	208.1 (4.0)	208.4 (1.5)	203.8 (4.4)	205.8 (2.7)	208.6 (3.2)	206.1 (2.4)
4	260.7 (4.1)	257.0 (4.5)	258.8 (2.1)	258.8 (1.7)	245.5 (3.4)	241.3 (1.6)	242.9 (5.4)	243.2 (2.1)	196.0 (5.5)	194.8 (3.4)	197.9 (4.7)	196.2 (1.6)	202.3 (3.8)	207.0 (3.8)	204.1 (3.6)	204.5 (2.4)
6	246.3 (3.5)	244.3 (2.4)	243.8 (4.4)	244.8 (1.3)	235.7 (2.3)	233.2 (3.6)	236.1 (1.8)	235.0 (1.6)	197.5 (2.8)	201.5 (4.1)	198.2 (4.6)	199.1 (2.1)	204.1 (2.3)	203.5 (3.9)	207.2 (4.0)	204.9
8	251.0 (1.9)	250.2 (2.8)	248.4 (4.6)	249.9 (1.3)	232.6 (2.6)	229.6 (2.4)	234.3 (4.1)	232.2 (2.4)	179.7 (6.3)	178.1 (4.7)	175.4 (5.1)	177.7 (2.2)	198.6 (2.7)	204.3 (2.4)	202.6 (2.9)	201.8 (2.9)
Natural 6.8-7	269.5 (2.3)	266.9 (4.5)	266.5 (5.0)	267.6 (1.6)	258.0 (4.6)	260.6 (2.2)	262.1 (4.2)	260.2 (2.1)	233.6 (3.9)	235.8 (3.9)	229.8 (6.4)	233.1 (3.0)	255.4 (2.8)	261.0 (3.6)	259.9 (3.7)	258.8 (3.0)

Results are the mean of triplicate readings, standard deviations are given in parentheses.

† No significant difference was found for the FPH sample from the same enzyme hydrolysis.

profiles had the same shape, thus indicating a general correlation between emulsifying capacity and solubility. The superior pH-emulsifying capacity of myofibrillar proteins of cod fish was also reported to show good correlation with pH-solubility with minimum emulsification at pH 4 and 5 [Grabowski and Sikorski, 1974]. It has also been reported that the soluble protein which acts as an emulsifier is involved in the entrapment of fat droplets [Pearson et al, 1965; Swift et al, 1961].

Table 31 shows the results of emulsion capacity (g oil/g protein) for 'average' samples and controls at various pH. It was found that all 'average' samples of FPH had a significantly lower ($P < 0.1\%$) emulsion capacity than EA and a higher value of emulsion capacity than ISB which reflects their solubilition at each pH.

Comparison between 'average' samples of FPH showed that trypsin-treated 'average' samples had a greater emulsifying capacity ($P < 1\%$) than bromelain-treated 'average' samples. This could be explained by the mode of action of two different enzymes but this observation does not correlate to the higher pH-solubility profile of bromelain-treated FPH compared to trypsin-treatment (see Section 4.2.1). This disparity could be explained by a molecular weight effect where a relatively larger proportion of small peptides (<1000 Daltons) of trypsin-treated FPH compared to the bromelain-treated FPH product leads to higher emulsification capacity. These small peptides may contribute to the emulsification capacity of the protein of trypsin-treated FPH.

TABLE 31: EMULSION CAPACITY (g oil/g protein) FOR FPH 'AVERAGE' SAMPLES AND CONTROLS AT VARIOUS pH

pHs	Control		'Average' Samples				Significance Level†													
	EA	ISB	T60	T20	B60	B20	T60 to EA	T20 to EA	B60 to EA	B20 to EA	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20	EA to ISB
2	285.5 (3.3)	188.3 (3.0)	256.8 (1.7)	236.9 (1.2)	206.1 (2.4)	208.4 (1.5)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)
4	274.7 (1.8)	122.3 (2.9)	258.8 (1.9)	243.2 (2.1)	204.5 (2.4)	196.2 (1.6)	(+++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)
6	304.5 (2.9)	174.4 (2.2)	244.8 (1.3)	235.0 (1.6)	204.9 (2.0)	199.1 (2.1)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(+++)	(++)	(+++)	(+++)	(+++)	(+++)	(+)	(+++)
8	360.1 (2.3)	257.1 (4.4)	249.9 (1.3)	232.2 (2.4)	201.8 (2.9)	177.7 (2.2)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(+++)	(+++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
Natural 6.8-7	316.6 (1.8)	264.8 (5.3)	267.9 (1.6)	260.2 (2.1)	258.8 (3.0)	233.1 (3.0)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(++)	(+++)

† Significance level is indicated as follows: not significant (-), significant(+), highly significant (++), very highly significant (+++)

Other factors which significantly reduce the emulsifying capacity of FPH and fish proteins are low molecular weight carbohydrate and storage conditions. In both cases loss of emulsion capacity was related to loss of protein solubility and factors which affect the solubility of myofibrillar proteins [Inklaar and Fortuin, 1969; Koury and Spinelli, 1975].

It was also found that 'average' samples of 60 minute hydrolysis had higher emulsion capacity than 'average' sample of 20 minute hydrolysis for both enzyme treatments. This again could be explained by the higher protein solubility of 60 minute hydrolysis than 20 minute hydrolysis. Spinelli et al [1972] showed a relationship between emulsion capacity of fish protein and time of hydrolysis (Rhozym P11 used for hydrolysis). It was found that there is not much variation after 15, 30, 60 minutes of hydrolysis which gave emulsion capacities of 225, 225 and 231 g oil/g protein respectively.

4.2.6 Viscosity

Viscosity and flow properties of protein dispersions are very important in relation to processing such as spray-drying, feed rate etc and new product development. It can be a useful index of structural changes in proteins and subsequently of the hydrodynamic/rheological properties of modified food proteins. Viscosity changes can be used to evaluate the properties of some foods such as thickening power of proteins, fluid foods, soups, beverages, batters etc (Kinsella, 1976; Kinsella, 1979). See also Section 2.2.3.6 and method of measuring viscosity in Section 3.3.6.

The results shown in Table 32 are viscosity (centipoise, Cp) of FPH samples at chosen temperature and concentration (% w/v). The viscosity value is a slope of a graph (shear stress vs rpm) of a straight line for a Newtonian fluid when shear stress is directly proportional to the shear rate (see Appendix 3 and Section 3.3.6 for viscosity formula).

It was found that the viscosity increases at a given temperature as the concentration increases for all the FPH samples. This could be explained by the fact that the viscosity of a protein solution is concentration independent. However the effect of protein concentration can be divided into three categories. The viscosity in dilute protein solution cannot be detected because of the absence of interaction of protein molecules. Thus this can be governed by the shape and size of the molecules. The viscosity in semi-concentrated protein solution is due to the presence of hydrodynamic interactions between the protein molecules up to a point where the critical concentration occurred. The viscosity in concentrated protein solution is also due to the hydrodynamic domains of the protein molecules and suspended particles interaction are of paramount importance (i.e. the above interaction in concentrated protein solutions show viscoelastic properties (Rha and Pradipasena, 1986).

The viscosity always decreases as the temperature increases at a given concentration. However, as the temperature increased, in addition to the decrease in viscosity due to the increase of kinetic energy, the

TABLE 32: VISCOSITY (Cp) OF FPH SAMPLES AT VARIOUS TEMPERATURES AND CONCENTRATIONS(% w/v)

Temp. °C	Conc. %	Sample T60/1	Sample T60/2	Sample T60/3	'Average' Sample T60	Sample T20/2	Sample T20/2	Sample T20/3	'Average' Sample T20	Sample B60/1	Sample B60/2	Sample B60/3	'Average' Sample B60	Sample B20/1	Sample B20/2	Sample B20/3	'Average' Sample B20
20	5	2.10	2.08	2.21	2.13 (0.10)	2.29	2.23	2.25	2.26 (0.03)	1.91	1.92	1.95	1.93 (0.02)	2.10	1.89	1.93	1.97 (0.02)
	10	3.18	2.52	3.01	2.9 (0.30)	3.82	3.71	3.85	3.79 (0.10)	2.43	2.39	2.53	2.45 (0.10)	2.81	2.90	3.15	2.95 (0.20)
	15	5.10	4.12	5.70	4.97 (0.8)	5.98	5.74	7.15	6.62 (0.60)	3.34	3.46	3.25	3.35 (0.10)	3.94	4.27	4.55	4.25 (0.31)
30	5	1.88	1.82	1.84	1.85 (0.01)	1.95	1.86	1.87	1.89 (0.10)	1.79	1.71	1.68	1.73 (0.10)	1.61	1.78	1.72	1.70 (0.10)
	10	2.35	2.10	2.57	2.34 (0.24)	2.97	2.79	2.64	2.80 (0.20)	2.01	2.08	2.21	2.10 (0.10)	2.34	2.18	2.68	2.40 (0.30)
	15	3.50	3.34	4.31	3.72 (0.50)	5.44	5.15	5.33	5.31 (0.200	3.19	2.98	2.99	3.10 (0.10)	3.27	3.36	3.68	3.50 (0.30)
40	5	1.60	1.43	1.57	1.53 (0.10)	1.73	1.76	1.70	1.73 (0.03)	1.53	1.56	1.54	1.54 (0.02)	1.52	1.55	1.54	1.54 (0.02)
	10	2.20	1.81	2.35	2.12 (0.30)	2.79	2.47	1.98	2.41 (0.40)	1.76	1.76	1.98	1.83 (0.13)	2.01	1.94	2.13	2.03 (0.10)
	15	3.30	2.66	3.85	3.27 (0.60)	4.70	4.20	4.41	4.44 (0.30)	2.29	2.70	2.28	2.45 (0.30)	2.91	3.09	3.09	3.03 (0.10)

thermal denaturation contributes to the rheology of protein containing systems. Proteins are sensitive to temperature and an irreversible denaturation (i.e. protein unfolding can occur as temperature increases as a result of the hydrodynamic volume increases).

It was also found that FPH samples after trypsin hydrolysis had a higher viscosity than FPH samples after bromelain hydrolysis at all temperatures and concentrations not significant. This could be due to the mode of action of the two enzymes in which trypsin-treated samples have lower solubility and slightly higher swelling properties than bromelain-treated samples with high solubility and low swelling properties.

It is known that viscosity is influenced by solubility and the swelling capacity of proteins. Mainly highly soluble, non-swelling proteins possess low viscosity (e.g. albumins, globulins). It is expected that viscosity will be inversely correlated with solubility. The soluble molecules with their large exposed hydrophobic molecules area should experience greater protein-protein interactions (Kinsella, 1976; Shen, 1981).

Table 33 shows the viscosity results of FPH 'average' samples and controls at various temperatures and concentrations (% w/v). It was found that egg albumin (EA) was not significantly different ($P > 5\%$) to all FPH 'average' samples at various temperatures and concentrations except

TABLE 33: VISCOSITY (Cp) OF FPH 'AVERAGE' SAMPLES AND CONTROLS AT VARIOUS TEMPERATURES AND CONCENTRATIONS (% w/v)

Temp. °C	Conc. %	Control		'Average' Sample				Significance Level†													
		EA	ISB	T60	T20	B60	B20	T60 to EA	T20 to EA	B60 to EA	B20 to EA	T60 to ISB	T20 to ISB	B60 to ISB	B20 to ISB	T60 to T20	T60 to B60	T60 to B20	T20 to B60	T20 to B20	B60 to B20
20	5	2.4	5.2	2.1 (0.1)	2.3 (0)	1.9 (0)	2.0 (0)	(-)	(-)	(-)	(-)	(++)	(++)	(++)	(++)	(-)	(-)	(-)	(-)	(-)	(-)
	10	3.1	n.d.	2.9 (0.3)	3.8 (0.1)	2.5 (0.1)	3.0 (0.2)	(-)	(-)	(-)	(-)					(-)	(-)	(-)	(-)	(-)	(-)
	15	3.6	n.d.	5.0 (0.8)	6.6 (0.6)	3.4 (0.1)	4.3 (0.3)	(+)	(+)	(-)	(+)					(-)	(-)	(-)	(-)	(-)	(-)
30	5	2.0	4.9	1.9 (0)	1.9 (0.1)	1.7 (0.1)	1.7 (0.1)	(-)	(-)	(-)	(-)	(++)	(++)	(++)	(++)	(-)	(-)	(-)	(-)	(-)	(-)
	10	3.4	n.d.	2.3 (0.2)	2.8 (0.2)	2.1 (0.1)	2.4 (0.3)	(-)	(-)	(-)	(-)					(-)	(-)	(-)	(-)	(-)	(-)
	15	3.3	n.d.	3.7 (0.5)	5.3 (0.2)	3.1 (0.1)	3.5 (0.3)	(-)	(+)	(-)	(-)					(+)	(-)	(-)	(+)	(+)	(+)
40	5	1.7	4.1	1.53 (0.1)	1.7 (0)	1.5 (0)	1.5 (0)	(-)	(-)	(-)	(-)	(++)	(++)	(++)	(++)	(-)	(-)	(-)	(-)	(-)	(-)
	10	2.0	n.d.	2.1 (0.3)	2.4 (0.4)	1.8 (0.1)	2.0 (0.1)	(-)	(-)	(-)	(-)					(-)	(-)	(-)	(-)	(-)	(-)
	15	2.5	n.d.	3.3 (0.6)	4.4 (0.3)	2.5 (0.3)	3.0 (0.1)	(-)	(+)	(-)	(-)					(+)	(-)	(-)	(+)	(-)	(-)

n.d. = Not determined

† Significance level is indicated as follows: not significant (-), significant (+), highly significant (++), very highly significant (+++)

T60, T20, B20 at 15% concentration. This again could be due to high solubility of egg albumin and non-swelling capacity which resulted in the low viscosity. It was also found that all FPH 'average' samples had a significantly lower viscosity ($P < 1\%$) than ISB. This correlated to solubility profiles because the ISB had a significantly lower solubility than the FPH product (see Section 4.2.1). However viscosity changes may be influenced by other parameters such as conformation, hydration, exposure of hydrophobic groups, change distribution which contribute to the intermolecular interaction. Thus it is not necessarily that the viscosity will correlate to solubility (Kinsella, 1976; Schmidt, 1981; Shen, 1981).

Jasim (1983) had reported values of viscosity (Cp) for fish plastein, FPH and egg albumin. It was found that the highest viscosities at 20°C were 2.2, 2.0 and 2.4 Cp respectively. The viscosity of FPC at different concentrations has been measured using an Ostwald viscometer and was to be in the range of 1-1.7 Cp for concentrations of 1-3.5% (Hindi, 1979).

Solubility and viscosity are non-equilibrium properties, the measured values are dependent upon measured conditions. It is difficult to compare data from other investigators.

4.2.7 Gel Formation

The capacity to form gels is an important property of many food proteins which provide a structural matrix for entrapment of water,

sugar, flavour and other food ingredients. However, protein gel formation usually involves heating a protein to cause at least partial denaturation or unfolding of the polypeptide chains and exposure of the reactive groups which then gradually link up to form the three dimensional network or gel matrix. This association involves disulphide, hydrogen, hydrophobic and ionic bonds. Protein gels are typically characterised by relatively high viscosity, plasticity, and elasticity in proteins such as gelatin jelly, coagulated egg white, soy bean tofu, milk casein curd and the myofibrillar gels formed by heating saline-soluble meat or fish proteins [Kinsella, 1976; Howell and Lawrie, 1985]. See also Section 2.2.3.7 and method of measuring in Section 3.3.7.

FPH samples (10%) were heated at 70°C and 80°C for 30 minutes at various pHs 2, 4, 6, 8 and natural (6.8-7) and ionic strength (2% and 3% salt solutions). All samples failed to form a gel at these pHs and ionic strength.

The lack of gel formation could be due to the temperature and concentration at which gelation occurs. The denatured protein can be formed only when a critical balance of attraction and repulsive forces is attained. Gelation of protein chains and protein chain-solvent interactions occurs mainly through hydrophobic amino acids rather than those of a hydrophilic nature to form a regular network or matrix. Crosslinking protein chains in order to form a gel structure needs a regular crosslinking between the protein chains. Therefore the

distribution of hydrophobic amino acids in a protein chain is more important than the amount of particular amino acids present [Kinsella, 1976; Hegg, 1982]. In the case of FPH possibly the required temperature, concentration and required hydrophobic/hydrophilic balance may not be achieved.

Hermansson [1982] reported that heating above an optimum or critical temperature (i.e. above 70 and 80°C) causes an increased tendency towards protein-protein interaction and partial disruption of the protein network due to local aggregation phenomena. Lawhon and Cater [1971] reported that protein isolated from cottonseed formed gels when heated at 100°C for 10 min. This may well explain that for FPH the optimum or critical temperature at which the gel will be formed had not been achieved.

Jasim [1983] also found that samples of fish plastein produced by bromelain and FPH hydrolysed by α-chytochrotrypsin gave no gel formation at different pHs (2, 4, 6, 8, 10, 12 and natural) and ionic strengths of 0.3, 0.6, 0.9, 1.2, 1.3, 1.8, 2M NaCl. However Miller and Groninger [1976] have shown that gelation of dispersions of acylated fish myofibrillar protein hydrolysed with bromelain is possible. It was found that the minimum concentration to form a gel was 3% solution of fish protein (acetylated and succinylated only) and 7% solution of fish proteins (i.e. acetylated and hydrolysed by bromelain).

Gossett et al [1984] have stated that the most common factor affecting gelation is electrostatic charge. The pH as well as the ionic strength of the protein environment can alter the charge distribution among the amino acid side chains and can either decrease or increase the protein-protein interactions.

4.3 FISHBURGER EVALUATION

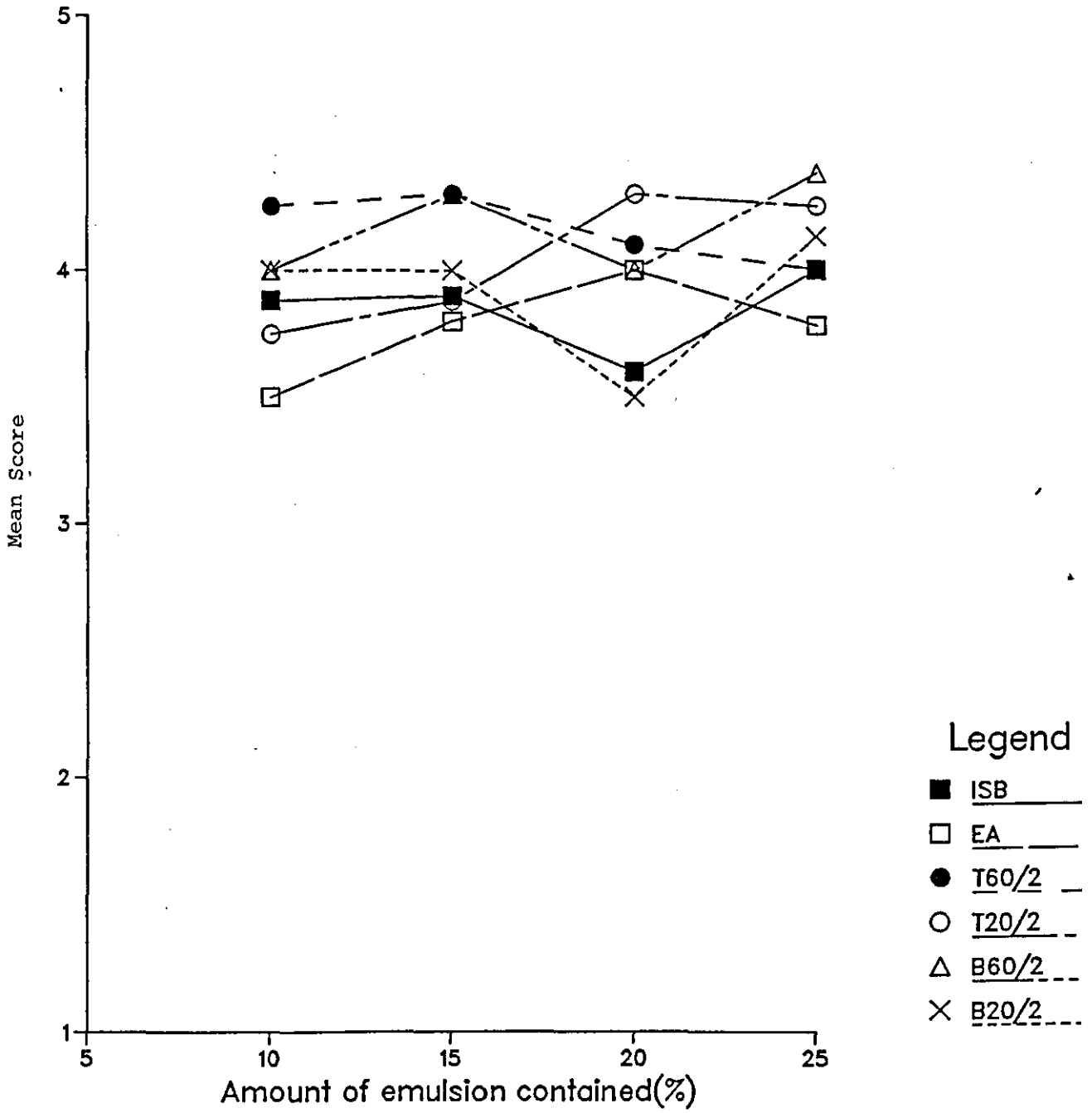
Weinberger [1983] showed that the reforming of various trimmings of fish into loaves or sticks might have an economical advantage. The binding properties of ten fish species were evaluated on the basis of a visual assessment score (9 = very strong binding, 1 = very poor binding) and by instrumental measurement of tensile strength using an Instron instrument. It was found that fish flesh with a high fat content (and low moisture content) did not bind well. Cod fish, among those species evaluated, scored 2 (only cod flesh) but on the addition of 1.5% sodium chloride scored 8. Suzuki [1981] reported that minced fish has a gel-forming capability because of myosin and actomyosin, but this is still not enough to hold fish particles together. Suzuki [1981] also showed the results of texture of marin beef (100%), minced beef (30%), minced fish (70%) and minced fish (100%). The texture pattern graphs were obtained by measuring hardness and stickiness (holding particles together) with a texturometer (power: 3V, plunger: tooth shape Ø = 10 mm).

Fish balls, made from minced fish, only were very fragile with low hardness and less stickiness compared to beef materials. It was also reported that on adding 1-3% sodium chloride to minced fish and kneading the actomyosin in muscles become sol as in the production of kamaboko (see Section 2.1.3.2). This addition of salt and kneading allowed the muscle fibres to be pulverised completely and become sol of actomyosin. It was shown that the higher the concentration of salt, the harder the texture becomes.

In this work only emulsions made from FPH of representative 'average' samples after trypsin/bromelain hydrolysis (T60/2, T20/2, B60/2 and B20/2) and controls (EA and ISB) were added to fresh minced fish (see Table 15 for chemical composition) to produce fishburgers (see Section 3.4.1). This was done to investigate the functional properties of FPH in food systems, in particular emulsifying properties. The functional properties of the FPH were assessed by taste panels on 'finished' products by assessing colour, odour, taste, texture and overall acceptability (see Section 3.4.3). Finished products means the deep-fried fishburgers.

Figure 7 shows the sensory evaluation of the colour of finished deep-fried fishburgers of representative samples of FPH and controls. A golden yellow-brown colour was predicted to be that which would be preferred by the consumer, therefore the highest acceptability score was given to this colour (see Table 14 taste panel score sheet). It was found that all the samples and the control fishburgers at all

Fig.(7) Sensory evaluation on colour of fishburger.

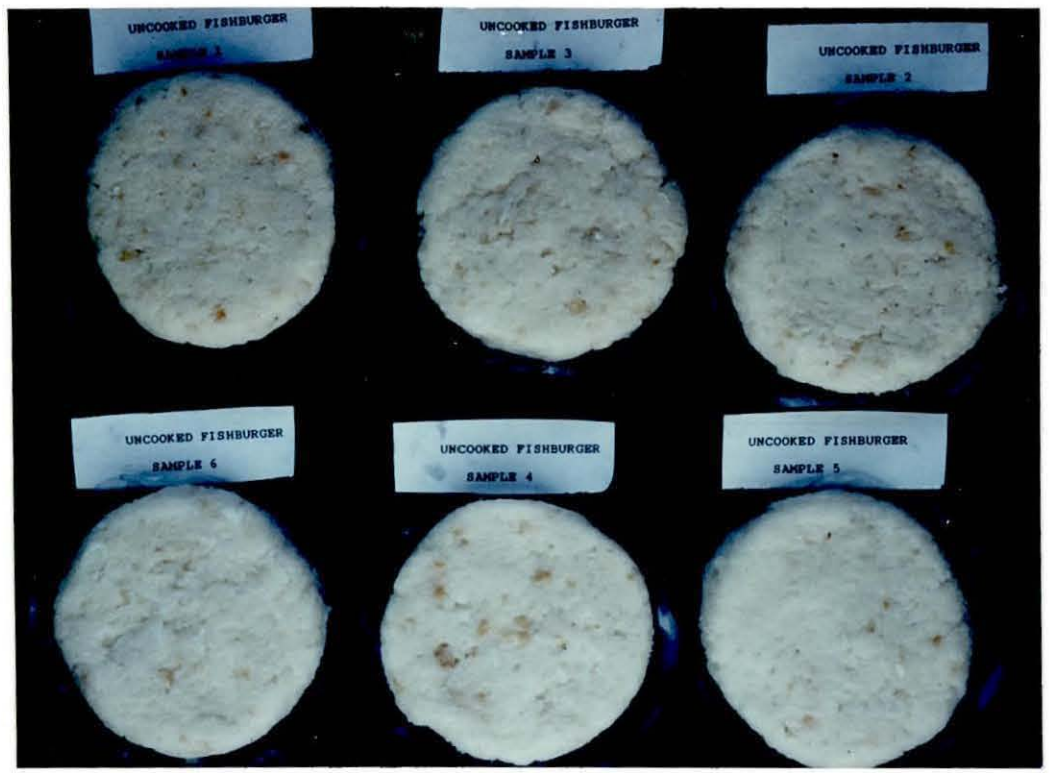


levels of emulsion addition had an acceptable colour score. The final colour of the fishburgers was due to naturally minced fish and emulsions bearing in mind no colour was added or batter used in the production of the fishburgers (see Figures 8 and 9). The samples of fishburger with added FPH-emulsion at different levels tended to have a higher colour score than the controls except sample T20/2 at a level of 10% addition and sample B20/2 at a level of 20% addition.

Figure 10 shows the sensory evaluation of the odour of finished deep-fried fishburgers of representative samples of FPH and controls. A strong fried-fish odour was predicted to be that which would be preferred by the consumer, therefore the highest acceptability score was given to it. All fishburgers with added FPH emulsion had a higher score than the controls. This could best be explained by the fact that FPH itself has a fishy odour (see Section 4.1) added to that of the fish mince on frying. Obviously the EA and ISB could not do this and may introduce other non-fishy cooked flavours.

Figure 11 shows the sensory evaluation of the taste of finished deep-fried fishburgers of representative samples of FPH and controls. The very strong (fried) fish taste was predicted to be that which would be preferred by the consumer, for fishburgers, therefore the highest acceptability score was given to it. All the fishburger samples with added FPH-emulsion had a higher acceptability score than the controls for reasons similar to those affecting the odour scores. The actual scores for the FPH samples were between 3-4 and tended to be lower as

FIGURE 8: RAW FISHBURGER SAMPLES AND CONTROLS WHICH CONTAINED 10% EMULSION ONLY



Sample 1: Uncooked T60/2

Sample 2: Uncooked B60/2

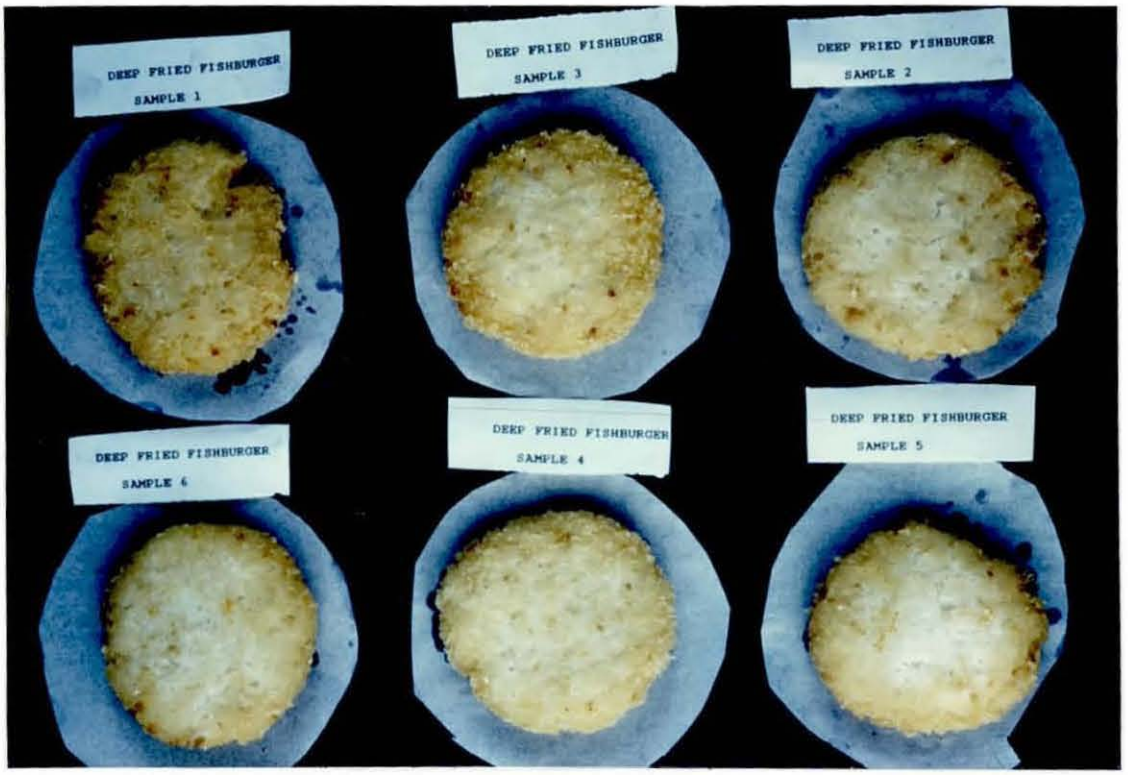
Sample 3: Uncooked T20/2

Sample 4: Uncooked B20/2

Sample 5: Uncooked EA

Sample 6: Uncooked ISB

FIGURE 9: COOKED FISHBURGER SAMPLES AND CONTROLS WHICH CONTAINED 10% EMULSION ONLY



Sample 1: Cooked T60/2

Sample 2: Cooked B60/2

Sample 3: Cooked T20/2

Sample 4: Cooked B20/2

Sample 5: Cooked EA

Sample 6: Cooked ISB

Fig.(10) Sensory evaluation on odour of fishburger.

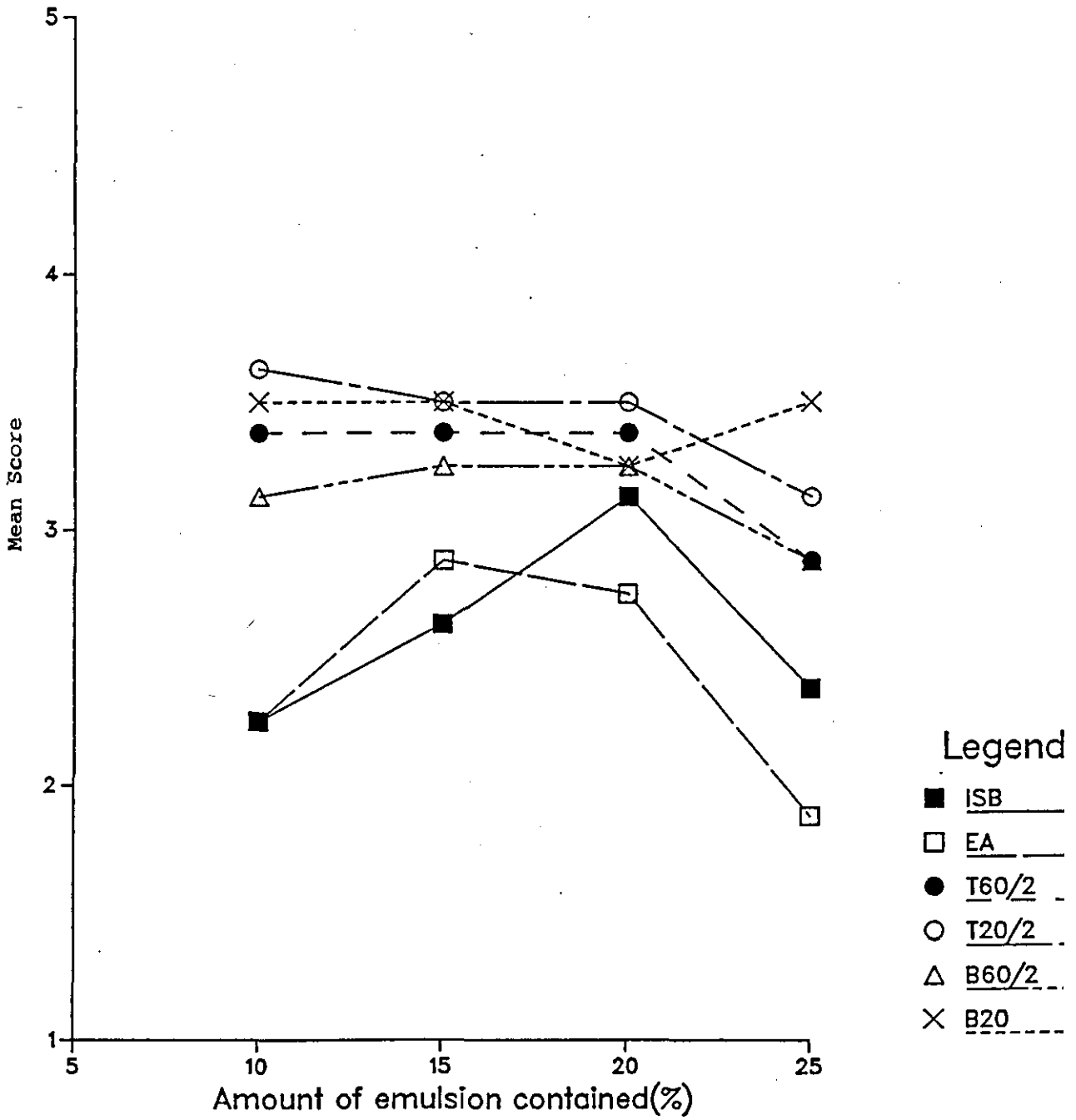
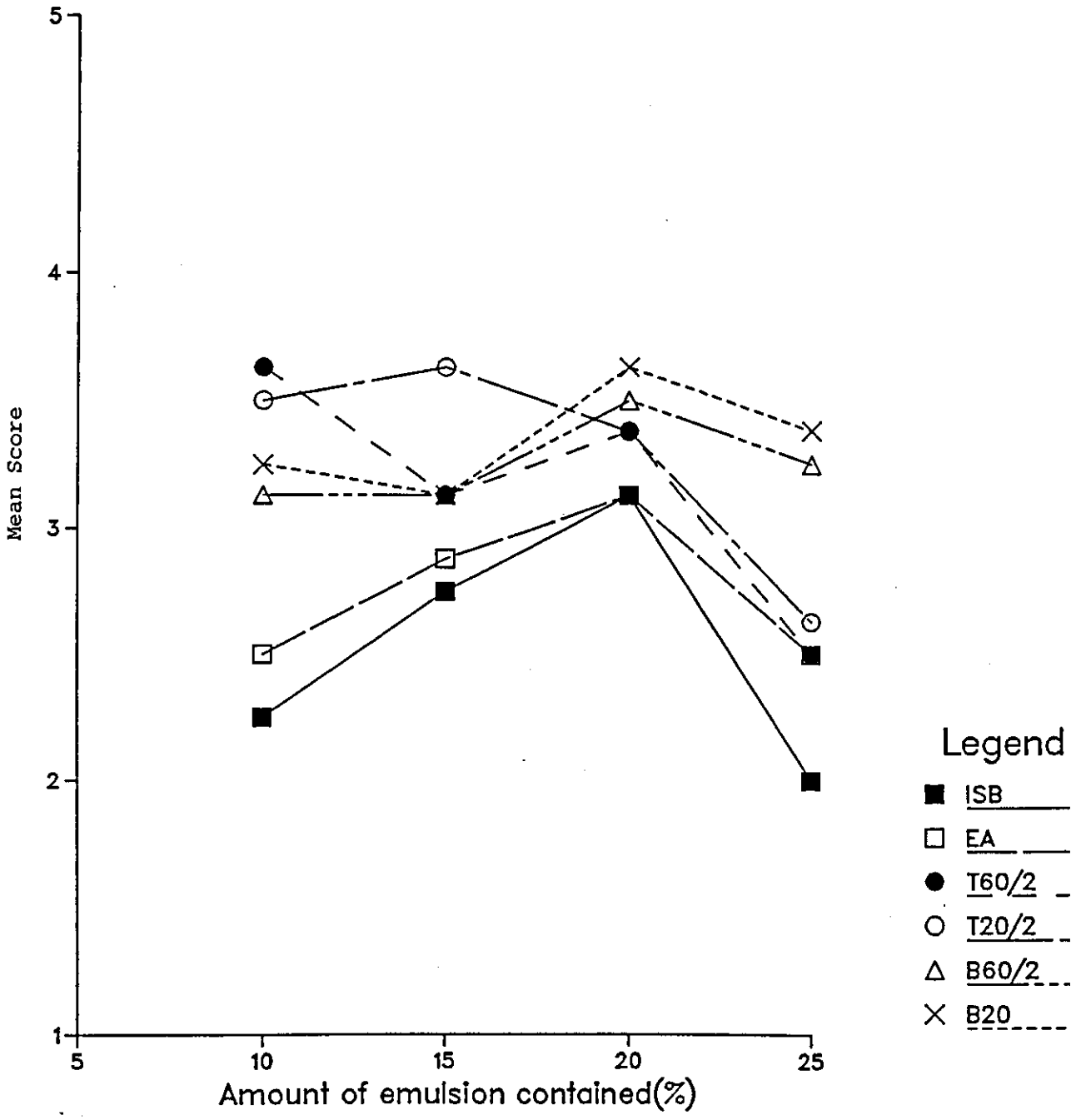


Fig.(11) Sensory evaluation on taste of fishburger.



the level of emulsion addition increased - this may be due to an increase in the amount of corn oil added at these levels which would modify the fish taste of the final product.

Figure 12 shows the sensory evaluation of the texture of finished deep-fried fishburgers of representative samples of FPH and controls. A juicy and tender texture was predicted to be that which would be preferred by the consumer for fishburgers, therefore the highest acceptability score was given to it. Almost all the samples of fishburgers with added FPH-emulsion had higher texture scores than the controls. It was also found that the highest texture score for both FPH and control fishburgers was at 25% level of addition. This could be due to the greater amount of emulsion added giving a juicier and tenderer product. From Figure 13 it is clear that all the FPH and control fishburgers gave the lowest Instron reading (kg force) at 25% added emulsion and highest reading at lower additions of emulsion. This is as expected as the tenderer texture score from the taste panel will be reflected in a lower force from the Instron to shear sections of fishburgers [Suzuki, 1981]. The functional properties of ingredients will be reflected in finished product attributes such as texture and on this basis the level of addition of various ingredients should be selected for the required formulation [Suzuki, 1981].

Figure 14 shows the sensory evaluation of overall acceptability of finished deep-fried fishburgers of representative samples of FPH and controls. "Like very much" was chosen to be a description which would

Fig.(12) Sensory evaluation on texture of fishburger.

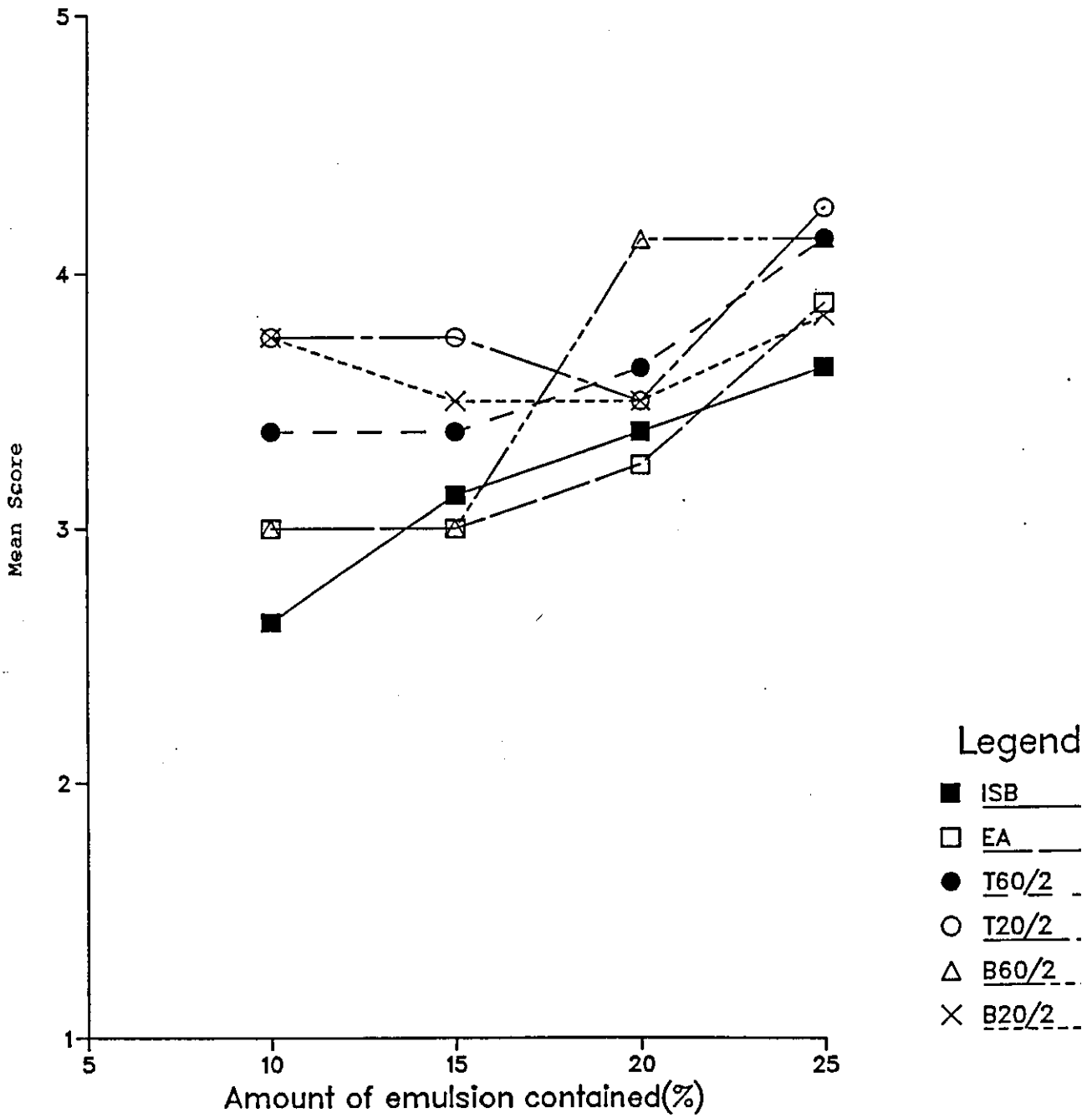


Fig.(13) Instron Reading(kg) V Emulsion(%) of fishburger.

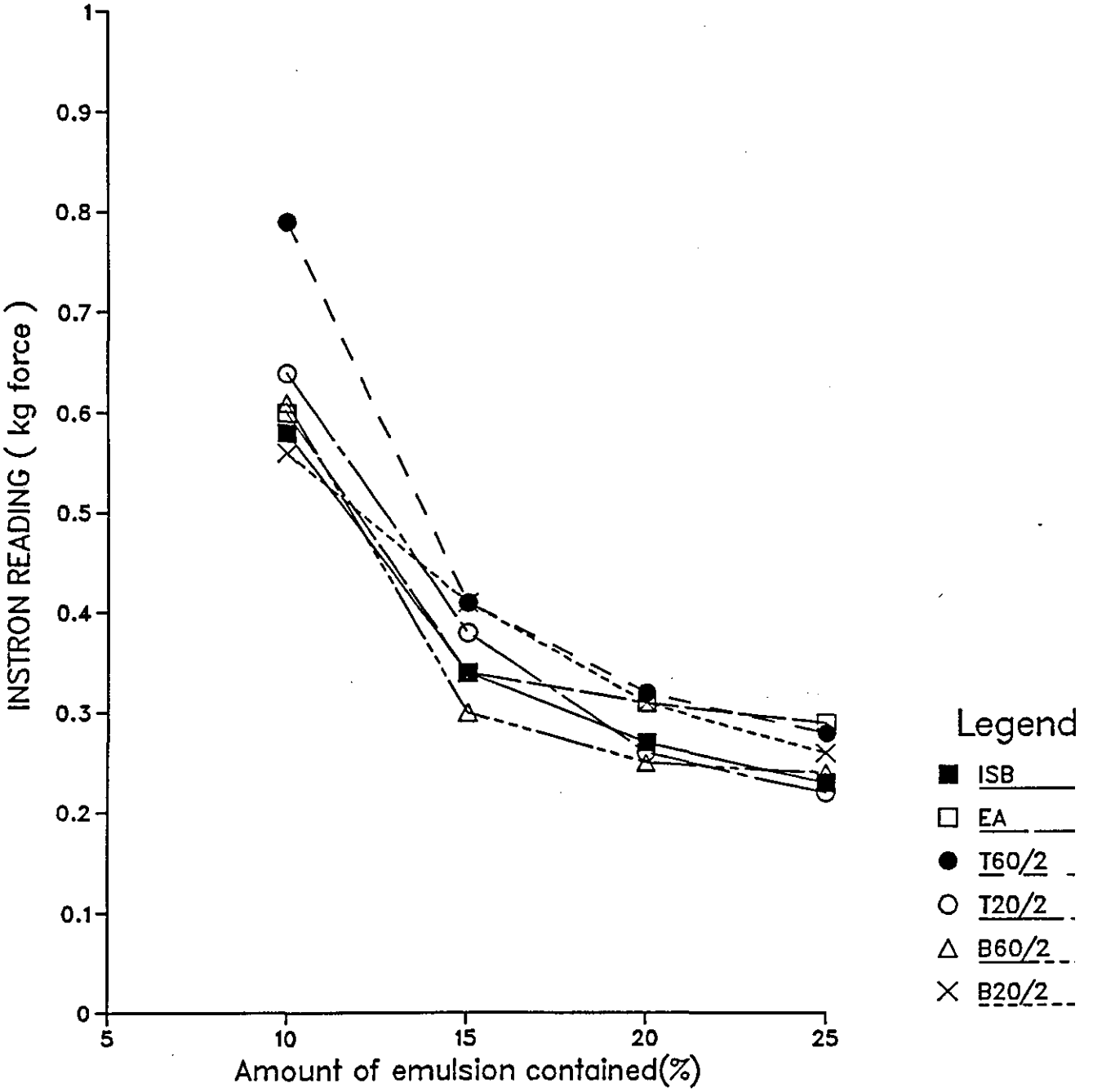
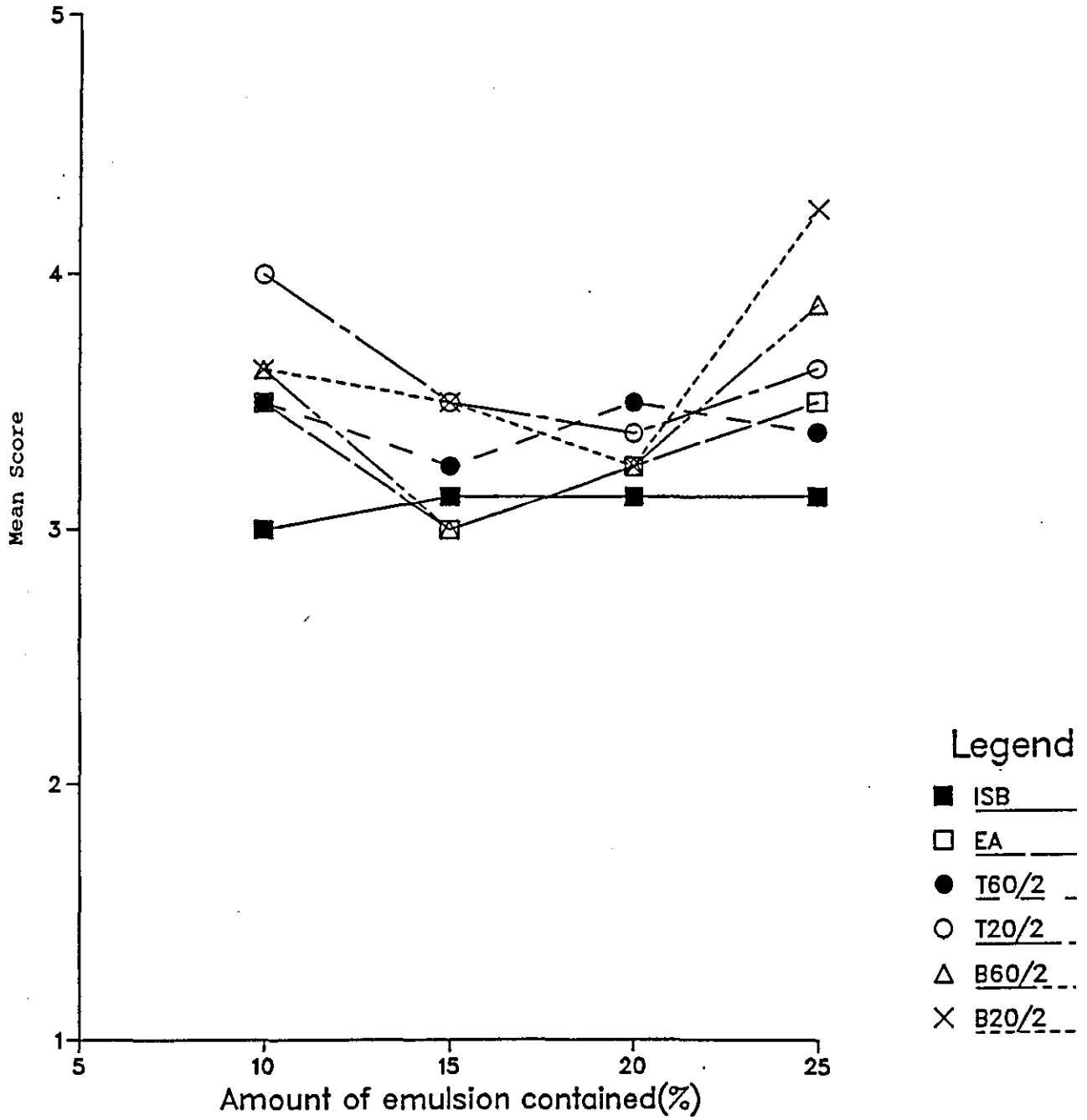


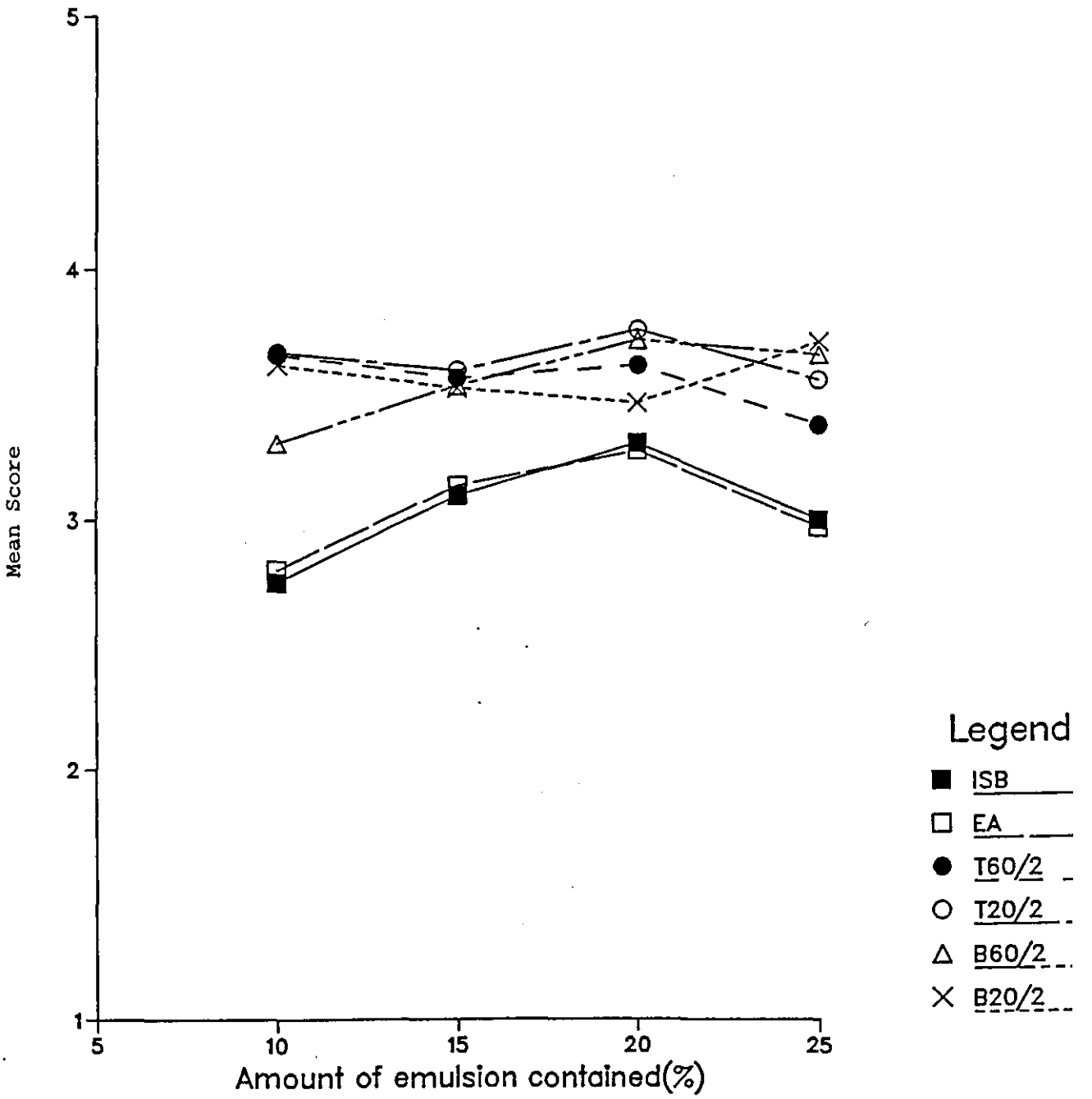
Fig.(14) Sensory evaluation on acceptability of fishburger.



be expressed by the consumer for good fishburgers, therefore the highest overall acceptability score was given to it. All the fishburger samples and controls had a reasonable overall acceptability score at all levels of emulsion addition. The samples containing FPH-emulsion tended to give higher scores than the controls. Fishburger samples including the control EA emulsion tended to have a higher overall acceptability than the fishburger containing ISB emulsion. These scores were given by the panellists taking into account all the parameters which had been scored individually and the panellists giving their own subjective individual weighting as to the importance of these other parameters.

Figure 15 shows the calculated acceptability of fishburger samples containing FPH and controls which have to be calculated by the averaging of four mean scores for colour, odour, taste and texture giving equal weighting to each of the parameters. All fishburgers which contained FPH-emulsion at all levels gave acceptability scores higher than controls. Comparing Figure 14 with Figure 15 shows a similar pattern for both the overall acceptability score and the calculated score with FPH fishburger being assessed better than control fishburgers. However, the difference between the FPH and the control samples was greater by calculation than by the taste panel overall preferences. The factors which control the weighting given by the panellists at the time of testing are based on immediate assessment which will be more subjective than for the calculated score. To reflect this subjective assessment would necessitate giving

Fig.(15) Calculated acceptability of fishburger.



different weightings to each parameter when doing the calculated scores which is not defensible as being literally more "calculated". Overall the two methods give a similar result.

Figure 16 shows weight loss (%) against amount of emulsion added for finished deep-fried fishburgers containing representative samples of FPH and controls. All samples of fishburgers with addition FPH-emulsion showed slightly greater weight loss than fishburgers containing the EA control at all levels of emulsion addition. Samples containing control ISB showed greater weight loss than those containing EA at all levels of emulsion addition and were more similar to the FPH emulsion fishburgers. For all samples the greatest weight loss occurred at emulsion level of 25%. This could reflect the higher amount of corn oil added at higher emulsion additions which could be released during cooking. However there was no significant difference ($P > 5\%$) in weight loss at all levels of emulsion addition between fishburger samples and controls (see Table 34).

4.4 ECONOMIC EVALUATION OF FPH

A simple evaluation for design and construction of pilot plant was used in the main pilot plant area of the Chemical Engineering Department (see Figure 17 for flow diagram of FPH processes). The production of FPH was as follows for raw materials and unit processes:

Fig. (16) Weight loss Vs Amount of emulsion of fishburger samples and controls

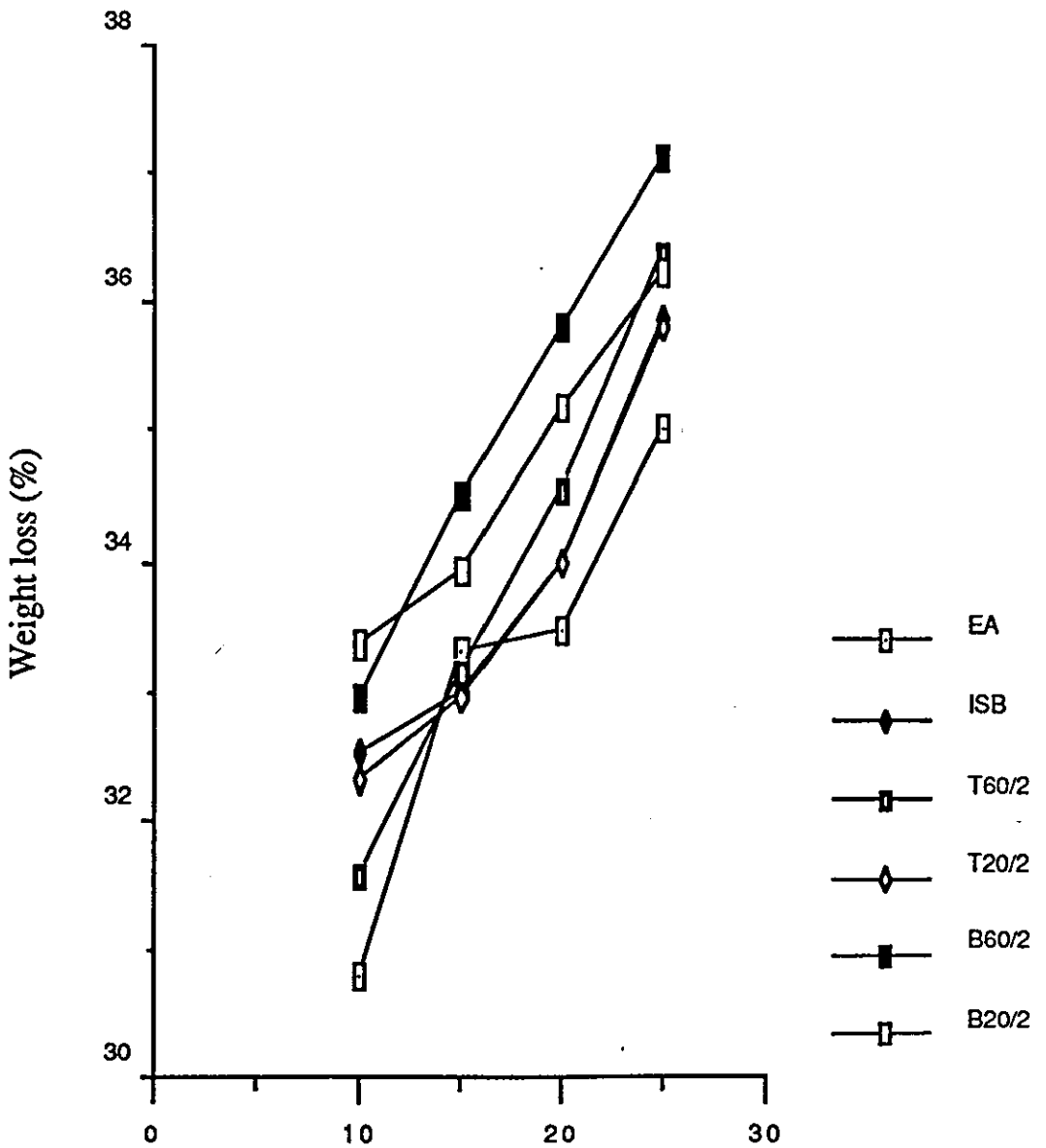
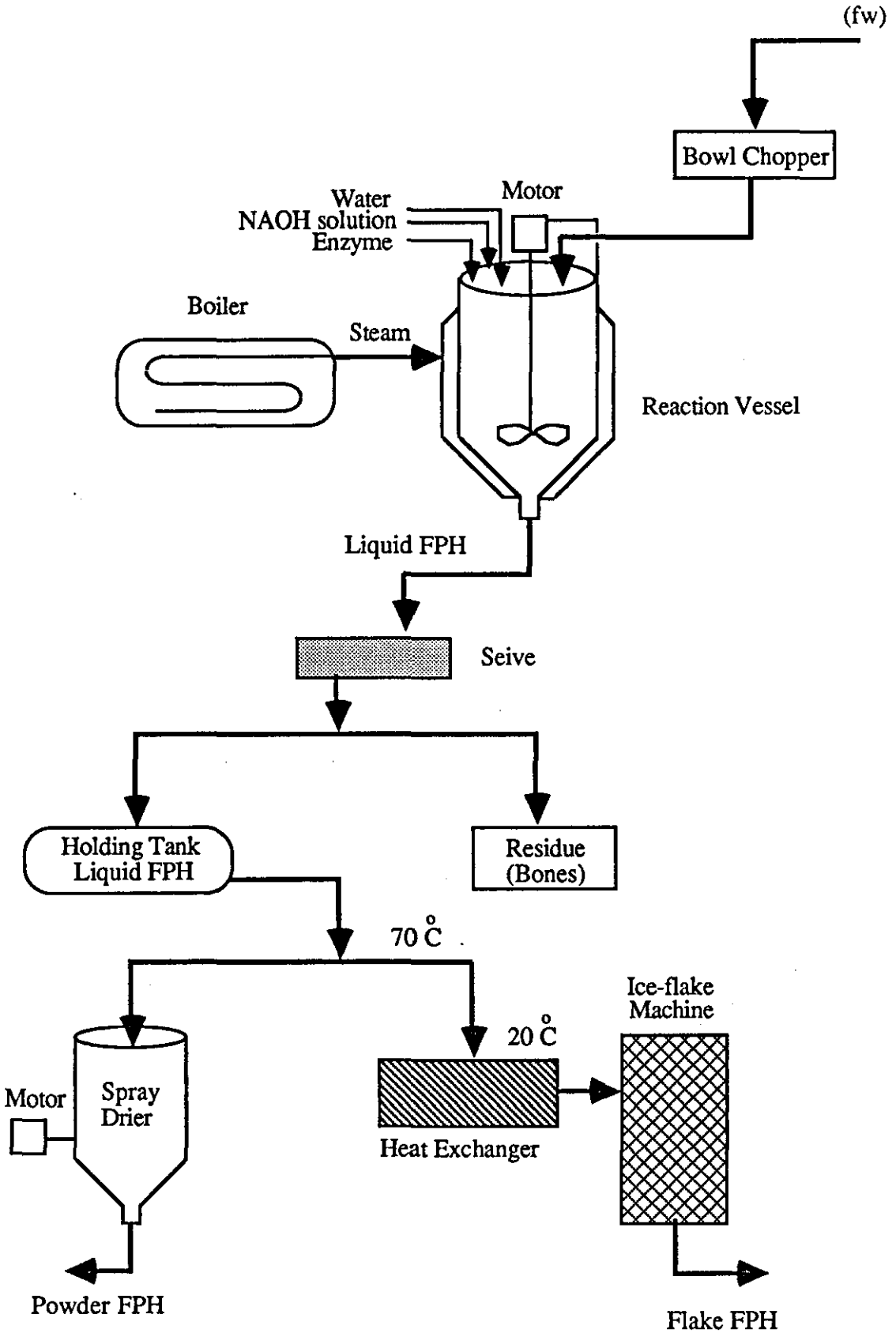


TABLE 34: WEIGHT LOSS (%) OF FISHBURGER WHICH CONTAINED AN EMULSION OF FPH SAMPLES AND CONTROLS

Emulsion (%)	Weight Loss (%)						Significance Level†														
	Controls		Samples				T60/2 to EA	T20/2 to EA	B60/2 to EA	B20/2 to EA	T60/2 to ISB	T20/2 to ISB	B60/2 to ISB	B20/2 to ISB	T60/2 to T20/2	T60/2 to B60/2	T60/2 to B20/2	T20/2 to B60/2	T20/2 to B20/2	B60/2 to B20/2	EA to ISB
	EA	ISB	T60/2	T20/2	B60/2	B20/2															
10	30.8 (0.9)	32.5 (0.8)	31.6 (1.2)	32.3 (0.5)	32.9 (1.3)	33.4 (0.7)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
15	33.3 (1.2)	33.0 (0.6)	33.1 (0.3)	32.9 (1.0)	34.5 (0.6)	33.9 (0.6)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
20	33.5 (0.6)	34.0 (1.14)	34.6 (1.0)	33.9 (0.5)	35.8 (0.5)	35.2 (0.5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
25	35.0 (0.9)	35.8 (0.8)	36.4 (1.5)	35.8 (0.8)	37.1 (0.3)	36.2 (0.8)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

† Significane level is indicated as follows: not significant (-); significant (+); highly significant (++); very highly significant (+++)

Fig. (17) Flow Diagram of FPH Process



1. Raw materials

A. Cod fish waste (fw), 500 kg/day in 5 batches of 100 kg. 60 minutes of hydrolysis was considered in this work. 1 tonne costs £56.

B. Trypsin enzyme preferred because 1 kg cost £34 i.e. cheaper than bromelain which 1 kg cost £237.

C. Sodium hydroxide (NaOH) 1 kg cost £0.74.

2. Equipment

A. The bowl chopper (Saanbrucken Muller) type MTK 10/70. Capacity 5 kg/minute.

B. The reaction vessel (see Figure 5) capacity 200 kg. Material stainless steel, fitted with stirrer (0.28 kWh motor).

C. Boiler. Capacity 140 lb steam/hr (2 kWh). Twin Industries Agencies Ltd, Camberley, Surrey.

D. Spray-drier. Capacity 12 kg/hr. Energy consumption 60,000 BTU/hr. Chemical Engineering Department.

E. Plate heater exchanger. Capacity 1000 kg/day (5 kWh). APV International Ltd, Crawley, West Sussex.

F. Ice flake machine. Capacity 37 kg/hr. (2.23 kWh).

Tables 35 and 36 show economic evaluations of production of FPH in the form of powder and flake respectively. It can be seen that the FPH powder costs £2.66/kg (see page 143 for calculation of production) which is not higher than the price of egg albumin (£2.5-3/kg) but is higher than soy bean isolate (£1-1.3/kg). Enzyme and spray drying

TABLE 35: ECONOMIC EVALUATION OF FPH POWDER PRODUCTION

Raw Materials	Annual Amount kg/year	Annual Cost £	Equipment	Cost (£)	Annual Energy Cost (£)	Annual Detail Production Cost	Cost (£)
Cod fish waste (fw)	150,000	8400	Bowl chopper 1.496 KWh (1.67 hr used)	3500	45	Total raw materials	16465
						Gas	10922
Water	150,000	150	Reactor vessel + stirrer (0.28 KWh)	2500	2445	Electricity	284
					38	Labour	1600
Sodium hydroxide (NaOH)	360	265	Boiler (12 KWh)	2700	-	Maintenance	2000
			Siever + tank	250	-	Rates and rent	6500
Enzyme (Trypsin)	225	7650	Spray-drier + motor (1.5 KWh - used 8 hr)	30000	8477	Other expenses	2000
					202	A - transportation B - Paper work C - Telephone	
	300,585†	16465		38950	11205		54171

† 83.6% FPH liquid recovery which will be 251289 kg and concentrated by centrifugation from 8.8% to 60%. Then the liquor will be 36856 kg/year to spray drying to get 22114 kg/year FPH powder

TABLE 36: ECONOMIC EVALUATION OF FPH FLAKE PRODUCTION

Raw Materials	Annual Amount kg/year	Annual Cost £	Equipment	Cost (£)	Annual Energy Cost (£)	Annual Detail Production Cost	Cost (£)
Cod fish waste (fw)	150,000	8400	Bowl chopper 1.496 KWh (1.67 hr used)	3500	45	Total raw materials	16465
						Gas	2445
Water	150,000	150	Reactor vessel + stirrer (0.28 KWh motor)	2500	2445	Electricity	284
					38	Labour (2 persons)	16000
Sodium hydroxide (NaOH)	360	265	Boiler (12 KWh)	2700	-	Maintenance	2000
			Siever + tank	250	-	Rates and rent	6500
			Heat exchanger 5.8 KWh (6.68 hr used)	520	217		
Enzyme	225	7650	Flake ice machine 2.23 KWh (24 hr used)	15000	899	Other expenses	2000
						A - transportation	
						B - paper work	
			Freezer storage 3 KWh (24 hr used)	25000	202	C - telephone	
	300,585†	16465		49470	3845		46810

† 83.6% recovery of FPH which will be 251,289 kg with 16.6% residue which will be 49897 kg/year with 8.8% total solid and 7.8% protein

costs still make the FPH production expensive. It can be seen also that FPH flake costing £0.21/kg is far cheaper to produce than FPH powder. FPH flake also can be used as well as the powder in food products because it has good functional properties especially emulsion capacity. The cold storage and melting energy were not counted in this process.

This method of estimation of the cost of FPH was based on the analysis of Backhurst and Harker (1973). In this method the major cost components are total annual manufacturing, labour, overheads and capital costs (including simple interest payments) and this total is divided by the annual production to give a cost/kg of product. Although this method takes a simple approach to the repayments on capital charges it gives a first approximation of the product cost from which it can be decided if more detailed calculations are worthwhile.

According to Merritt (1982), from 2.4 ealier, fish meal cost £493/tonne and FPH £1025/tonne based on similar sized plants of 7500 tonnes of raw material p.a. Production costs, in 1990, of fish meal were estimated as £281/tonne based on selling prices (Anon, 1991) from the largest plants in Chile. The present work gives a production cost of £2,600/tonne for the spray dried powder based on a plant using 150 tonne p.a. The production costs of meal and FPH are affected by many factors such as: scale, process type, labour, energy costs (and savings!), effluent treatment and particularly raw material type and cost. It is difficult to compare like with like. However, allowing for the volatility in the price of meal, as an internationaly traded product and the fact that FPH will never be made on the same scale, it seems that the cost of FPH is still considerably higher than meal and this must be reflected in the applications to which it is put. It is unlikely to be viable as a bulk animal feed but its functional properties may allow it to find a niche in some human foods or as a specialist animal feed for veal calves.

CALCULATION OF PRODUCTION COSTS

FPH POWDER

Capital cost for FPH powder = 38950 (£) (see Table 35).

Loan from bank over 10 years (17% interest) = £4557.

Therefore:

1. Total production cost = (annual running cost + bank instalment)
   (i.e. production cost)

   = 54171 + 4557 = 58728 (£)

2. FPH powder production/year = 22,113 kg.

Therefore the cost of FPH powder = $\frac{1}{2} = \frac{58728 \text{ (£)}}{22113 \text{ (kg)}}$

= 2.66 (£)

FPH FLAKE

Capital cost for FPH flake production = 46810 (£) (see Table 36)

Loan from bank over 10 years (17% interest) = 5788 (£).

Therefore:

1. Total production cost = (annual running cost + bank instalment)

   = 46810 + 5788 = 52598 (£)

2. Total FPH flake production/year = 251289 kg (see Table 36).

Therefore cost of 1 kg FPH flake = $\frac{(1)}{(2)} = \frac{52598 \text{ (£)}}{251289 \text{ (kg)}}$

= 0.21 (£)

CHAPTER 5

CONCLUSION

A large proportion of the fishery by-catch and by-product from processing is wasted every year and has not been effectively processed into a good quality product. The main type of products derived from fish waste materials have not been found suitable for human consumption up to now. Edible fish meal and FPC were successfully incorporated into food systems in some countries in products such as bread, biscuits, pasta, beverages and soups. Major difficulties were encountered, in particular flavour, when a faint fishy taste and odour were detectable and there was a lack of functional properties leading to a gritty texture, poor binding and low dispersibility. Furthermore, the cost of these products was high because of considerable processing such as solvent extraction to remove the "fishy" components which also destroyed functionality. The products used were in small quantities on a commercial basis for fortification of established acceptable foods but this inevitably leads to a high cost for the nutritional value imparted to these products.

The FPH produced as a suspension by enzymic hydrolysis from fw showed that FPH possesses functional properties such as solubility, oil and water binding capacity, swelling and emulsion capacity but showed no gelling properties. However, these functional properties were very dependent on pH but high values of functionality were noted at natural

pHs. Generally this pH is highly desirable and nothing needs to be altered (i.e. no expenses incurred) when FPH is used with fish mince because it has the same pH as fish mince (6.8-7.3). Additionally, pH is a very important factor in fish products such as kamaboko to form a gel i.e. care should be taken not to lower the pH below 6.0 otherwise gel will not be formed. FPH could have a role in stabilising such products.

The molecular weight distribution showed that an FPH with a small proportion of large molecular weight components (> 5000 Daltons) and a small proportion of low molecular weight components (< 1000) had good functional properties compared with a large proportion of high or low molecular weight components. The idea of removing large and small molecular components by ultrafiltration could help to achieve better functional properties by selecting the appropriate MW range polypeptides.

FPH functional properties, in particular solubility and emulsion capacity, have been demonstrated by incorporating FPH-stabilised emulsions with fish mince to produce fishburgers with a good overall acceptability compared with EA and ISB-stabilised fishburgers. So these functional properties of FPH suggest a means of utilising fish mince in a variety of products (stabilised by the FPH) thus making use of a resource which is at present difficult to form into good quality products. Products such as fish cake, fish fingers, fish balls, fish sausages, fish pasta, soup and fishburgers are considered

to be of poor quality and "non-pure" fish mince products because of other added ingredients such as flour, egg, starch, potato and soy bean etc. Thus FPH could be an appropriate product to increase the usage of fish mince and bring about the development of fish mince products up to high standard with a good nutritional status. Recent publications indicate that FPH could be used in very high quality fish products such as surimi and kamaboko.

Results showed that FPH could be produced at a reasonable cost compared with EA and ISB.

CHAPTER 6

SUGGESTIONS FOR FURTHER WORK

The work described has been aimed to produce FPH by enzymic hydrolysis as an alternative to other competitors as a functional protein. There are many other aspects which remain to be explored. The more important areas are suggested below:

1. Production of FPH from other fish species should be considered.

2. In performing enzymic hydrolysis cheaper enzymes or multi-enzyme systems, particularly if they contain endo- and exo-peptidases, could be considered.

3. An attempt to examine in detail the molecular weight distribution and fractionation of FPH should be tried. This information could consider the mechanisms involved in enzyme cleavage of proteins and form a guide for future research efforts towards 'designer' protein hydrolysates.

4. More products such as fish balls, fish sausage and fish slices should be produced by incorporation of FPH and these food systems studied as for fishburgers.

5. More work should be carried out to find other uses for FPH such as for microbial growth media. Some basic work has been done on production of microbial growth media successfully from FPH, but the results were not included here.

6. Finally, more work should be attempted to lower the price of FPH.

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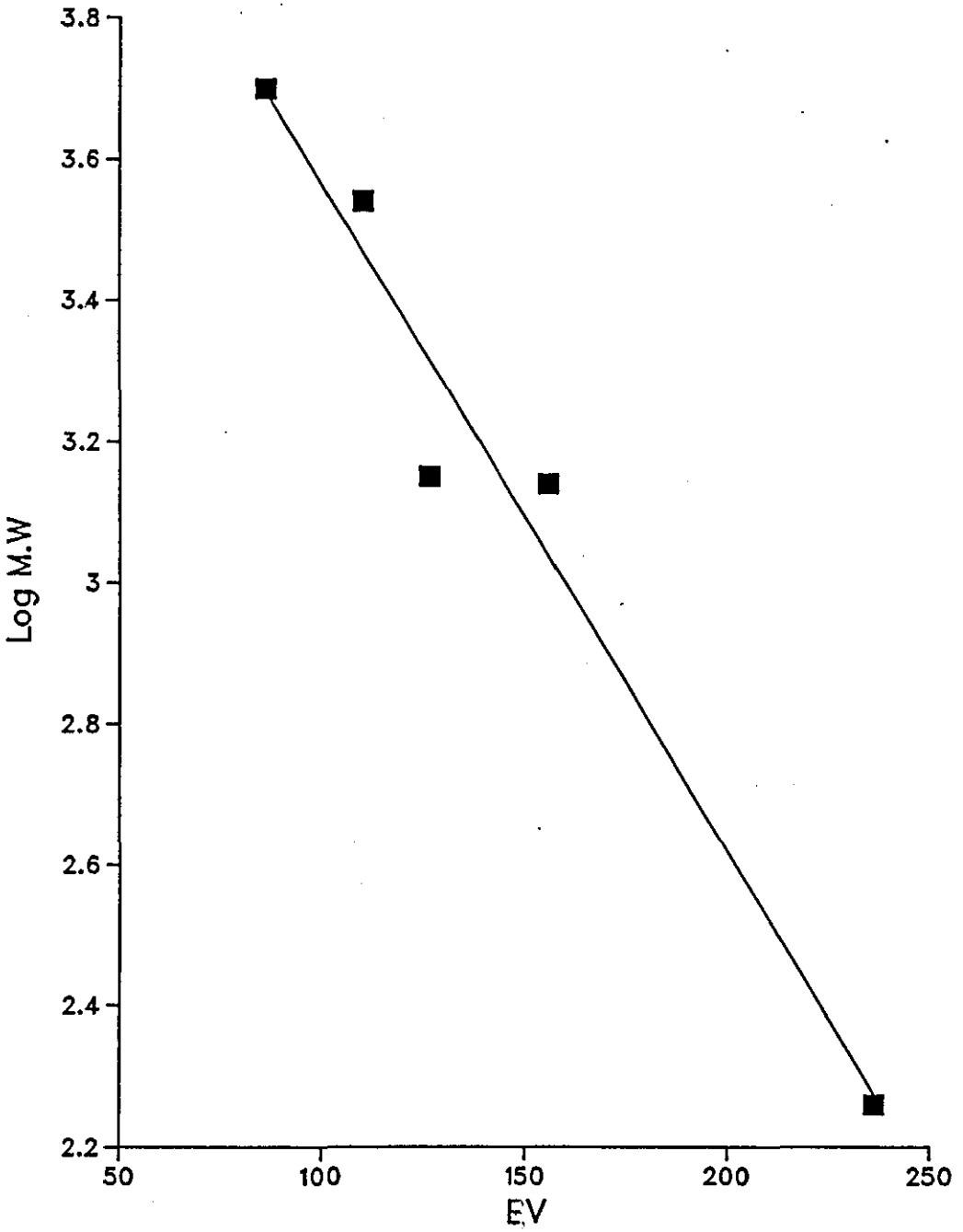
APPENDIX 1

STANDARD CURVE FOR GEL PERMEATION

(see Section 3.5.9)

Standard curve for gel permeation



APPENDIX 2

PARTICLE SIZE DISTRIBUTION FOR FPH SAMPLES AND CONTROLS

(see Section 3.5.10)

2.1 Individual T60 FPH samples

2.2 Individual T20 FPH samples

2.3 Compiled T60 and T20 FPH samples

2.4 Individual B60 FPH samples

2.5 Individual B20 FPH samples

2.6 Compiled B60 and T20 FPH samples

2.7 Controls EA and ISB

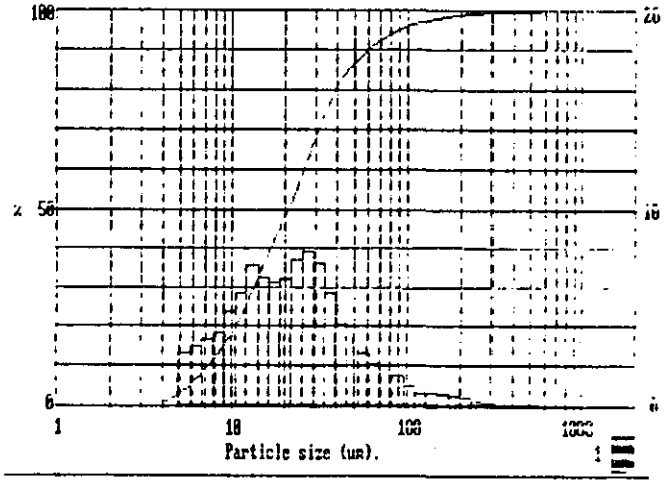
2.1 INDIVIDUAL T60 FPH SAMPLES

MALVERN Instruments M6.82 Date 14-07-1989 Time 10:27

CLIENT : Food Lab.
SAMPLE : FPH (T60/1
Our REF : 89/190

System number 1988 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.82 Date 13-07-89 Time 10:27



Size microns	% under	% in band	Size microns	% under	% in band
564.0	100.0	0.0	53.1	88.1	3.2
487.0	100.0	0.0	45.8	84.9	4.2
420.0	100.0	0.0	39.5	80.7	5.0
362.0	100.0	0.0	34.1	74.4	7.1
312.0	100.0	0.0	29.4	67.0	7.9
270.0	100.0	0.2	25.4	59.7	7.4
233.0	99.7	0.3	21.9	52.6	6.5
201.0	99.5	0.5	18.9	45.8	6.3
173.0	98.9	0.6	16.3	39.5	6.0
149.0	98.3	0.6	14.1	33.7	5.4
129.0	97.7	0.6	12.1	28.7	5.0
111.0	97.1	1.0	10.5	23.9	4.8
95.9	96.1	1.5	9.0	19.5	4.4
82.7	94.6	1.5	7.8	15.4	3.7
71.4	93.1	1.5	6.7	11.7	3.6
61.6	91.6	2.6	5.8	8.1	3.6

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Mean length = 14.3 mm.
Obscuration =0.3418
Volume Conc. = 0.0147 %
Log. Diff. =2.94
Model indp

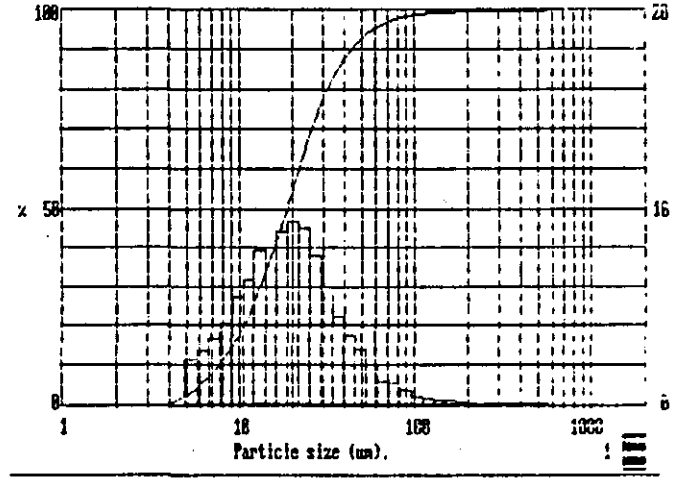
Div.0.5) = 20.2 um
Div.0.9) = 50.1 um
Div.0.1) = 7.6 um
D(4,3) = 24.4 um
D(3,2) = 16.2 um
Span = 2.1
Spec. surf. area
0.4183 sq.m./cc.

MALVERN Instruments M6.82 Date 07-07-1989 Time 12:17

CLIENT : Food Lab.
REFERENCE : F.P.H. (T 60/2)
OUR REF. : 89/191

System number 1988 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.82 Date 07-07-89 Time 12:19



Size microns	% under	% in band	Size microns	% under	% in band
564.0	100.0	0.0	53.1	94.0	2.5
487.0	100.0	0.0	45.8	91.1	3.0
420.0	100.0	0.0	39.5	87.6	4.5
362.0	100.0	0.0	34.1	83.0	6.0
312.0	100.0	0.0	29.4	77.0	7.6
270.0	100.0	0.0	25.4	69.4	9.0
233.0	100.0	0.0	21.9	60.3	9.4
201.0	100.0	0.1	18.9	50.9	8.8
173.0	99.9	0.1	16.3	42.1	8.0
149.0	99.7	0.2	14.1	34.1	7.5
129.0	99.6	0.2	12.1	26.6	6.8
111.0	99.4	0.4	10.5	19.8	5.5
95.9	99.0	0.7	9.0	14.3	4.2
82.7	98.3	1.1	7.8	10.1	3.9
71.4	97.2	1.2	6.7	6.6	3.5
61.6	96.0	2.1	5.8	4.1	

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Mean length = 14.4 mm.
Obscuration =0.3469
Volume Conc. = 0.0154 %
Log. Diff. =3.15
Model indp

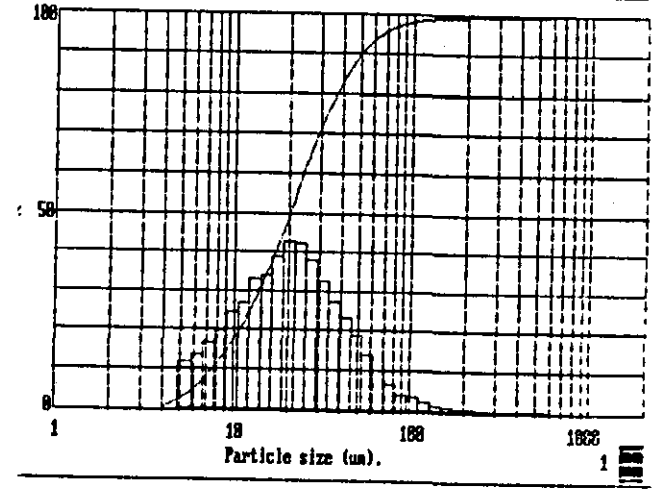
Div.0.5) = 16.6 um
Div.0.9) = 45.7 um
Div.0.1) = 7.1 um
D(4,3) = 22.6 um
D(3,2) = 13.5 um
Span = 2.8
Spec. surf. area
0.4324 sq.m./cc.

MALVERN Instruments M6.82 Date 28-06-1989 Time 17:05

CLIENT : Food Lab.
REFERENCE : F.P.H. (T 60/3)
OUR REF. : 89/192

System number 1988 Diode CT1306

alvern Instruments EASY Particle Sizer M6.82 Date 28-06-89 Time 17:05



Size microns	% under	% in band	Size microns	% under	% in band
564.0	100.0	0.0	53.1	91.3	3.8
487.0	100.0	0.0	45.8	87.6	4.7
420.0	100.0	0.0	39.5	82.8	5.5
362.0	100.0	0.0	34.1	77.3	6.6
312.0	100.0	0.0	29.4	70.8	7.6
270.0	100.0	0.0	25.4	63.2	8.5
233.0	100.0	0.1	21.9	54.6	8.6
201.0	99.9	0.1	18.9	46.0	7.8
173.0	99.8	0.1	16.3	38.2	6.9
149.0	99.7	0.2	14.1	31.4	6.6
129.0	99.4	0.5	12.1	24.8	5.4
111.0	99.0	0.7	10.5	19.4	4.9
95.9	98.3	0.8	9.0	14.4	4.0
82.7	97.5	1.3	7.8	10.5	3.4
71.4	96.2	2.1	6.7	7.1	2.8
61.6	94.2	2.8	5.8	4.3	

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Mean length = 14.3 mm.
Obscuration =0.3418
Volume Conc. = 0.0147 %
Log. Diff. =2.94
Model indp

Div.0.5) = 20.2 um
Div.0.9) = 50.1 um
Div.0.1) = 7.6 um
D(4,3) = 24.4 um
D(3,2) = 16.2 um
Span = 2.1
Spec. surf. area
0.4183 sq.m./cc.

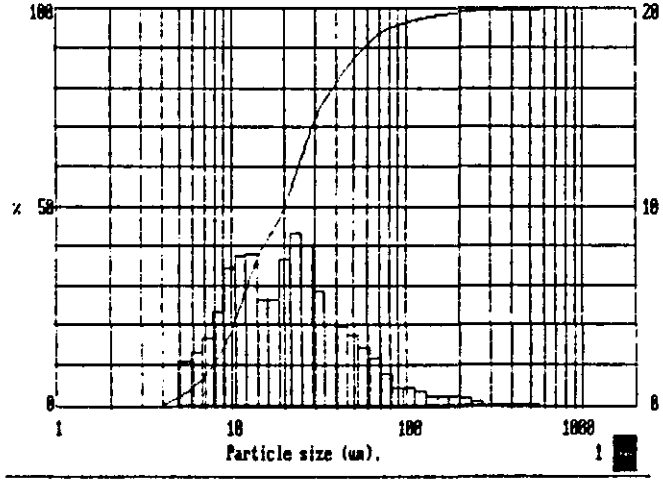
4.2 INDIVIDUAL T20 FPH SAMPLES

MALVERN Instruments M6.02 Date 18-07-1989 Time 15:09

CLIENT : Food Lab.
SAMPLE : FPH (T20/1)
LAB REF: 89/199

System number 1986 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 18-07-89 Time 15-18



Size : microns	under	% in band	Size : microns	under	% in band
564.0	100.0	0.0	53.1	88.6	3.6
487.0	100.0	0.0	45.8	85.2	3.9
420.0	100.0	0.0	39.5	81.6	4.1
362.0	100.0	0.0	34.1	77.5	4.7
312.0	100.0	0.0	29.4	71.4	6.7
270.0	100.0	0.2	25.4	63.4	8.6
233.0	99.8	0.2	21.9	54.8	7.4
201.0	99.4	0.4	18.9	47.4	6.5
173.0	99.0	0.4	16.3	42.1	5.3
149.0	98.6	0.5	14.1	36.8	4.9
129.0	98.1	0.5	12.1	32.6	4.0
111.0	97.6	0.9	10.5	21.7	6.9
95.9	96.8	0.7	9.0	14.8	4.7
82.7	96.1	1.6	7.8	10.1	3.4
71.4	94.1	3.2	6.7	6.7	2.7

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Beam length = 14.3 mm.
Obscuration =0.3671
Volume Conc. = 0.0127 %
Log. Diff. =2.89
Model indp

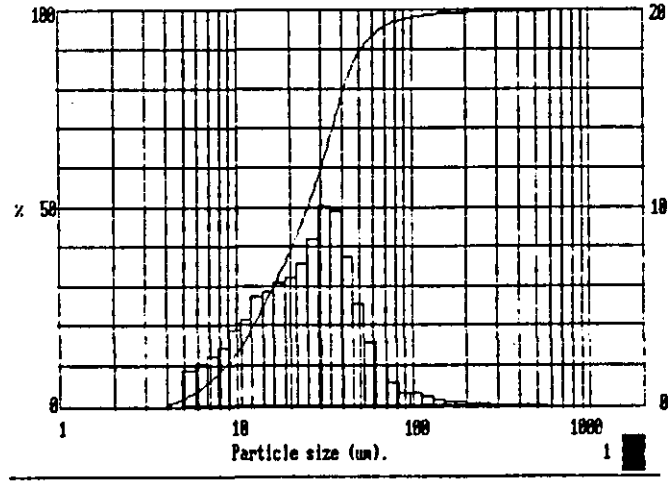
D(v,0.5) = 28.0 um
D(v,0.9) = 56.2 um
D(v,0.1) = 7.8 um
D(4,3) = 25.5 um
D(3,2) = 15.9 um
Span = 2.4
Spec. surf. area

MALVERN Instruments M6.02 Date 28-07-1989 Time 15:88

CLIENT : Food Lab
SAMPLE : FPH (T20/2)
LAB REF: 89/200

System number 1986 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 28-07-89 Time 15-89



Size : microns	under	% in band	Size : microns	under	% in band
564.0	100.0	0.0	53.1	91.3	5.1
487.0	100.0	0.0	45.8	86.2	7.6
420.0	100.0	0.0	39.5	78.6	9.8
362.0	100.0	0.0	34.1	68.7	10.1
312.0	100.0	0.0	29.4	58.6	8.4
270.0	100.0	0.1	25.4	50.2	7.2
233.0	99.9	0.1	21.9	43.0	6.4
201.0	99.8	0.1	18.9	36.6	6.2
173.0	99.7	0.2	16.3	30.3	5.8
149.0	99.5	0.2	14.1	24.5	5.3
129.0	99.3	0.4	12.1	19.1	4.4
111.0	98.9	0.6	10.5	14.7	4.8
95.9	98.3	0.7	9.0	10.9	3.9
82.7	97.6	1.2	7.8	7.9	2.5
71.4	96.4	2.0	6.7	5.4	2.1
61.6	94.4	3.2	5.8	3.3	

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Beam length = 14.3 mm.
Obscuration =0.2842
Volume Conc. = 0.0135 %
Log. Diff. =3.40
Model indp

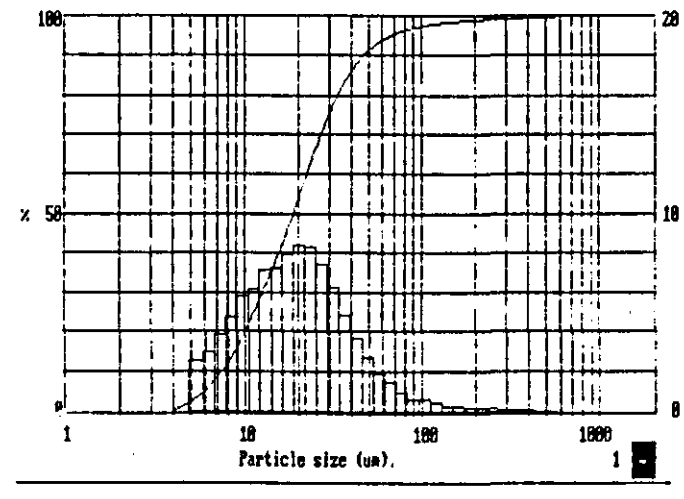
D(v,0.5) = 28.3 um
D(v,0.9) = 58.0 um
D(v,0.1) = 8.7 um
D(4,3) = 27.6 um
D(3,2) = 16.8 um
Span = 1.7
Spec. surf. area
0.3611 sq.m./cc.

MALVERN Instruments M6.02 Date 28-07-1989 Time 16:38

CLIENT : Food Lab
SAMPLE : FPH (T20/3)
LAB REF: 89/201

System number 1988 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 28-07-89 Time 16-39



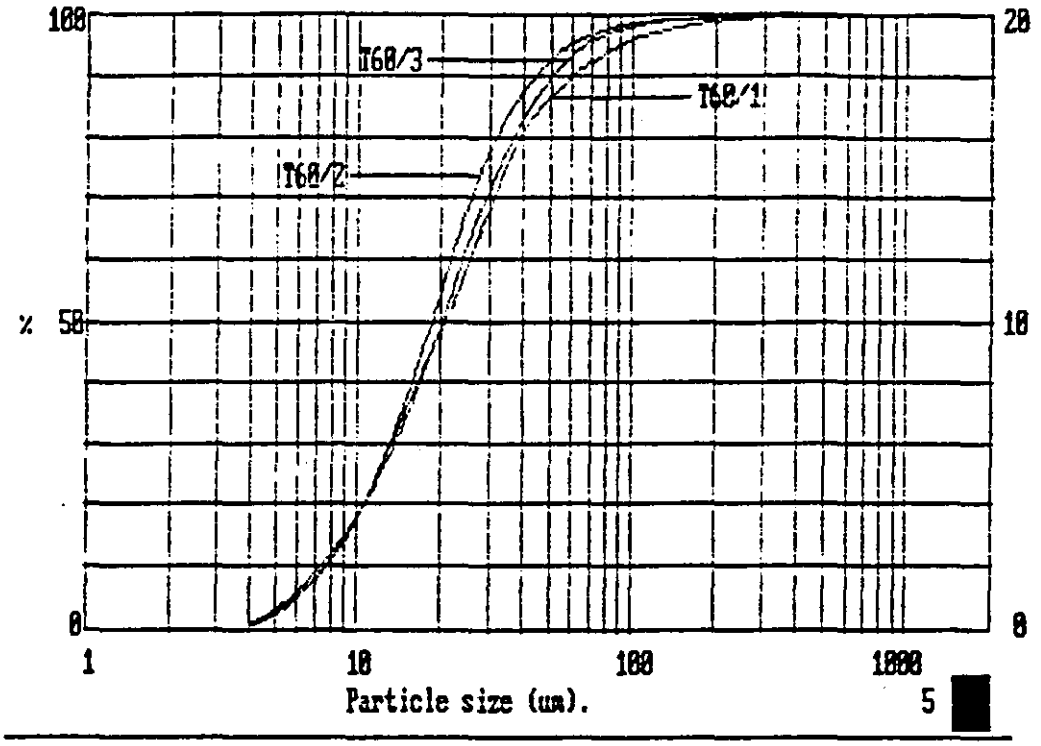
Size : microns	under	% in band	Size : microns	under	% in band
564.0	100.0	0.0	53.1	92.5	5.7
487.0	100.0	0.0	45.8	89.8	3.7
420.0	99.9	0.1	39.5	86.1	4.9
362.0	99.9	0.1	34.1	81.2	6.3
312.0	99.8	0.1	29.4	74.9	7.4
270.0	99.6	0.2	25.4	67.5	8.3
233.0	99.5	0.2	21.9	59.2	6.4
201.0	99.3	0.2	18.9	52.8	7.9
173.0	99.1	0.2	16.3	45.0	7.2
149.0	98.8	0.3	14.1	38.8	7.2
129.0	98.6	0.4	12.1	32.6	6.4
111.0	98.2	0.6	10.5	22.2	5.9
95.9	97.6	0.6	9.0	15.4	6.5
82.7	96.9	1.0	7.8	11.6	3.9
71.4	95.9	1.5	6.7	7.8	3.0

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Beam length = 14.3 mm.
Obscuration =0.2439
Volume Conc. = 0.0092 %
Log. Diff. =3.45
Model indp

D(v,0.5) = 16.6 um
D(v,0.9) = 45.1 um
D(v,0.1) = 7.3 um
D(4,3) = 22.9 um
D(3,2) = 14.2 um
Span = 2.1
Spec. surf. area

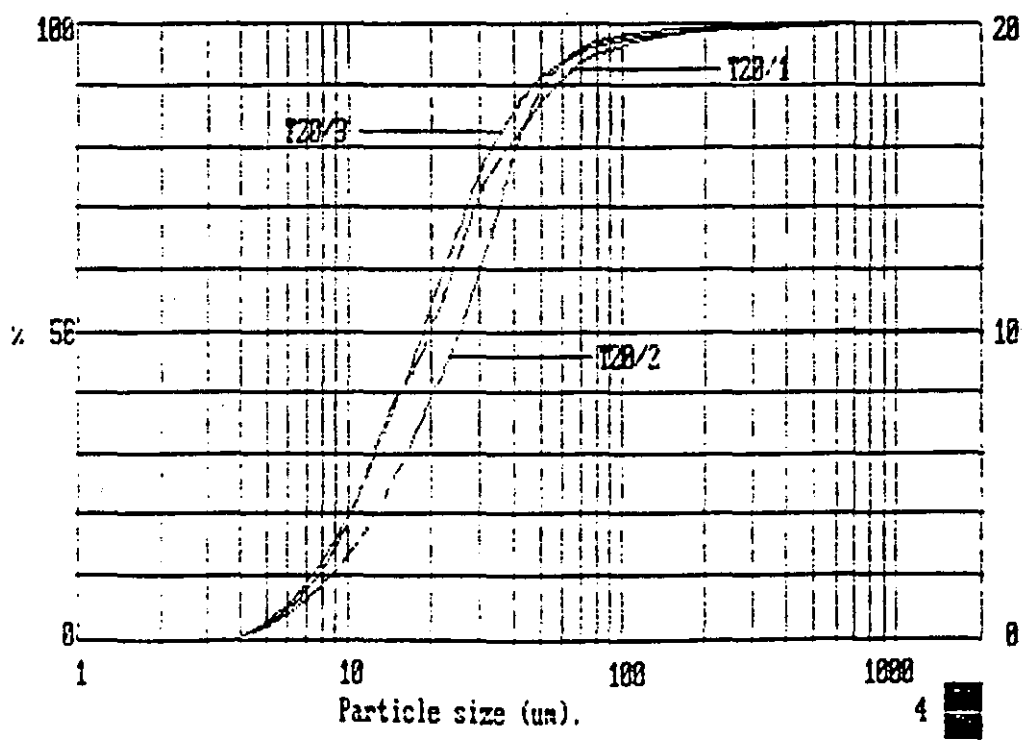
2.3 COMPILED T60 and T20 FPH SAMPLES

Malvern Instruments MASTER Particle Sizer M6.02 Date 17-07-89 Time 16-34



System number 1988 Diode CT1306

Malvern Instruments MASTER Particle Sizer M6.02 Date 21-07-89 Time 16-43



System number 1988 Diode CT1306

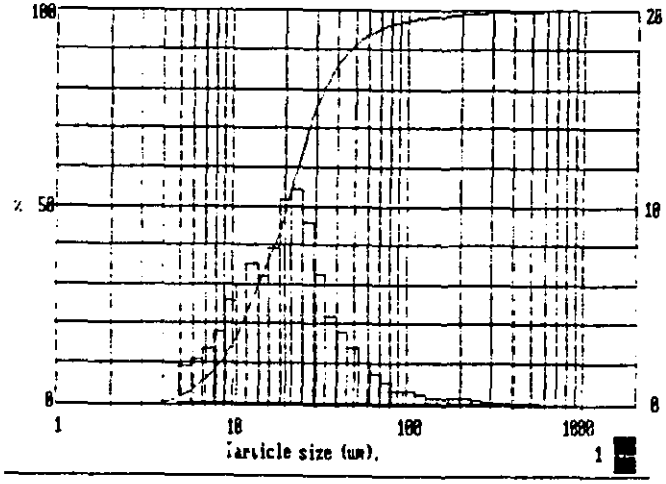
2.4 INDIVIDUAL B60 FPH SAMPLES

MALVERN Instruments M6.02 Date 21-07-1989 Time 18:58

CLIENT : Food Lab
SAMPLE : Fph (B60/1)
LAB REF: 89/196

System number 1966 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 21-07-89 Time 19-51



Size microns	% under	% in band	Size microns	% under	% in band
564.	100.	0.	53.	92.	2.6
467.	100.	0.	45.6	89.4	3.0
420.	100.	0.	39.5	85.8	4.4
362.	100.	0.	34.1	81.4	6.5
312.	100.	0.	29.4	74.9	9.6
270.	99.	0.	25.4	65.7	13.
233.	99.	0.	21.9	54.6	16.8
201.	99.	0.	18.9	44.5	7.9
173.	98.	0.	16.3	36.6	6.5
149.	98.	0.	14.1	30.1	5.1
129.	98.	0.	12.	25.0	6.
111.	97.	0.	10.	19.5	5.
95.	97.	0.	9.	14.5	3.
82.	96.	0.	7.	11.6	3.6
71.	96.	0.	6.	8.1	0.7

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Beam length = 14.3 mm.
Obscuration =0.3446
Volume Conc. = 0.0153 %
Log. Diff. =3.35
Model indp

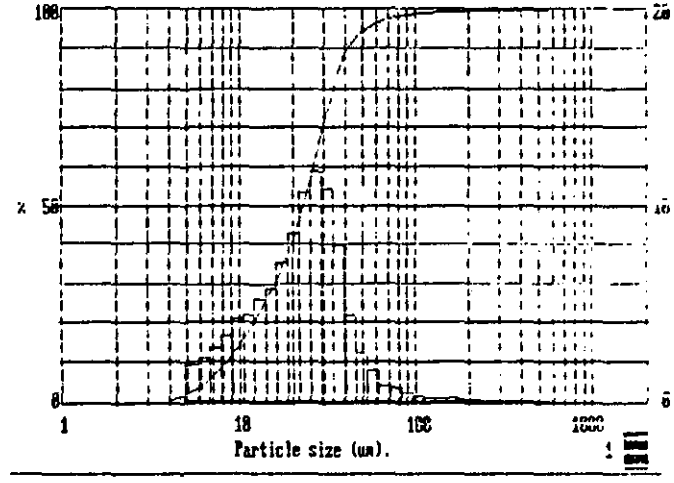
Div,0.5) = 26.5 um
Div,0.9) = 47. um
Div,0.1) = 8.5 um
D(4,3) = 24. um
D(3,2) = 16.6 um
Span = 1.9

MALVERN Instruments M6.02 Date 14-07-1989 Time 15:16

CLIENT : Food Lab
SAMPLE : Fph (B60/2)
LAB REF: 89/197

System number 1966 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 13-07-89 Time 15-17



Size microns	% under	% in band	Size microns	% under	% in band
564.0	100.0	0.0	53.1	55.	2.5
467.0	100.0	0.0	45.6	52.	3.4
420.0	100.	0.0	39.5	48.	4.
362.0	100.0	0.0	34.1	44.	10.5
312.0	100.	0.0	29.4	33.	11.5
270.0	100.0	0.0	25.4	21.	10.7
233.0	100.0	0.1	21.9	10.	9.0
201.0	99.9	0.1	18.9	1.	7.1
173.0	99.8	0.	16.	3.	5.
149.	99.	0.	14.	2.	4.
129.	99.	0.	12.	2.	4.
111.	99.	0.	10.	1.	4.
95.	99.	0.	9.	1.	3.
82.	99.	0.	7.	1.	2.
71.	97.	0.	6.	1.	2.

Result source= Averaged
Record No. = 0
Focal length = 300 mm.
Experiment type pil
Volume distribution
Beam length = 14.3 mm.
Obscuration =0.3460
Volume Conc. = 0.0155 %
Log. Diff. =3.35
Model indp

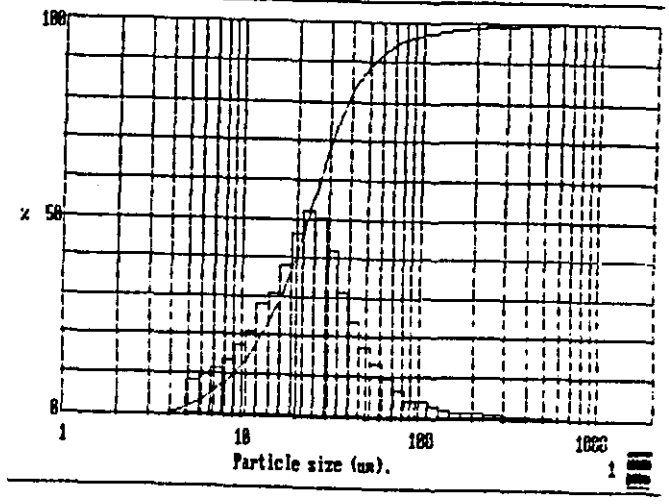
Div,0.5) = 25.0 um
Div,0.9) = 47. um
Div,0.1) = 8.5 um
D(4,3) = 24. um
D(3,2) = 16.6 um
Span = 1.9

MALVERN Instruments M6.02 Date 06-07-1989

CLIENT : Food Lab.
SAMPLE : FPH (B60/3)
LAB REF: 89/198

System number 1988 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.02 Date 06-07-89 Time 11-38



Size microns	% under	% in band	Size microns	% under	% in band
564.0	100.0	0.0	53.1	90.2	3.6
467.0	100.0	0.0	45.6	86.7	4.6
420.0	100.0	0.1	39.5	81.8	6.3
362.0	99.9	0.1	34.1	75.5	8.4
312.0	99.8	0.1	29.4	67.1	10.1
270.0	99.7	0.2	25.4	57.0	10.6
233.0	99.5	0.3	21.9	46.4	9.3
201.0	99.2	0.3	18.9	37.	7.6
173.0	98.9	0.3	16.3	29.5	6.2
149.0	98.6	0.3	14.1	23.5	5.6
129.0	98.3	0.3	12.1	17.9	4.9
111.0	97.8	0.5	10.5	13.0	3.4
95.9	97.0	0.8	9.0	10.1	2.5
82.7	96.1	1.0	7.8	7.4	2.1
71.4	94.9	1.2	6.7	5.0	2.0

Result source= Averaged
Record No. = 7
Focal length = 300 mm.
Experiment type pil
Volume distributio
Beam length = 14.3 mm.
Obscuration =0.3060
Volume Conc. = 0.0145 %
Log. Diff. =2.70
Model indp

Div,0.5) = 25.0 um
Div,0.9) = 52.5 um
Div,0.1) = 9.0 um
D(4,3) = 27.0 um
D(3,2) = 18.6 um
Span = 1.9
Spec. surf. area

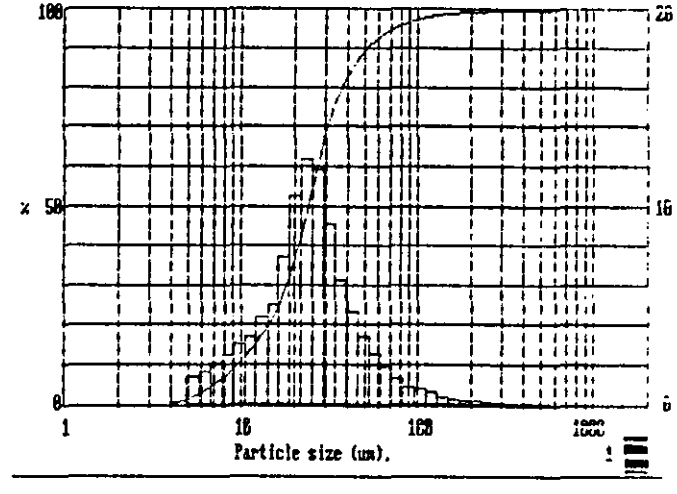
2.5 INDIVIDUAL B20 FPH SAMPLES

MALVERN Instruments M6.82 Date 18-07-1989 Time 13:51

CLIENT : Feed Lab
SAMPLE : FPH (B20/2)
LAB REF: 89/194

System number 1986 Diode CT1306

Malvern Instruments EASY Particle Sizer M6.82 Date 18-07-89 Time 13:51



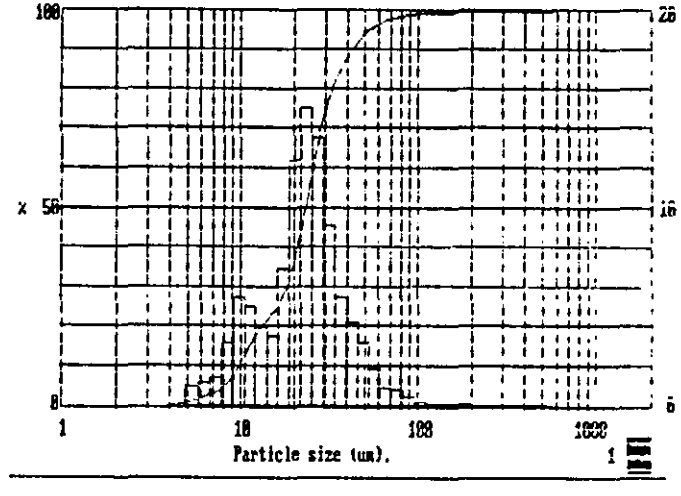
Size : microns : under	% in band:	Size : microns : under	% in band:	Result source= Averaged
564.0 : 100.0	0.0 :	53.1 : 90.6	3.9 :	Record No. = 0
487.0 : 100.0	0.0 :	45.8 : 87.3	4.7 :	Focal length = 300 mm.
420.0 : 100.0	0.0 :	39.5 : 82.7	6.5 :	Experiment type pil
362.0 : 100.0	0.0 :	34.1 : 76.4	9.1 :	Value distribution
312.0 : 100.0	0.0 :	29.4 : 67.3	11.8 :	Mean length = 14.3 mm.
270.0 : 99.9	0.1 :	25.4 : 55.6	12.8 :	Obscuration =0.2408
233.0 : 99.8	0.2 :	21.9 : 43.0	10.5 :	Volume Conc. = 0.0118 %
201.0 : 99.7	0.2 :	18.9 : 32.5	7.4 :	Log. Diff. =3.90
173.0 : 99.4	0.3 :	16.3 : 25.0	5.1 :	Model indp
149.6 : 99.2	0.4 :	14.1 : 19.9	4.4 :	Div.0.5 = 23.6 um
129.0 : 98.8	0.6 :	12.1 : 15.5	3.5 :	Div.0.9 = 51.2 um
111.0 : 98.2	0.8 :	10.5 : 12.0	3.1 :	Div.0.1 = 9.5 um
95.5 : 97.4	0.9 :	9.0 : 8.9	2.5 :	Div.Sr = 27.6 um
82.7 : 96.5	1.3 :	7.8 : 6.4	2.1 :	Div.2 = 15.5 um
71.2 : 95.2	1.3 :	6.7 : 4.3	1.6 :	Span = 1.7
61.3 : 93.9	1.3 :	5.8 : 2.7	1.1 :	Conc. corr. appl.
52.8 : 92.6	1.3 :	5.0 : 1.6	0.7 :	

MALVERN Instruments M6.82 Date 18-07-1989 Time 15:02

CLIENT : Feed Lab
SAMPLE : FPH (B20/3)
LAB REF: 89/195

System number 1988 Diode CT1306

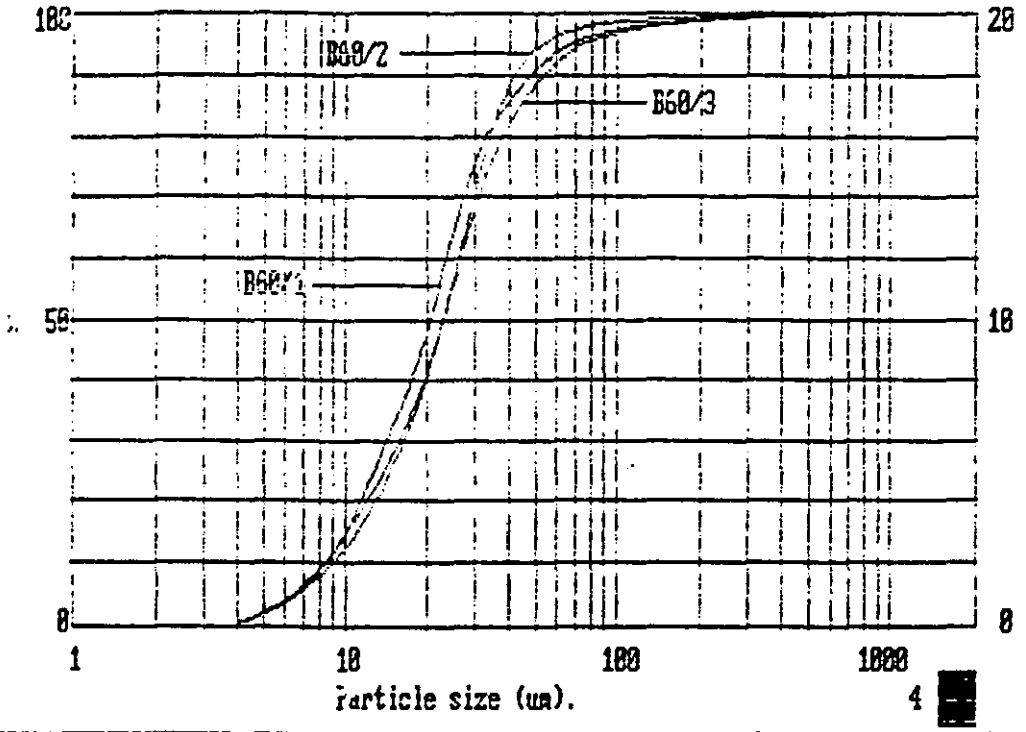
Malvern Instruments EASY Particle Sizer M6.82 Date 18-07-89 Time 15:03



Size : microns : under	% in band:	Size : microns : under	% in band:	Result source= Averaged
564.0 : 100.0	0.0 :	53.1 : 95.3	3.1 :	Record No. = 15
487.0 : 100.0	0.0 :	45.8 : 92.1	4.3 :	Focal length = 300 mm.
420.0 : 100.0	0.0 :	39.5 : 87.5	5.5 :	Experiment type pil
362.0 : 100.0	0.0 :	34.1 : 82.4	7.4 :	Value distribution
312.0 : 100.0	0.0 :	29.4 : 73.2	13.0 :	Mean length = 14.5 mm.
270.0 : 100.0	0.0 :	25.4 : 59.6	15.0 :	Obscuration =0.3201
233.0 : 100.0	0.0 :	21.9 : 44.6	12.4 :	Volume Conc. = 0.0164 %
201.0 : 100.0	0.1 :	18.9 : 32.2	9.9 :	Log. Diff. =3.41
173.0 : 99.9	0.1 :	16.3 : 23.3	5.6 :	Model indp
149.6 : 99.8	0.1 :	14.1 : 17.8	4.0 :	Div.0.5 = 22.1 um
129.0 : 99.7	0.1 :	12.1 : 13.8	3.1 :	Div.0.9 = 49.3 um
111.0 : 99.6	0.2 :	10.5 : 10.7	2.5 :	Div.0.1 = 9.0 um
95.5 : 99.4	0.5 :	9.0 : 8.2	2.1 :	Div.Sr = 24.5 um
82.7 : 98.9	0.5 :	7.8 : 6.1	1.6 :	Div.2 = 14.6 um
71.2 : 98.4	0.5 :	6.7 : 4.5	1.1 :	Span = 1.6
61.3 : 97.9	0.5 :	5.8 : 3.4	0.7 :	Conc. corr. appl.
52.8 : 97.4	0.5 :	5.0 : 2.7	0.5 :	

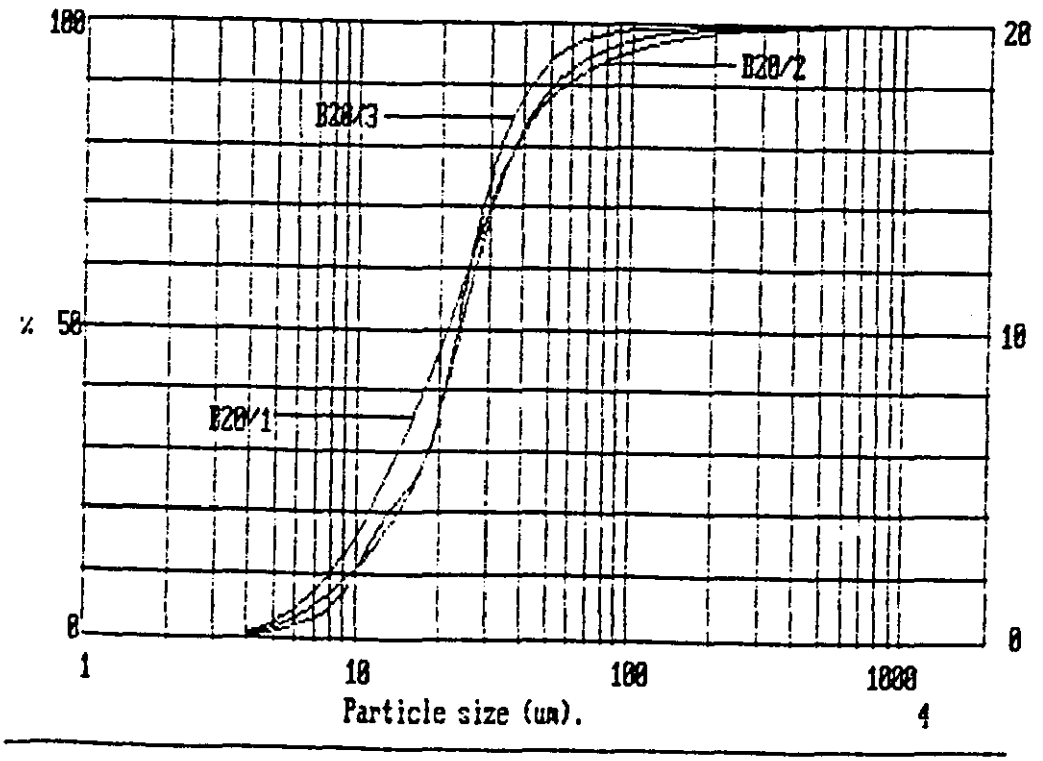
2.6 COMPILED B60 and T20 FPH SAMPLES

Malvern Instruments MASTER Particle Sizer M5.02 Date 21-07-89 Time 14-16



System number 1988 Diode CT1386

Malvern Instruments MASTER Particle Sizer M5.02 Date 17-07-89 Time 16-55



System number 1988 Diode CT1386

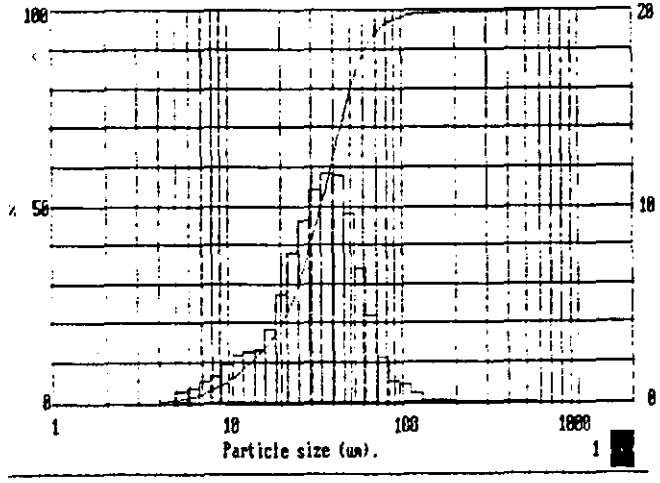
2.7 CONTROLS EA AND ISB

MALVERN Instruments Mk.82 Date 25-07-1989 Time 16:35

CLIENT : Food Lab
SAMPLE : CONTROL (EGG ALBUMEN)
LAB REF: 89/208

System number 1986 Diode CT1306

Malvern Instruments EASY Particle Sizer Mk.82 Date 25-07-89 Time 16-36



Size : microns :	under	% : in band:	Size : microns :	under	% : in band:
[illegible]	100.0	0.0	53.1	83.7	9.7
[illegible]	100.0	0.0	45.8	74.0	11.5
[illegible]	100.0	0.0	39.5	62.4	11.6
[illegible]	100.0	0.0	34.1	50.8	10.7
[illegible]	100.0	0.0	29.4	39.9	9.7
[illegible]	100.0	0.0	25.4	30.5	7.6
[illegible]	100.0	0.0	21.9	22.9	5.9
[illegible]	100.0	0.0	18.9	17.4	3.7
[illegible]	[illegible]	[illegible]	16.3	13.7	[illegible]
[illegible]	[illegible]	[illegible]	14.1	11.5	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	8.4	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	6.0	[illegible]
[illegible]	[illegible]	[illegible]	9.0	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	7.8	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	6.7	[illegible]	[illegible]
[illegible]	97.	0.0	5.8	[illegible]	[illegible]

Result source= 89_208
Record No. = 7
Focal length = 300 mm.
Experiment type pi
Volume distribution
Beam length = 14.5 mm.
Obscuration =0.000
Volume Conc. = 0.0000 %
Log. Diff. =0.00
Model indp

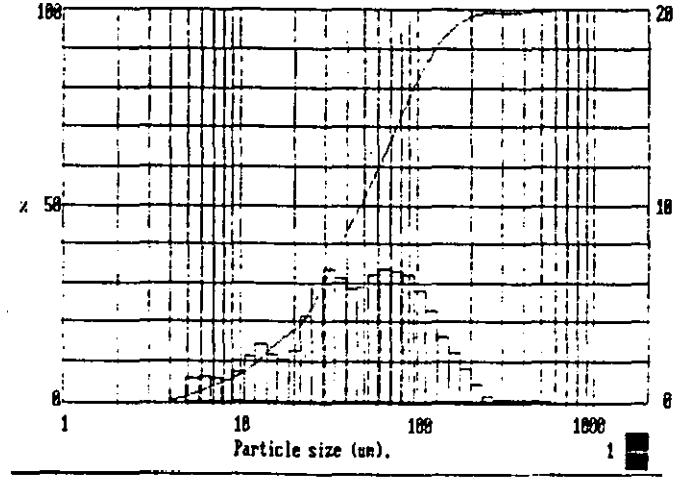
D(v,0.5) = 35.6 um
D(v,0.9) = 84.[illegible] um
D(v,0.1) = 12.5 um
D(4,3) = 55.0 um
D(3,2) = 27.1 um
Span = 1.[illegible]
Spec. surf. area
0.2433 sq.m./cc.

MALVERN Instruments Mk.82 Date 25-07-1989 Time 16:43

CLIENT : Food Lab
SAMPLE : CONTROL (ISOLATE SOY BEAN
LAB REF: 89/209

System number 1986 Diode CT1306

Malvern Instruments EASY Particle Sizer Mk.82 Date 25-07-89 Time 16-43



Size : microns :	under	% : in band:	Size : microns :	under	% : in band:
564.0	100.0	0.0	53.1	54.7	5.9
487.0	100.	0.1	45.8	48.8	5.7
420.0	99.9	0.1	39.5	43.1	6.3
362.0	99.9	0.1	34.1	36.6	6.7
312.0	99.9	0.0	29.4	30.[illegible]	6.1
270.0	99.7	0.3	25.4	24.[illegible]	6.2
233.0	99.5	0.3	21.9	19.7	4.8
201.0	98.6	1.0	18.9	17.1	2.1
173.0	9[illegible]	[illegible]	16.3	14.[illegible]	[illegible]
149.0	9[illegible]	[illegible]	14.1	12.[illegible]	[illegible]
129.0	9[illegible]	[illegible]	12.1	9.[illegible]	[illegible]
111.0	86.[illegible]	[illegible]	10.5	[illegible]	[illegible]
95.9	81.0	6.4	9.0	[illegible]	[illegible]
82.7	74.[illegible]	[illegible]	7.8	[illegible]	[illegible]
71.4	67.[illegible]	[illegible]	6.7	[illegible]	[illegible]
61.[illegible]	[illegible]	[illegible]	5.8	[illegible]	[illegible]

Result source= 89_209
Record No. = 7
Focal length = 300 mm.
Experiment type pi
Volume distribution
Beam length = 14.5 mm.
Obscuration =0.2570
Volume Conc. = 0.0190 %
Log. Diff. =3.66
Model indp

D(v,0.5) = [illegible].2 um
D(v,0.9) = [illegible].5 um
D(v,0.1) = [illegible] um
D(4,3) = 57.8 um
D(3,2) = 31.1 um
Span = [illegible]
Spec. surf. area
0.2291 sq.m./cc.

APPENDIX 3

SHEAR STRESS VS RPM OF FPH SAMPLES AND CONTROLS AT VARIOUS TEMPERATURES AND CONCENTRATIONS USING HAAKE VISCOMETER

(see Section 3.3.6)

3.1 Individual T60 FPH samples

3.2 Individual T20 FPH samples

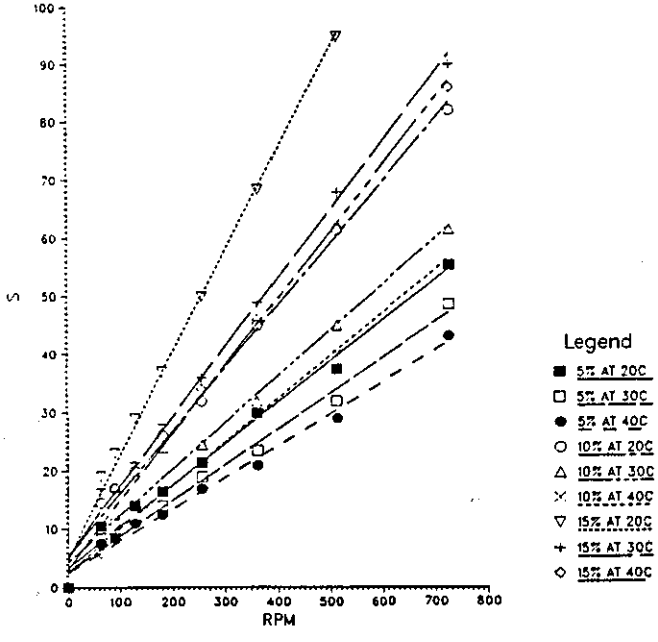
3.3 Individual B60 FPH samples

3.4 Individual B20 FPH samples

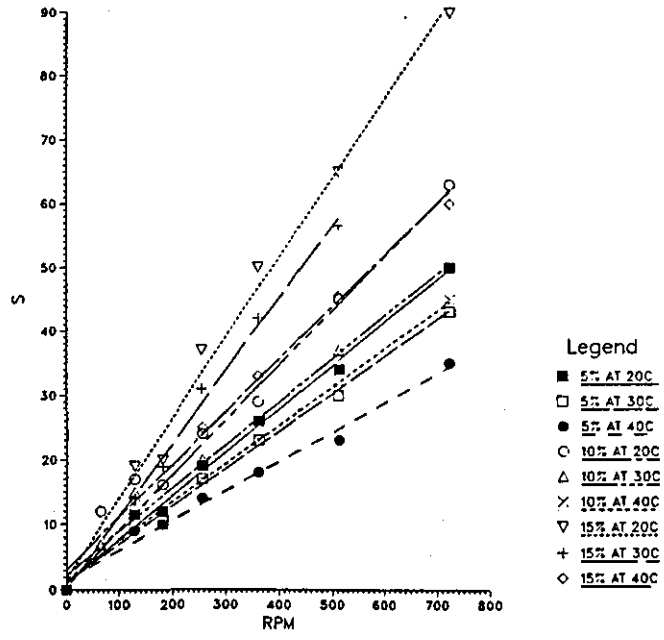
3.5 Controls EA and ISB

3.1 INDIVIDUAL T60 FPH SAMPLES

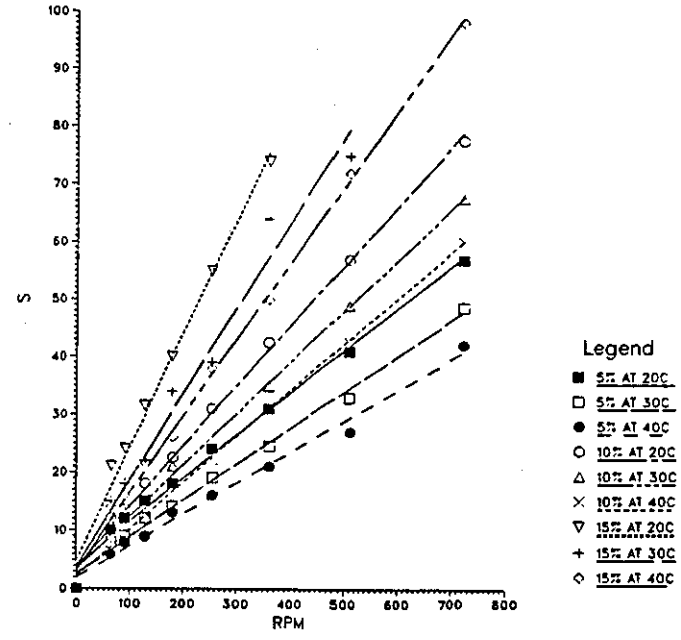
Shear Stress V RPM of T60/1 at various Temp. & Conc.



Shear Stress V RPM of T60/2 at various Temp. & Conc.

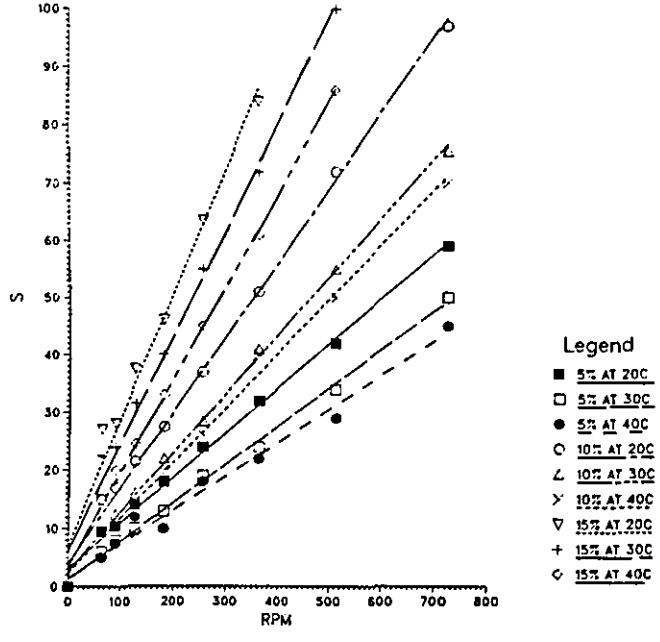


Shear Stress V RPM of T60/3 at various Temp. & Conc.

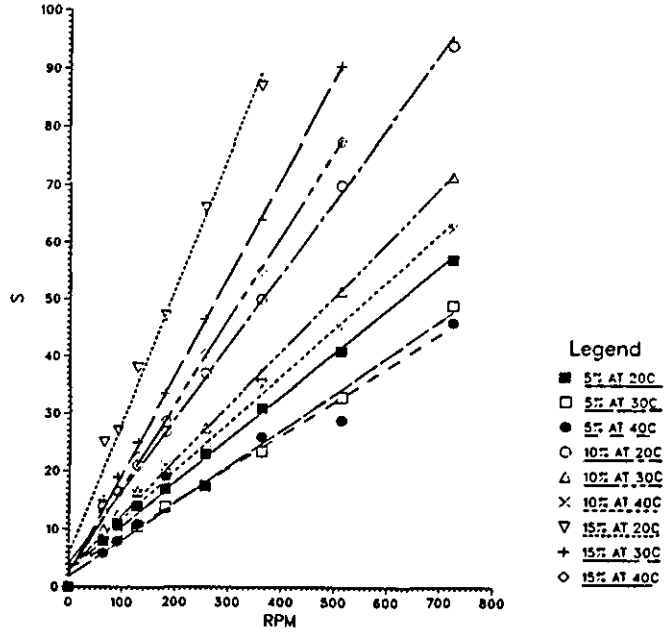


3.2 INDIVIDUAL T20 FPH SAMPLES

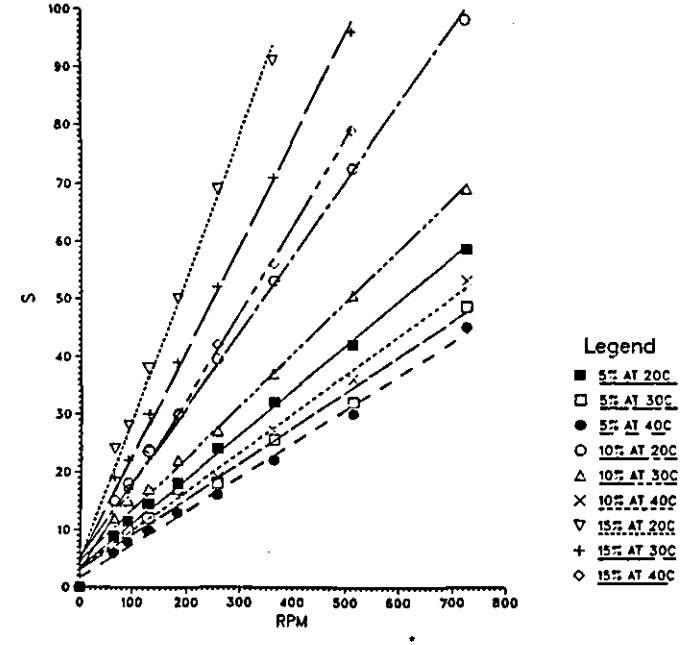
Shear Stress V RPM of T20/1 at various Temp. & Conc



Shear Stress V RPM of T20/2 at various Temp. & Conc.

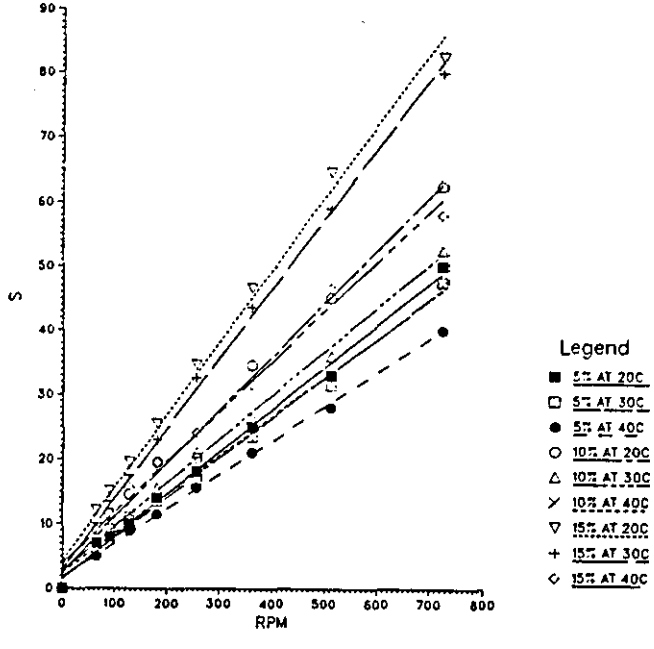


Shear Stress V RPM of T20/3 at various Temp. & Conc.

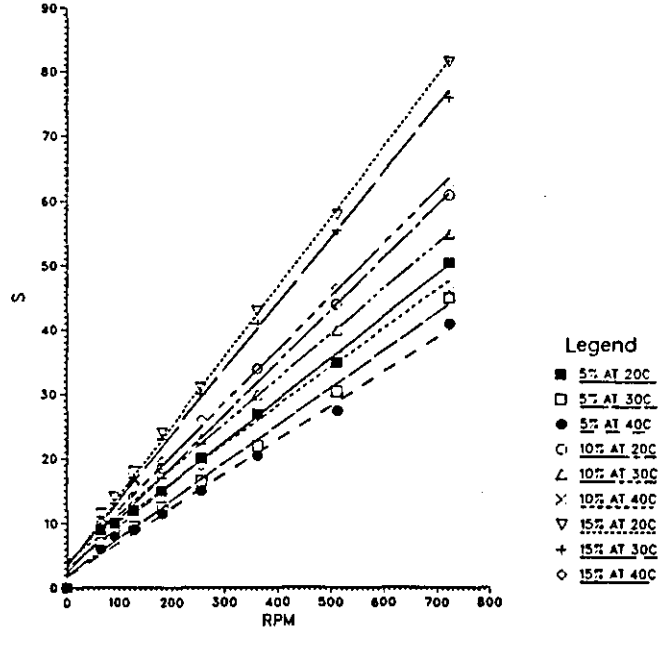


3.3 INDIVIDUAL B60 FPH SAMPLES

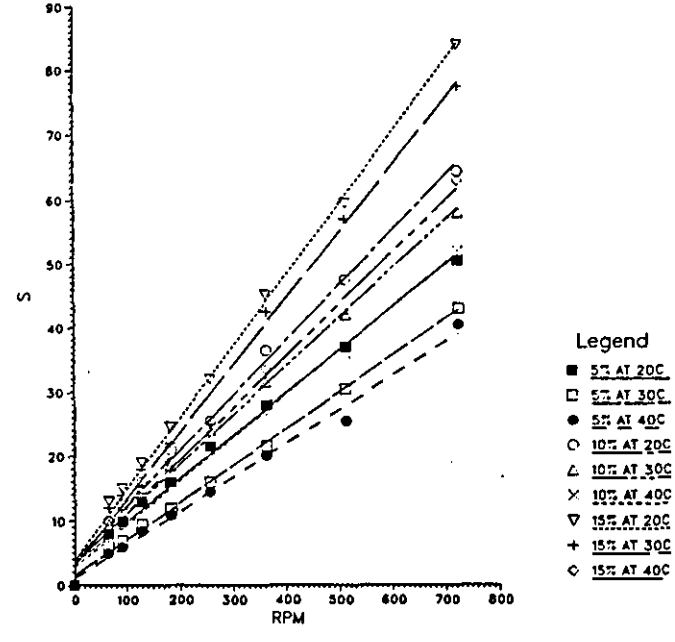
Snear Stress V RPM of B60/1 at various Temp. & Conc.



Shear Stress V RPM of B60/2 at various Temp. & Conc.

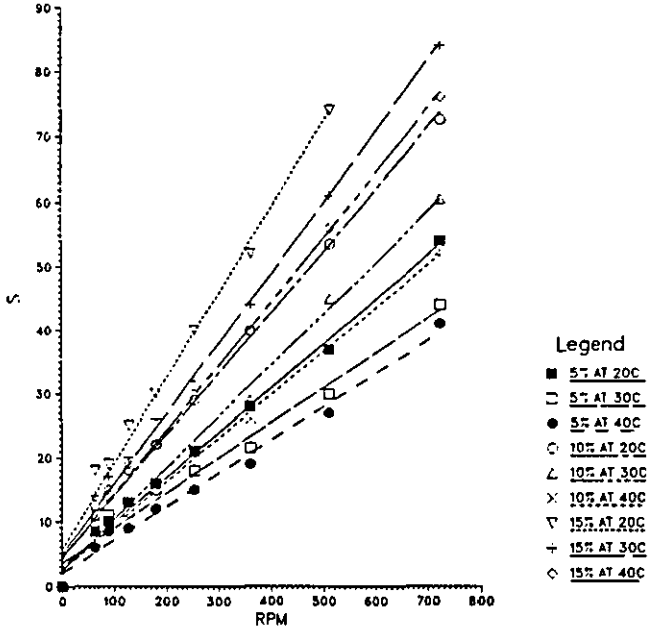


Shear Stress V RPM of B60/3 at various Temp. & Conc.

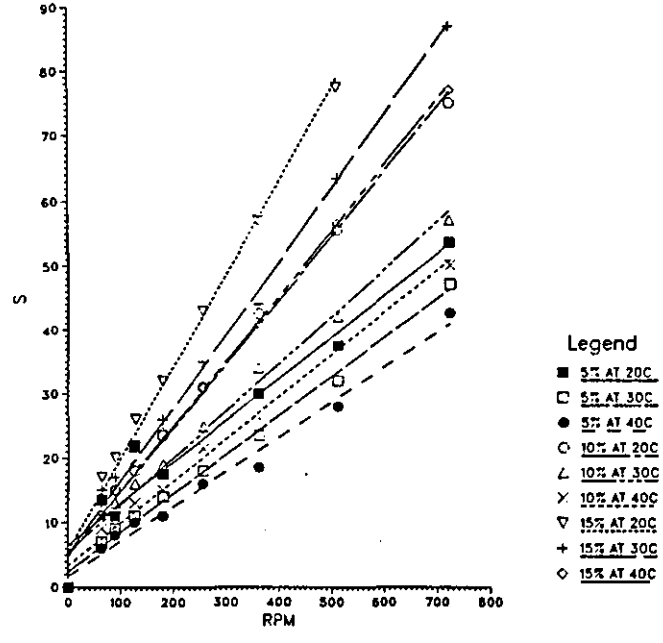


3.4 INDIVIDUAL B20 FPH SAMPLES

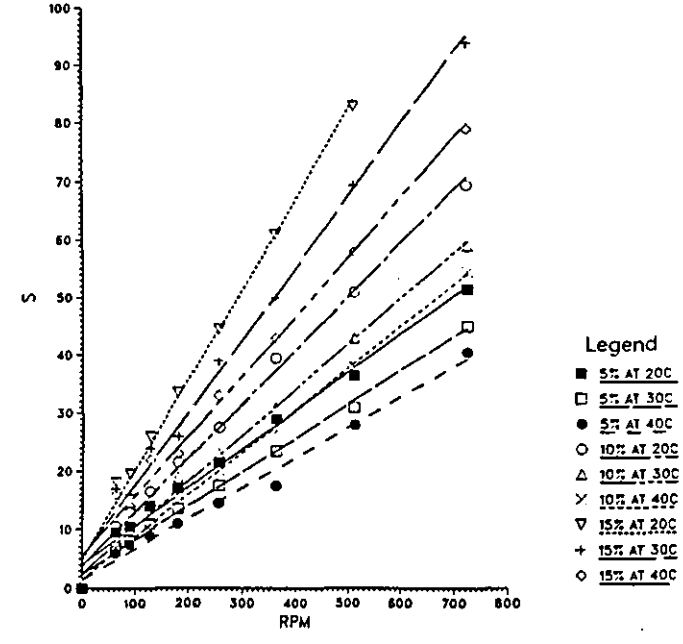
Shear Stress V RPM of B20/1 at various Temp. & Conc.



Shear Stress V RPM of B20/2 at various Temp. & Conc.

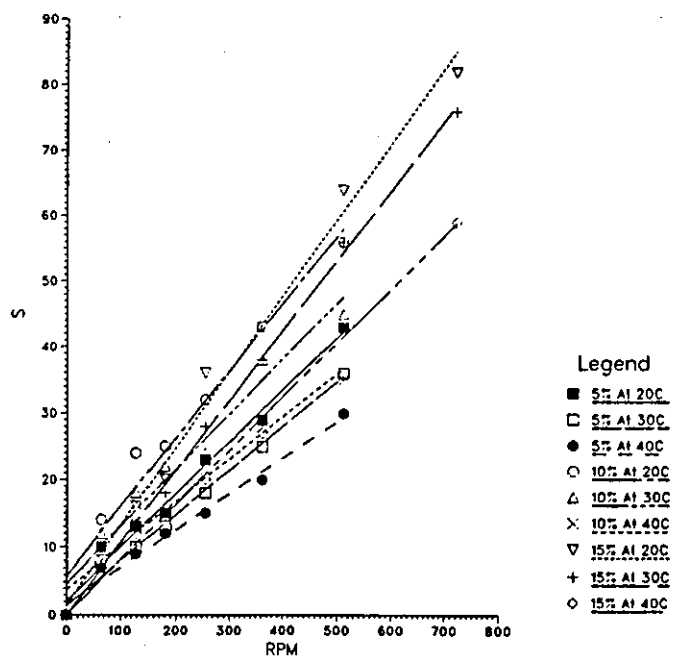


Shear Stress V RPM of B20/3 at various Temp. & Conc.



3.5 CONTROLS EA AND ISB

Shear Stress V RPM of EA at various Temp. & Conc.



Shear Stress V RPM of ISB at various Temp. & Conc.