

CHICKPEA PROTEINS FOR FOOD APPLICATIONS

A thesis

presented for the Degree of Doctor of Philosophy

by

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STATEMENT

This thesis contains no material previously submitted for a degree or diploma in any University and to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference is made in the text.



Liu Li Hui

PUBLICATIONS

Part of this work has been published in the following papers:

1. Liu, L.H.; Hung, T.V.; Black, R.G. and Trehwella, M.A. A study on solubility of grain legume proteins by Infrared Spectroscopy, *ASEAN Food J.*, 1994, 9: 24-29.
2. Hung, T.V.; Liu, L.H.; Black, R.G. and Trehwella, M.A. Water absorption in chickpea (*C. arietinum*) and field pea (*P. sativum*) cultivars using the Peleg model. 1993, *J. Food Sci.*, 58: 848-852.
3. Liu, L.H.; Hung, T.V.; Black, R.G. and Trehwella, M.A. Chickpea protein for human consumption. *Proceeding AIFST Convention*, Adelaide, May, 1993, 28.
4. Liu, L.H.; Hung, T.V.; Panozzo, J. and Black, R.G. Functional properties of native and modified chickpea proteins for food applications, *Chem. Aust.*, 1994, 506.
5. Liu, L.H.; Hung, T.V.; Panozzo, J., Black, R.G. and Trehwella, M.A. Rheological characteristics of wheat doughs supplemented with soy, native and modified chickpea proteins, *Proceedings of the 44th Australian Cereal Conference*, Ballarat, Sept., 1994, 228-235.
6. Liu, L.H.; Hung, T.V. and Panozzo, J. Pasting characteristics of wheat flour supplemented with chickpea proteins, *Australian Cereal Conference*, Adelaide, Oct., 1995.
7. Liu, L.H.; Hung, T.V.; Panozzo, J. and Bason, M. Factors affecting the viscosity of wheat flour pastes, supplemented with non wheat proteins, *Handbook, 46th Australian Cereal Conference*, Sydney, 1996, 115.

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SUMMARY

The potential applications of chickpea proteins (*Cicer arietinum L.* cv Kaniva) as food ingredients have been investigated and presented in three major parts of this thesis.

The first part (chapter 3) identified different extraction conditions and their effects on the extraction yield and characteristics of the isolated proteins.

The water absorption of chickpea seeds was defined. Chickpea protein extractabilities were basically pH dependent. The combined effects of several factors affecting the extraction yield were examined in forty eight different extractions. Extraction pH and particle size of the grits were the most significant factors but extraction time and temperature had only a minor effect. Salt solutions did not improve the extraction yield. Extraction under different conditions gave different extraction yields and different types of proteins. Gel filtration patterns, electrophoretic and densitometric analysis and enzymatic *in vitro* digestion studies indicated that the water extracted proteins were characteristically more similar to those extracted at alkaline pH than proteins extracted at acidic pH.

The relative amount of albumin and globulin in chickpea depended on extraction conditions and on variety. Fractionation with K_2SO_4 solutions produced highly purified albumins. Depending on the procedure employed, albumin (>30% of the total protein) could be a major protein fraction. This casts doubt on the perception that the albumin fraction of chickpea only accounted for a minor proportion (10%) of the total proteins.

The structural differences of Kabuli and Desi chickpea albumins and globulins were identified. The albumins were comprised of subunits with high molecular weight while the globulin contained of low molecular weight subunits. Also, the level of essential amino acids in albumin was always higher than that of globulin. Globulins prepared under different conditions possessed a similar structure, but their *in vitro* digestibility was different, reflecting the effect of processing conditions on their functional performance. Several pilot scale extractions (500 kg) were conducted with commercial equipment, based on the concepts developed in the laboratory and the isolated proteins were used for further investigations.

The second part (chapter 4) defined the functional properties of these isolated proteins and relevant factors affecting their behaviour.

Extraction at different pHs affected the functional properties of chickpea proteins such as solubility, oil and water absorption, emulsifying capacity and stability were more soluble and also absorbed more oil and water than . These proteins were more soluble than soy protein isolate at certain pHs. Emulsion capacity of three chickpea protein isolates showed a good relationship between their solubility and emulsion capacity. Their pH/emulsion capacity curves resembled those of their pH/solubility. Alkaline and water extracted proteins were more sensitive to heat treatment than acidic extracted proteins. Salt could decrease or increase the emulsion capacity of alkaline and acidic extracted proteins. With salt addition, all chickpea protein isolates were less stable than soy protein isolate but their foaming capacity was higher than that of soy protein isolate.

To measure protein contents of chickpea and other grain legumes in aqueous solutions, a quick, non-destructive and easy to perform procedure was successfully developed by using infrared spectroscopy. The different response of the three chickpea protein isolates toward infrared measurement reflected their different amino acid composition and conformation, due to the effects of different extraction conditions.

The third part (chapter 5, 6 and 7) investigated the effects of chemical and enzymatic modifications on the composition, structural characteristics, functional and flow properties of the modified proteins, particularly, their rheological properties in wheat flour dough systems.

Chickpea proteins were acylated by succinic and acetic anhydride and hydrolysed by papain and trypsin. The levels of acylation varied with the type of reagent, its concentration and also types of proteins involved. Acylation did not significantly alter the gel filtration profile of the modified proteins but the SDS-PAGE patterns showed their electrophoretic mobility was retarded. Densitometric scanning of SDS-PAGE confirmed some structural changes occurred in chickpea proteins due to protein aggregations. Acetylation slightly reduced the lysine but improved the *in vitro* digestibility of the modified proteins by papain, pepsin and chymotrypsin while succinylation only improved the pepsin protein hydrolysate.

Acetylation improved the solubility of chickpea proteins at high alkaline pHs and their water and oil absorption capacities. The emulsion capacity of acetylated chickpea proteins was higher than that of the native protein but these emulsions were less stable than those of unmodified chickpea proteins. Compared with the unmodified proteins, succinylated proteins were more soluble at pHs above its pI and less soluble at pHs below its pI (pH 4.8). Dilute salt solutions (0.2M NaCl) depressed the solubility of all succinylated proteins and shifted its pI to pH 4.0. Succinylation greatly improved their water and oil absorption, making them greater than those of unmodified and acetylated proteins. Similarly, succinylation also greatly

improved their emulsifying capacities and stabilities. The different extent of succinylation showed very moderate influence on these properties.

Chickpea protein dispersions possessed Newtonian flow behaviour at low protein concentrations (<4%). At high concentrations (>8%), non-Newtonian flow behaviour became more progressive with increasing concentration. The increasing non Newtonian behaviour followed the order of unmodified, acetylated and succinylated proteins.

The flow behaviour of chickpea protein dispersions varied with salt concentration, type of proteins and extent of acylation. The Newtonian flow behaviour of unmodified chickpea protein dispersions remained intact only in a dilute salt solution (0.2M NaCl) but their flow behaviour index decreased with salt concentrations up to 1M then increased. In contrast, the flow behaviour of succinylated protein dispersions increased proportionally with increasing salt concentrations. The effect of salt addition on the flow behaviour index of acetylated proteins varied greatly with the extent of acetylation. The remarkable effect of salt addition, even at a very low concentration (0.2M), on the consistency coefficient (m), Casson yield stress and apparent viscosities of the acylated proteins reflected the structural changes. The effect of dissolving pHs reflected a correlation between protein solubilities and the apparent viscosities.

For unmodified proteins, the effects of thermal treatments were demonstrated at 55°C. For acetylated proteins, the flow index of each protein dispersion was affected differently by a similar thermal treatment. Apparent viscosities of unmodified protein dispersions increased but those of all acetylated protein dispersions decreased with increasing temperature. The apparent viscosities of succinylated proteins increased at 55°C. An equation to illustrate the effect of temperature on the apparent viscosities and degrees of succinylation was suggested .

The behaviour of unmodified and modified chickpea proteins as food ingredients in fortified wheat flour doughs was evaluated, using farinograph, extensigraph and RVA measurements. Final quality of the resulting breads from these fortified doughs was also studied. The study provided a useful means to investigate the rheological properties of wheat flour dough and the structural changes of the modified chickpea proteins.

Chickpea proteins influenced the fortified breads in terms of loaf volume and texture. The effects depended on the type of protein used and the level of supplementation. Native chickpea protein or enzymatic hydrolysates showed a very mild reduction in the quality of their fortified breads and can be compared favourably with soy protein as a source of protein enrichment for bakery products.

CHAPTER 1

FOOD PROTEIN - GENERAL INTRODUCTION

FOOD PROTEIN - GENERAL INTRODUCTION

1.1 FOOD PROTEINS AND THE BODY'S NEEDS

1.1.1 PROTEINS AND ESSENTIAL AMINO ACIDS

Proteins are a constituent of every living cell and are essential to life as a vital part of the nucleus and protoplasm. Proteins combine with nucleic acids to form nucleoproteins in the cell nucleus which are responsible for the transmission of genetic information. As enzymes or some parts of many hormones, proteins are essential in the regulation of body processes. Proteins are important for the growth and repair of body tissues and can also be used as a source of energy.

Proteins are continuously degraded and resynthesised to fulfil the body's needs for growth, maintenance and function. Proteins are long chains of amino acids linked by peptide bonds. Amino acids are the basic structural units from which proteins are synthesised and into which they are degraded. Proteins can be synthesised by plant and animal cells.

Based on functional and nutritional basis, amino acids are classified into two groups: essential and non essential. An amino acid that cannot be synthesised by the body *at a rate sufficient to* meet the needs for growth and maintenance is defined as an essential amino acid and must be provided by the diet. Nine essential amino acids (EAA) cannot be synthesised by humans. Consequently, the ultimate value of a food protein lies in its amino acid composition and proteins in foods are evaluated by their amino acid profiles.

1.1.2 PROTEIN REQUIREMENT

In 1992, sufficient food was available to feed all human being with a diet of 9,823 kJ /day. The body needs amino acids, nitrogen, and organic acids rather than protein *per se* to synthesise its own required proteins. Consequently the term protein requirement does not strictly reflect the body requirement for proteins (Harper, 1977; William *et al.*, 1974). Nevertheless, this term has been widely used. FAO (Food and Agriculture Organisation, 1973) as well as the Food and Nutrition Board of the National Research Council in the USA (1980) recommended a safe level of protein intake to meet all physiological needs of healthy

individuals. The adequate level of protein requirement depends on age, sex, activities and special needs such as pregnancy or lactation (Table 1.1).

For the infants and children with rapid increase in body weight, protein intake is recommended at a higher rate per unit of body weight (2-2.2g / kg of body weight). Additional amount of protein is also suggested for pregnant women and lactating mothers.

Table 1.1 Recommended protein levels for different groups (g) by WHO and USA standard (g / kg body / day)

Group	Age (Year)	USA. NRC ^a	WHO ^b
Infant	0 - 1	2.0 - 2.2	1.53
Children	1 - 10	1.8 - 1.2	1.2 - 0.9
Male	11 - 14	1.0	0.81
	14 - 50+	0.8	0.60
Female ^c	11 - 14	1.0	0.76
	15 - 50+	0.8	0.63

^a National Research Council, Food and Nutrition Board, National Academy of Sciences, Washington DC, 1980.

^b FAO/WHO Expert group. Protein requirements FAO, Nutrition Meeting Report Series, 52, Rome, 1973.

^c Pregnant and lactating. additional 30 g and 20 g per day respectively (NRC, 1980).

1.2 PROTEIN CLASSIFICATION

Protein can be classified by many different criteria. *Complete proteins* are proteins containing all essential amino acids capable of growth when used as a sole source of protein in the diet. In contrast, *incomplete proteins* are those which lack or have limiting amounts of one or more essential amino acids.

Proteins can also be classified by their source of origin. Egg, meat, fish, and milk provide major *animal proteins* while cereal, oilseed and grain legumes are major sources of *vegetable or plant proteins*. Animal and vegetable proteins have been used for human consumption and animal feed for centuries. In addition to these *conventional proteins*, novel sources of protein

have been extensively sought for the past twenty years. Some of these *novel proteins* were produced by microorganisms such as yeast, fungi, bacteria and algae or by leaves and grasses.

1.3 WORLD FOOD SUPPLY AND PROTEIN CONSUMPTION

1.3.1 WORLD FOOD SUPPLY

In 1992, sufficient food was produced to provide all human being with a diet of 9,823 kJ /day/person. Based on this average calorie requirement, the current food production could even support an extra 15% of the world's population (Uvin, 1994).

This estimation, however, only confirms that the world has sufficient knowledge and resources to produce enough food for the whole population, but it does not provide an equitable access to food supply. In the World Declaration on Nutrition in Rome, it is estimated that 780 million people in developing countries still do not have access to enough food to meet their basic nutritional needs (Rome, 1992). This inadequate status of food distribution is not only a serious survival problem in the developing countries, but also a social problem in affluent countries including the USA (Bistran *et al.*, 1974).

These figures reflect only the food energy but not the food quality. Although the 1992 world food supply could satisfy up to 115% of current world population, it could only provide a healthy diet for between 59 and 77% of the world population. This would be for a diet where 15-20% of the calories are derived from animal sources, including animal proteins.

1.3.2 PROTEIN CONSUMPTION AND AFFLUENCE

Global food production has greatly increased but food consumption per head in developing countries has not increased proportionately because of high birth rates. Protein consumption in the affluent world and the developing world is also markedly different. In 1980, the developing countries shared 16-20% of the world's animal protein. In contrast, people in all industrialised countries consumed about five times more animal protein per person than those of the developing countries. In North America a person consumed about 77 g of protein while some Africans had only 4-5g per day as an extreme comparison (Hill and Patterson, 1989).

In 1990, plant proteins still accounted for 80% of proteins consumed in poor countries while animal sources were the main proteins consumed in the economically developed countries (Fig 1.1). The difference in the consumption of plant and animal proteins is not only related to the stage of economic development between these countries but also to different groups within a country. Surprisingly, even in the 1980's the Kwashiorkor form of protein-energy malnutrition still occurred in the United States (Bistran *et al.*, 1974; Rossouw, 1989). Proteins from plant origin are still important sources for many low income populations.

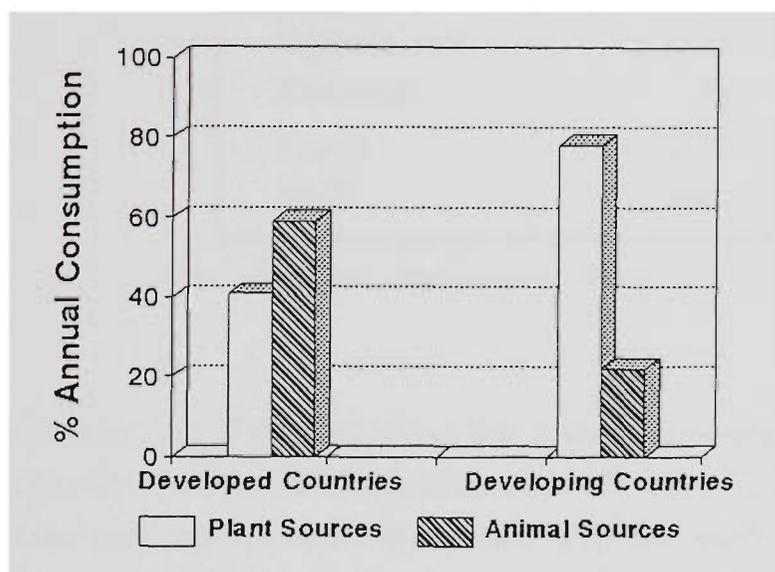


Fig. 1.1 Protein consumption in developed and developing countries.

1.4 VEGETABLE PROTEINS

1.4.1 AN ECONOMIC SOURCE

Although there are sufficient proteins to feed the world population, proteins from animal sources are still beyond the financial capacity of many millions of people. Compared with protein produced from plant sources, animal protein production requires tremendous inputs of energy, land and labour and is therefore much more expensive (Pimentel *et al.*, 1976). As shown in Table 1.2, beef protein is approximately 10 times more expensive than soy protein.

In 1975, up to 91% of total cereal, legume and plant proteins (26.5×10^6 tonnes) which could be used for human consumption was fed to livestock in the USA to produce 5.3 metric tonnes of animal proteins. The conversion rate was only 20%. Among the livestock systems, milk production is considered as the most efficient in converting plant into animal protein. A

dairy cow needs 350g of plant protein to produce 100g milk protein. In terms of energy input, even with this most efficient system, for every kjoule of milk protein produced 30 kjoule of plant protein energy are required. The ratio is about 30:1 (Pimentel and Pimentel, 1977).

Table 1.2 Price of some major commercial proteins (USD/tonne)^a

Protein	Price
Soybean meal	175 - 450.5
Fish meal	600
Casein	1,250 - 2,000
Meat	2,000 - 4,000

^a Source - Prave *et al.*, Interdisciplinary Science reviews 1980.

Christiensen (1948) pointed out that soybean produced on one acre of land in the USA could provide a person with sufficient protein for up to 2,224 days, but beef produced from the same acre could provide only 77 days of protein needs (Fig 1.2). Today, despite a remarkable improvement in livestock production, vegetables still remain an economically viable source of protein.

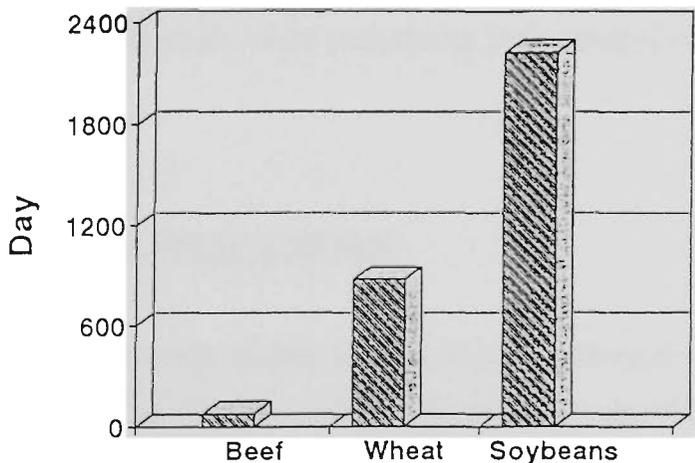


Fig. 1.2 The capacity to produce different proteins by one acre of land.

1.4.2 ANTI NUTRITIONAL FACTORS

Anti-nutritional factors associated with vegetable protein sources have affected its utilisation

for human consumption. These anti-nutritional factors fall into two major groups: protein and non protein factors (Chavan and Kadam, 1989).

Although grain legume seeds are a rich source of dietary protein, they are also a rich source of anti nutritional proteins consisting mainly of proteinase inhibitors, phytohaemagglutinins and food allergens. Proteinase inhibitors are polypeptides and proteins which inhibit proteolytic enzyme activity. Two well characterised proteinase inhibitors found in soybean are the Kunitz and Bowman-Birk inhibitor (Liener, 1976). Other inhibitors have also been isolated from field pea, winged bean, cowpea and kidney bean. Phytohaemagglutinins are carbohydrate binding proteins which can agglutinate erythrocytes. These glycoproteins are found in the seed's cotyledons of many grain legumes. They have different molecular weights and varying toxicities (Jaffe, 1980). Grain legumes are a common source of food allergens. Castor bean and cottonseed are the most highly potent allergens while peanut is the most highly allergenic grain legume consumed directly by man. In addition to these main anti nutritional proteins, lipoxygenases in soybean and other grain legumes catalyse fat oxidation to produce typical beany flavour. Myrosinase in rapeseed hydrolyses glucosinolates to harmful compounds such as isothiocyanates and oxazolidinethiones (Sosulski, 1983). Grain legume seeds also contain several non-protein anti nutritional factors. For example, the seeds of some lupin varieties have high levels of the alkaloid quinolizidines (Aquilera and Trier, 1978) and manganese (Hung *et al.*, 1986). Cottonseed contain toxic polyphenolic compound - gossypol (Jadhav *et al.*, 1989) while phytic and erucic acid in rapeseed adversely affect its utilisation (Reddy *et al.*, 1982).

However, these undesirable factors have been greatly reduced or eliminated by either successful breeding programs or by processing technologies to improve the nutritional value of vegetable proteins.

1.4.3 DIVERSE APPLICATIONS

Plant proteins are not merely an economic source of protein for low income populations, they are increasingly used as valuable ingredients in many processed food systems. Processing technology stimulated many swift and dramatic changes in the utilisation of vegetable proteins, resulting in a tremendous increase in their production and use since the end of World War II in 1945.

Traditional foods based on grain legumes, particularly soybean, had been initially limited to China and other Asian communities. However, they have currently been produced by modern technology thanks to extensive research and development efforts. UHT soy milk, long shelf

life aseptically packed tofu (a form of soy protein concentrate) and soy milk powder are available worldwide. In addition to these traditional foods, vegetable proteins have also been used as ingredients in many food systems. For example, soy proteins have been used in soups, confectionery products, simulated meats, peanut butter-like spread, high protein puddings and meaty flavoured products.

Like animal proteins, plant proteins have been used in many and diverse food applications because, like animal proteins, they can interact with other ingredients to produce desirable properties. This phenomenon is a result of extensive research activities on the functional properties of vegetable proteins and their behaviour in food systems. These successful applications have stimulated further investigations of functional properties to expand their food uses.

1.5 FUNCTIONALITY OF VEGETABLE PROTEINS

Nutritional value is the primary criterion for a new protein. However, the nutritional value is of limited use only if the protein or the food in which it is incorporated is not acceptable. The successful application of a protein as a food ingredient is greatly dependent on its critical functional characteristics or functional properties. Solubility, gelling, emulsifying and foaming properties, thermal stability and rheological properties are some functional properties which must be considered before proteins can be incorporated into foods. Functionality or functional properties of a protein are broadly defined as any physicochemical properties which affect the processing and behaviour of protein in food systems (Kinsella, 1976). These functional properties are consequences of the inherent physical and chemical properties of the protein molecules. Consequently, it is possible to predict, control and produce desirable functional properties for specific end use applications if there is sufficient understanding of these basic molecular properties.

Vegetable proteins have been successfully used as ingredients in many food systems. Therefore there is a need to investigate extensively the functionality of vegetable proteins for both academic understanding and commercial applications.

1.6 OBJECTIVES OF THIS INVESTIGATION

This thesis is a study of the food applications of proteins isolated from chickpea (*Cicer arietinum* L.). Chickpea is a protein rich grain legume which is widely grown around the world and has become an important grain legume in Victoria, Australia. The study is focussed on the preparation of chickpea protein isolates under different processing conditions followed by the characterisation of these proteins. A pilot scale production of chickpea protein isolates was conducted. The functional properties of chickpea concentrates or isolates, prepared on a laboratory and pilot scale were extensively studied. The investigation was particularly oriented towards an examination of chemical and enzymatic modification of the isolated chickpea proteins. The effects of those modifications on the functional properties of chickpea proteins and their consequent behaviours in some food systems were also investigated.

CHAPTER 2

VEGETABLE PROTEINS and PROTEIN ISOLATION LITERATURE REVIEW

VEGETABLE PROTEINS and PROTEIN ISOLATION LITERATURE REVIEW

As a main source of protein, grain legumes and oil seeds have played an important role in human nutrition in China and other Asian countries. Cereals are widely used for bread and bakery products but have a low level of protein. Pulses, however, not only have higher protein content but can also be used for various applications. Since the nutritional and economic values of grain legume proteins and particularly of soybean proteins were realised, extensive investigations on many aspects of these proteins have been conducted. Among pulses, soybean (*Glycine max.*) is the most economically important crop which has unique characteristics of an oil seed and a grain legume. Extensive knowledge and technology accumulated on this crop would assist the development of other grain legumes and oil seeds for food applications. Consequently, a brief review of technological knowledge and various food applications of soybean proteins is firstly presented. Then, the current research status and food applications of proteins isolated from other grain legumes and oil seeds, particularly of chickpea seeds are also reviewed.

2.1 SOYBEAN

Soybean (*Glycine max (L) Merrill Leguminosae*) is the most important crop in the US and perhaps in the world due to its wide applications in animal feed, human foods and industrial utilisations. Soybean contributes more fat and protein to the American diet than any other single source (Smith and Circle, 1972). As shown in Fig 2.1, world production of major grain legumes is about 150 million tonnes per year with 90 million tonnes of soy bean, accounting for about 60% (Uebersax and Ruengsakulrach, 1989). In 1994, the U.S. produced a record of 62 million tonnes of soybean, an increase of about 600% over 50 years (Fig 2.2) (Buffet, 1994).

In the Orient, preparation of soy bean based products is a fine art. By experience, the Chinese have developed many simple and yet effective processes for making a great variety of wholesome and nutritious soybean foods for thousands of years. From ancient times, before the role of proteins in human nutrition had been defined, the Chinese had already mastered the early art of extraction and precipitation of soybean protein. Soybeans were soaked, ground and the aqueous extracts were coagulated to form a solid curd with high protein content.

Numerous traditional fresh, dried, liquid, moist, paste, fermented or non fermented foods were also prepared from soybean. These arts performed skilfully by the ancient Chinese and other Asian food technologists were further developed by today's technology for mass production. Some typical traditional soy based products are illustrated in Fig 2.3.

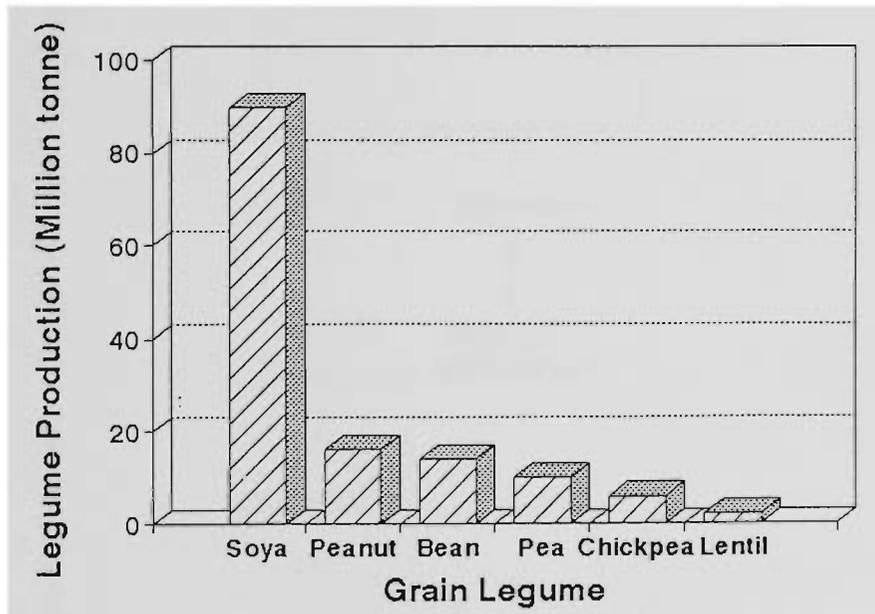


Fig 2.1 World production of major grain legumes.

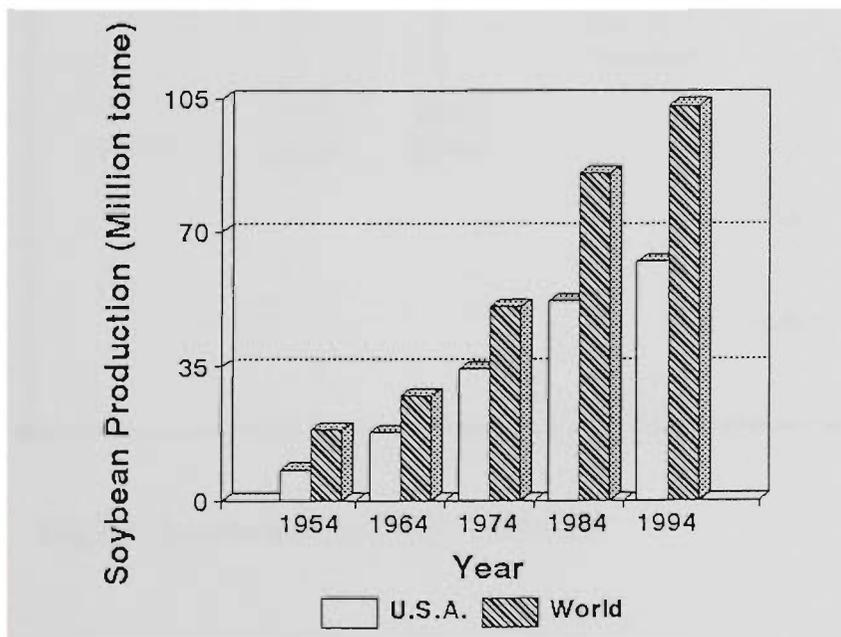


Fig 2.2 Soybean production in U.S.A from 1954 to 1994.

In contrast to the Orient, soybean is relatively new to Europe and America. The total harvested soybean crop in the US in 1922 amounted to only 4×10^6 tonnes but since World War II, there has been a tremendous increase in production and use of soybean. Initially, the soybean was only considered as an oil seed because the defatted meal had very little economic value, being mainly used for animal feed. A dramatic change occurred as the value of protein

content in soybean was fully recognised. For the last two decades in America, soy proteins have been used as food ingredients in increasing amounts due to its low cost, high nutritional value and important functional properties (Buffett, 1994; Haumann, 1984).

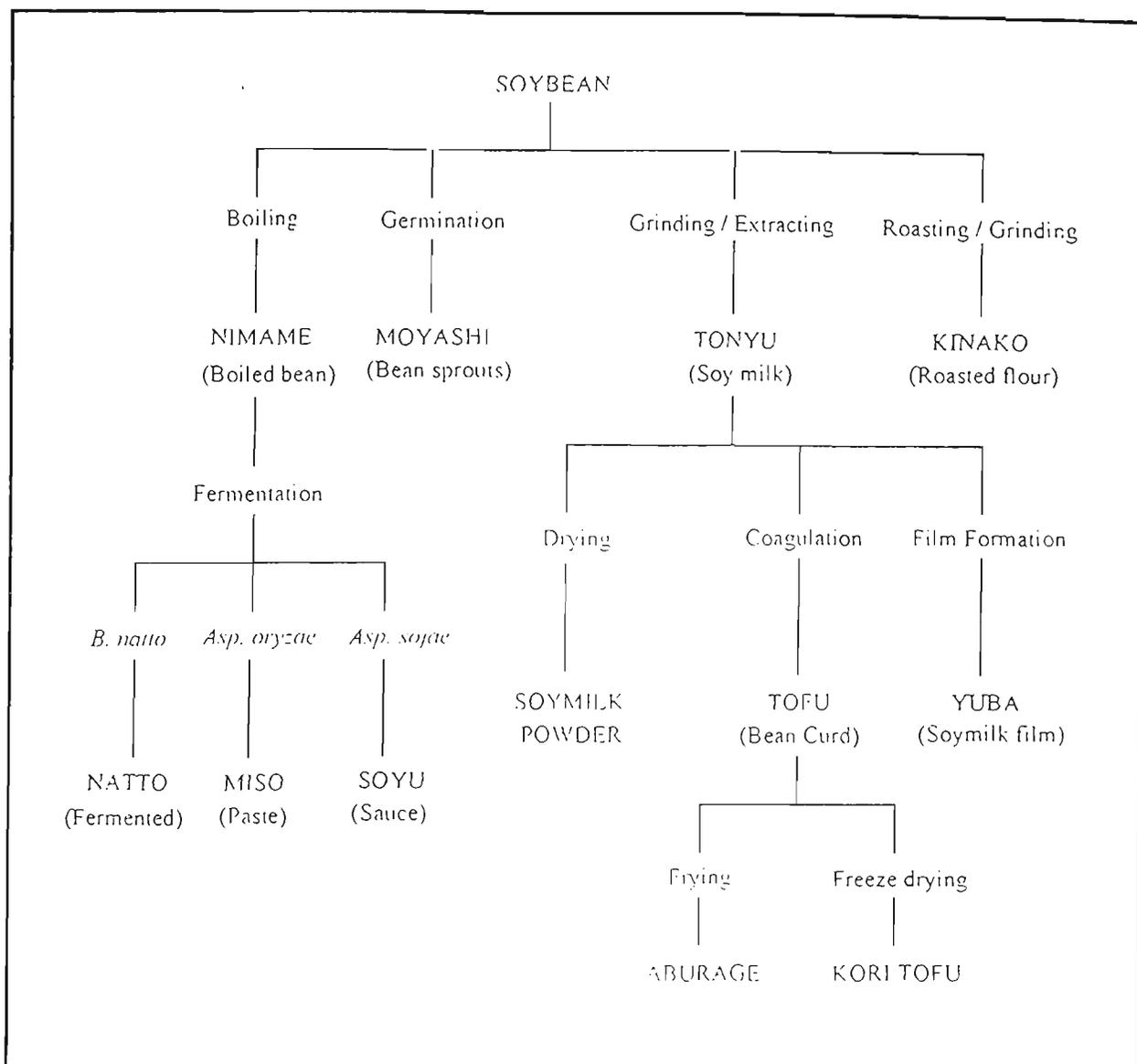


Fig 2.3 Traditional soy based products.

2.1.1 SOY PROTEIN PRODUCTS

Today, various protein rich products can be produced from soy bean. Typical protein based products derived from this crop are presented schematically in Fig 2.4.

a. Flour

Two types of full fat flours can be prepared from dehulled soybeans for the baking industry. The *enzyme active full fat flour* increases the whiteness of wheat flour due to carotene oxidation associated with lipoxygenase activity.

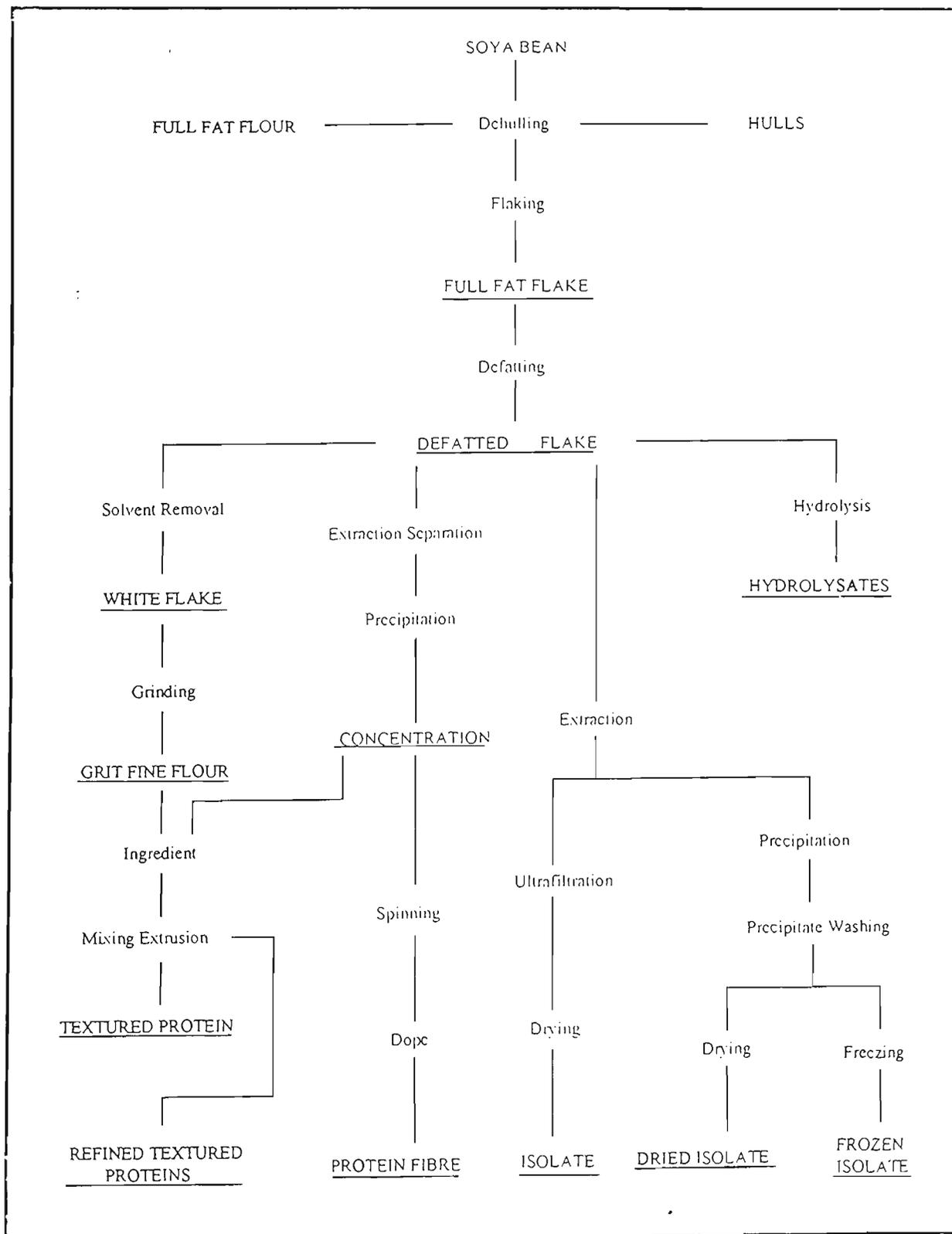


Fig 2.4 Major protein based products prepared from soybean.

The flour also improves the machinability of a dough. The *enzyme deactivated full fat flour* can be made from system treated and dried, dehulled soybeans. The protein content in the full fat flours is about 25-50%. The shelf life of a flour is affected by the oil content. A high oil flour will have more risk of fat oxidation and a shorter shelf life. Consequently, soybean flour with low, medium and high oil levels are now prepared. Also, *lecithinated soy flour* is produced by mixing lecithin at a 15% level with low or defatted soy flour. Defatted flour can be made from defatted conditioned soy meal or soy flakes. The oil extraction with hexane, at a low temperature, will cause little denaturation of the protein. The temperature and process used to extract oil affects the functional properties of soy protein.

b. Flakes and Grits

Dehulled soybeans are conditioned, pressed and rolled to thin flakes with 250-370 μ m in thickness, before solvent extraction. The flaking process assists the oil extraction by disrupting intact cotyledon cells, increasing the solvent penetration capacity by forming suitable beds for solvent flow. The defatted flakes can be made into grits of various sizes: coarse (750-3350 μ m), medium (400-750 μ m) and fine (170-400 μ m) particles. Soy grits have been used in the largest volume by the food industry since grits are the most economic source of protein concentrate with desirable functional properties. In fact, grits and defatted soy flour, are differentiated only by particle size. Like defatted soy flours, the quality and functional properties of grits are affected by the particle size and the degree of heat treatment.

c. Protein Concentrates

Soy protein concentrates with at least 65% protein content (FAO, 1987) are produced from defatted flakes by removing non-protein soluble components such as carbohydrates, ash and peptides. There are three principal processes to insolubilise or concentrate proteins based on heat, acid or alcohol treatment (Berry, 1987). For the heat treatment, the defatted flours or flakes are extracted with hot water (65-95°C) at pH 5.5-7.5 and the solids are separated by centrifugation. For acid extraction, proteins are insolubilised at their isoelectric points (pH 4.5) and soluble carbohydrates, minor proteins and other non-protein materials are removed. For ethanol extraction, defatted flours are mixed with 60-80% concentration aqueous ethanol. Most of the protein is insoluble and soluble oligosaccharides are removed. All three processes give soy protein concentrates with similar composition but different functional properties for different food systems.

d. Protein Isolates

Protein isolates contain over 90% protein, produced also from defatted flakes. The proteins

are initially solubilised with dilute alkali (pH 10). The soluble proteins are then separated from insoluble carbohydrates and non-protein components by filtration or by centrifugation. The clarified extracts can be concentrated by either isoelectric precipitation or by membrane filtration. The concentrated curd or precipitate is then washed several times to remove more non protein components, neutralised, freeze dried or spray dried.

e. Textured Soy Proteins

Texturized soy proteins can be made from soy flours, grits or concentrates. These soy components are mixed with other ingredients (flavours, colours, nutrients) then the mixture is passed through a cooker-extruder. The wet protein mixture is converted into a plastic mass by an elevated temperature and pressure inside the extruder, before being extruded through a die. Due to the drop of pressure and temperature through the die, the plastic mass expands and forms a fibrous product. When the dried fibrous product is hydrated, a chewy textured protein is obtained.

f. Spinning Protein Isolates

Fibres are produced by extruding a soybean protein dispersion through spinnerets, into an acidic coagulating bath. The fibres are then heated, neutralised, washed, and cut into different lengths. Soy proteins can also be mixed with other proteins such as milk protein or caseinate to enhance the nutritional value or to improve the performance of this process.

g. Protein Hydrolysates

Soy proteins can be hydrolysed by enzymes or acids to give a mixture of proteins with smaller molecular weights, larger peptides and amino acids. The hydrolysed proteins or hydrolysates are flavouring ingredients. They have also been used for infant formula.

2.1.2 VEGETABLE PROTEIN ISOLATION

Protein concentrates and protein isolates from animal sources such as milk or fish and vegetable sources such as soybean or other oil seeds and non oilseeds could be prepared by several procedures (Fig 2.5). Basically, there are two major approaches to fractionate plant proteins: dry and wet methods. The former has rather limited application because it fails to fractionate oil seeds or seed with low starch level (Sosulski and McCurdy, 1987). The wet method has wider application.

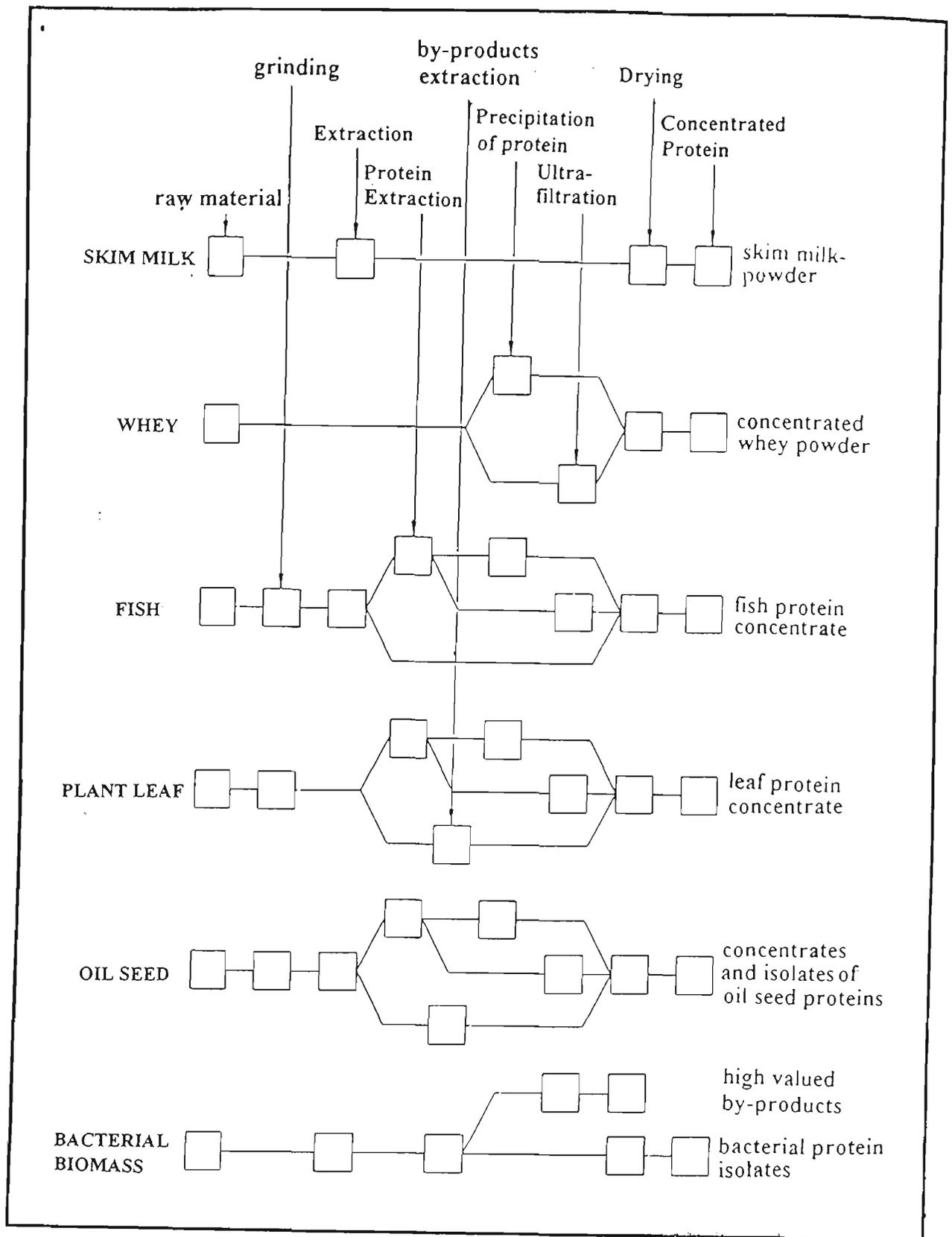


Fig 2.5 Outline of common key steps in the preparation of food proteins.

Various wet procedures were developed or modified to fractionate and isolate proteins, depending on the composition of the raw material as well as the end use. All of these protein isolation procedures have been based on three common basic steps.

1. Solubilisation of proteins from raw materials
2. Concentration of solubilised proteins
3. Purification of isolated proteins

An extraction process is usually based on the specific protein solubility of the investigated protein which is a function of extraction pH. For all grain legumes, proteins are most soluble at alkaline or acidic pHs but almost insoluble at isoelectric pHs. A typical process includes the solubilisation of proteins at pH 8.5-9 in aqueous medium followed by the concentration of soluble proteins by precipitation at isoelectric pH (4.2-4.5). Finally, the concentrated protein-curd or protein precipitate will be purified by washing several times to remove more carbohydrates and non-protein components, before drying. This is a very common process, employed for extraction of both oil seeds such as soybean (Johnson and Kikuchi, 1989), cottonseed (Berardi *et al.*, 1969), peanut (Cater *et al.*, 1974) or non oil seeds such as field pea (Madsen and Buchbjerg, 1987), lupin (Manrique and Thomas, 1976) and faba bean (Gueguen, 1991).

Recently, ultrafiltration (UF) or membrane technology has also been used to fractionate and concentrate solubilised proteins. Soy protein isolates have been produced by using a combined UF and continuous diafiltration process (Pompeic *et al.*, 1976). A soy protein isolate with about 90% purity was produced by discontinuous diafiltration or UF process (Lawhon, 1983) while a soy flour extract was concentrated to produce an isolate with about 88% protein by direct ultrafiltration.

For oilseed such as soybean or rapeseed, the process was modified to simultaneously extract and recover both oil and protein (Eapen *et al.*, 1969; Lawson *et al.*, 1981). The protein solubilisation step can also be combined with other treatments to remove anti nutritional factors. To deactivate proteinase inhibition, defatted soy flour was treated with heat before being fractionated with aqueous alcohols to produce protein fractions with different compositions and characteristics. Heat treatment was also used to remove gossypol from cotton seeds (Lusas and Jividen, 1987) or inactivate myrosinase from rapeseed (Eapen *et al.*, 1969). Sosulski *et al.* (1973) diffused chlorogenic and quinic acids from sunflower kernels using aqueous solvents. Chango *et al.* (1993) debittered lupin proteins with calcium alginate.

The solubilised proteins could be concentrated mainly by coagulating at isoelectric pHs, using acids. The precipitation usually occurs at a single pH but El Nockrasky *et al.* (1977) improved the yield of rapeseed protein isolate by a two stage precipitation procedure at pH 6.0 and 3.6. Salts were also used as coagulants, either calcium sulphate or nigari (seawater salts) was used to produce tofu or bean curd.

There are several factors associated with various procedures presented in this review, which could affect the protein extraction yield and the functional properties of the isolated proteins. Information on the physicochemical properties and the effects of processing conditions on the functional properties of the isolated proteins is essential for their application in food systems.

2.1.3 FUNCTIONAL PROPERTIES OF SOY PROTEINS

As shown in Fig 2.3 and Fig 2.4, various traditional and convenience foods have been prepared from whole soybean seeds or from soybean proteins. These products are well accepted. Novel applications of soybean protein in food and related industries have been extensively sought. Nutritional quality and relatively low cost are two major factors contributing to the great increase in soybean protein production and diverse protein based food products. Another important factor affecting the utilisation of soybean proteins is its functional attributes. Functional properties or functionality encompass any physicochemical property which affects the behaviour of protein in a food system (Kinsella, 1976). The major functional properties of soybean proteins are solubility, water absorption, hydration and swelling, dispersity, coagulation and gelation, oil absorption, emulsifying capacity and stability, foam forming capacity and stability, flow properties and cohesion. As food ingredients, soy proteins have to provide one or more of these key functional attributes in the storage, preparation and utilisation of a food product. The functional contribution and modes of action of soy protein in different food systems are given in Table 2.1.

Like other food proteins, the functional properties of soybean protein are a consequence of its inherent physical and chemical properties. The physical and chemical properties affecting the functional properties of a protein are ultimately related to amino acid sequence. The three dimensional structure of a protein is dictated by its amino acid sequence. The thermodynamic stability, charge distribution and arrangement of hydrophilic and hydrophobic patches are also influenced by the amino acid sequence. Since the thermodynamic requirement dictates the folding of a protein, in order to achieve the lowest possible level of the global free energy, hydrophobic groups are buried inside while hydrophilic groups and charged residues are exposed on the protein surface. However, not all hydrophobic groups are completely buried in the interior. Some are located on the protein surface and the distribution of these hydrophobic cavities influences the shape of a protein and also its functional properties (Damodaran, 1994).

The physical and chemical properties of a protein are susceptible to the processing conditions or environmental changes that ultimately affect the amino acid sequences. Consequently, the functional properties of soy and other food proteins can be altered and improved to meet specific requirements by affecting the amino acid sequences.

Plant proteins are relatively cheap but their application in fabricated foods is rather limited because these proteins lack desirable functional performance (Damodaran, 1994). In addition, no single protein can provide all the functional properties required in various food systems (Kinsella, 1984). To improve the performance of these proteins including soybean protein, enzymatic, chemical and other forms of modification have been extensively investigated (Kinsella and Shetty, 1979; Morr, 1990; Rhee, 1994).

Table 2.1 Functional Attributes of Soybean Proteins in Food Systems

Function	Mechanism	Food System
1. Solubility	Hydrophilicity	Beverages
2. Viscosity	Water binding/ hydrodynamic size/shape	Soups, gravies, salad dressing
3. Water binding	Hydrogen bonding	Meat sausages, cakes, breads
4. Gelation	Water entrapment and immobilization, network	Meats, gels, cakes, bakeries, cheeses
5. Cohesion/Adhesion	Hydrophobic/ionic and hydrogen bonding	Meats, sausages, pasta, baked goods
6. Elasticity	Hydrophobic bonding/ disulfide cross-links	Meats, bakery
7. Emulsification	Absorption at interfaces/film formation	Sausages, bologna, soup, cakes, dressing
8. Foaming	Interfacial adsorption/film formation	Whipped toppings/ ice cream, cakes, desserts
9. Fat and flavour binding	Hydrophobic bonding/entrapment	Simulated meats, bakery, doughnuts

Source: Kinsella *et al.*, 1985

The spectacular growth of soybean production and its enormous economic value has attracted extensive research, particularly on protein fractions which were initially considered only as a by product. This dramatic success has stimulated substantial investigations of other protein bearing grain legumes as a new source of vegetable proteins (Hulse, 1991). The search for novel applications of these vegetable proteins is expanding rapidly and the current research and development status of these proteins is summarised in the following section.

2.2 OTHER PROTEIN SOURCES

2.2.1 PEANUT

Peanuts or ground nut (*Arachis hypogae L.*) account for 14% of oilseeds (2.8×10^7 tonnes) and 4% of protein meal produced annually in the world (4.4×10^6 tonnes) (Lusas *et al.*, 1989). India and China are the two major peanut producers. Interest in peanut proteins as nutritional and functional ingredients in foods has increased due to the bland flavour and low level of some anti nutritional factors (Ayres and Davenport, 1977; Conkerton and Ory, 1976). Aflatoxin contamination is the only problem which requires attention.

Peanut protein concentrates and isolates with over 90% protein were obtained by conventional processes including alkali extraction and isoelectric precipitation (McLean, 1941; Bock, 1968; Cater *et al.*, 1974). An ultrafiltration membrane technique was also developed (Lawhon and Lucas, 1984). Simultaneous aqueous extraction of oil and protein was investigated (Rhee *et al.*, 1972, 1973). The effect of pretreatments of peanut meal and protein extraction conditions on the quality and functional properties of peanut proteins have been extensively studied (McWalters *et al.*, 1976; Ramanatham *et al.*, 1978; Ihekoronye, 1987). Aboagye and Stanley (1985) reported that high temperature, high feed moisture and low screw speed affected the characteristics of extruded peanut products.

The essential limiting amino acids in peanut protein are lysine, threonine and methionine. Mild heat treatment can inactivate anti nutritional factors and enhance digestibility of peanut proteins (Ananatharaman and Carpenter, 1969). The functional properties (Ramanatham *et al.*, 1978; McWalters *et al.*, 1976) and the effects of chemical and biological modification of peanut proteins (Beuchat, 1977) have been investigated.

For food use, peanut flours and proteins have been incorporated in snack food (Toft, 1986), cake type doughnuts (McWalters, 1982), Chinese noodles (Chompreeda *et al.*, 1987), breads

(Khalil and Chushtai, 1984), hamburger and texture proteins (Ayres and Davenport, 1977; Aboagye and Stanley, 1985).

2.2.2 COTTONSEED

Cotton seed (*Gossypium Hirsutum L.*, *Aboreum L.*, *Barbadense L.* or *Herbaceum L.*) is the second major oilseed in the world, (2.8×10^7 tonnes), with the potential to provide enough protein at 45g / day / person for approximately 350 million people (Lusas and Jividen, 1987a). Although cottonseed contains about 22.5% protein with good nutritional quality, a high level of gossypol in the gland forms a major obstacle for food uses of this protein source (Yoo and Hsueh, 1985; Valle *et al.*, 1986)). Gossypol is a yellow green polyphenolic compound, toxic to man and monogastric animals (Gray and Leffler, 1981). Consequently, cottonseed and its derivatives have been limited primarily to cattle and other ruminants. The American FDA and FAO/WHO have set a tolerance level of free gossypol for food use at 450-600 mg (Lusas and Jividen, 1987b).

Promising work has been done by McMichael and other workers (Miraville, 1972) to provide glandless cottonseeds free of gossypol, using genetic engineering approaches or by plant breeding. The level of total gossypol was reduced from 1.2% to 0.02% in glandless cotton seeds (Lusas and Jividen, 1987). Other processes to remove gossypol include solvent extraction, heat treatment or selectively binding gossypol with ionic salts (Lusas and Jividen, 1987; Gardner *et al.*, 1976; Hanumantha Rao *et al.*, 1987; Valle *et al.*, 1985).

Cottonseed proteins were prepared from defatted glandless cottonseed flour. The proteins consisted of two fractions. Storage protein was soluble at high and low pHs while non storage protein was more soluble at neutral pH. The essential amino acids of each fraction were slightly different (Martinez *et al.*, 1969; Lawhon *et al.*, 1975). Protein concentrates were prepared by aqueous acidic extraction (Lawhon *et al.*, 1972) and spray drying. Concentrates were also prepared by pin milling and air classification (Martinez *et al.*, 1969). Isolates were prepared by dilute alkali extraction and acidic precipitation (Martinez and Hopkins, 1974) or by leaching the proteins soluble at neutral pH with water, followed by acidification (Berardi *et al.*, 1969; El Tinay *et al.*, 1980; El Tinay *et al.*, 1988). Other processes reported include aqueous extraction processing based on gravity separation (Rhee *et al.*, 1973) or by industrial ultrafiltration membrane processing (Lawhon *et al.*, 1972; Lawhon *et al.*, 1974; Hensley *et al.*, 1977). Lawhon (1983) combined the classical alkali extraction of bland glandless cottonseed with the membrane separation of the extracts to give a retentate of 7S and 11S type proteins. These were spray dried to yield the protein isolate.

Functional properties of cottonseed protein such as water holding, oil holding, emulsifying and foaming capacities were greatly improved by acylation or enzymatic modification (Choi *et al.*, 1983; Childs and Forte, 1976).

The potential application of cottonseed proteins for food use has not been extensively investigated. The proteins, however, have been used in bread (Khan and Rooney, 1977; Khan *et al.*, 1976), children's foods or high protein curds (Hayes *et al.*, 1986; Choi and Rhee, 1984), meat products (Lawhon *et al.*, 1972; Terrell *et al.*, 1981), beverages (Lawhon *et al.*, 1974) and desserts (Simmons *et al.*, 1980).

2.2.3 RAPESEED

Rapeseed or Canola (*Brassica campestris L.*, *B. napus L.*) is a major oil seed, accounting for 10% (2×10^7 tonnes) of world's oilseed production and 10% (1.1×10^7 tonnes) of world protein meal (Lusas *et al.*, 1987). The leading producers of rapeseed are China, Canada, the European Economic Community (EEC) and India.

The protein content in the defatted rapeseed meal is low (34%) but its protein contains an excellent essential amino acid profile with 6% lysine and 3-4% methionine and cystine (Sosulski, 1983). Phytic acid, erucic acid and glucosinolates in rapeseed have adversely affected its utilisation. Phytic acid forms stable complexes with basic amino acid residue, decreasing the solubility of rapeseed proteins and affecting their functional properties. The glucosinolates are hydrolysed by the enzyme myrosinase, under some circumstances, to produce physiologically harmful compounds such as isothiocyanates and oxazolidinethione (Van Etten *et al.*, 1969; Kozłowska, 1983). Excess erucic acid in the diet may cause liver diseases. New varieties of rapeseed developed in Canada and in Europe are almost free of erucic acid (less than 1%) in the seed oil.

Several procedures were developed to remove glucosinolates and phenolic compounds including aqueous extractions of rapeseed meal or ground rapeseed or by diffusion from intact rapeseed (Ballaster *et al.*, 1970; Sosulski *et al.*, 1972). A typical process to produce rapeseed protein includes mild heat treatment, myrosinase inactivation, solvent oil extraction and glucosinolate removal (Eapen *et al.*, 1969; Mieth *et al.*, 1983). Sosulski and Bakal (1969) prepared rapeseed isolates by alkali extraction and acid precipitation. The process gave a low protein recovery (50%) and inferior quality products. El Nockrasky *et al.* (1977) improved the yield by a two stage precipitation procedure at pH 6.0 and pH 3.6, with the isolates containing up to 98.6% proteins. Recently, Tzeng *et al.* (1990) designed a process consisting of extraction of oil free meal at pH 10-12, isoelectric precipitation to recover

proteins and ultrafiltration followed by diafiltration to concentrate and purify the acid soluble proteins. Compared with soy protein, rapeseed protein concentrates exhibit a lower solubility, oil emulsification, whippability and viscosity (Sosulski *et al.*, 1976).

Rapeseed proteins have been used in bread (Kodagoda *et al.*, 1973) and meat products (Sosulski *et al.*, 1976). However, the use of rapeseed proteins in foods is limited (Tzeng *et al.*, 1990), due to their adverse organoleptic properties, the level of glucosinolates and complex extraction process.

2.2.4 SUNFLOWER SEEDS

Sunflower seeds (*Helianthus annuus L. var. marcoparpus DC.*) account for 10% of the world's oil seed production and 7% of vegetable proteins is produced from the meal of sunflower seeds (Lucas *et al.*, 1989). The defatted flour contains about 60% protein which, except for low lysine content, has an excellent amino acid balance (Lawhon *et al.*, 1982; Sosulski and Fleming, 1977).

Sunflower proteins have unique organoleptic and functional properties (Sosulski, 1979). A most unique characteristic of sunflower is its very low level of toxic components (Lusas, 1985). The only major deterrent to the use of sunflower seed is its chlorogenic acid content. This colourless phenolic compound, under alkali extraction conditions, will change to an irreversible olive green colour (Sabir *et al.*, 1974). As a result of the oxidation of polyphenolic compounds, either autolytically under alkaline conditions or by polyphenoxidase, stable bonds are formed between phenolic compounds and proteins and the green colour is irreversible (Loomis and Battaile, 1966; Sosulski *et al.*, 1972).

Several methods were developed to prevent this oxidation by controlling three factors thought to be responsible for the colour development: alkaline pH, oxygen, and polyphenoxidase. Sosulski *et al.* (1973) extracted chlorogenic and quinic acids from sunflower kernels using aqueous solvents. Oxygen expulsion and exclusion techniques were also investigated (Lawhon *et al.*, 1982).

Functional properties of native (Brueckner *et al.*, 1986; Lin and Humbert, 1974), chemically (Schwenke and Ranschall, 1983) and enzymatically modified (Jones and Tung, 1983) sunflower proteins have been investigated. Sunflower proteins have excellent fat absorption, oil emulsification and whipping properties. Succinylation and acetylation of sunflower protein improved water solubility, water absorption and oil emulsification (Kabirullah and Wills,

1982). Protein solubility and water hydration capacity was increased by trypsin hydrolysis (Jones and Tung, 1983).

Sunflower proteins are relatively low in lysine but rich in other essential amino acids, particularly methionine and cysteine (Sosulski and Fleming, 1977). Sunflower proteins were compared favourably with soybean flour and casein in terms of PER, feed consumption and weight gain in rat feeding trials. There were significant differences in feed consumption and weight gain but the PER of sunflower protein was much lower than that of soybean.

For food use, sunflower flour and proteins have been used in meat products (Brueckner *et al.*, 1982), in biscuits (Wills *et al.*, 1984) and in bread (Sosulski and Mahmoud, 1979).

2.2.5 LUPIN

Lupin (*Lupinus spp*) is a valuable source of protein for both animal feed and human consumption because of the high yield of protein in the seed (up to 48%), which compares favourably with soybeans (Cerletti, 1983). Australia is the world's major lupin producer, producing 916,000 tonnes per year over 6 years to 1992 (Petterson and MacKintosh, 1994).

Although lupin is similar to soybean in terms of protein content and amino acid profile, its application in human foods was limited due to bitter and toxic alkaloids (up to 2.5%) (Hudson, 1979) and, to some extent, a high level of manganese (up to 4,500 ppm) (Hung *et al.*, 1988). New sweet varieties, developed in Western Australia, almost free of quinolizidine alkaloids have enhanced the food use prospect of lupin (Aquilera and Trier, 1978).

Lupin proteins have been extensively investigated. Early works revealed different types of globulin which constitute the major protein component (87%). The albumin fraction accounts for only 5-13% of the total protein, which varied with varieties (Cerletti *et al.*, 1978; Blagrove and Gillespie, 1978). The globulin fraction was extensively studied by several investigators (Blagrove and Gillespie, 1975; Casero *et al.*, 1983).

Typically, lupin protein isolates were prepared by alkali extraction and acid precipitation from commercial flours. The extract was dissolved at pH 7 and spray dried. Manrique and Thomas (1976) employed various isolation procedures to prepare lupin protein concentrates and isolates from lupin flour or whole seed. This included single and double extraction processes in either alkaline or acidic conditions. The effect of extraction conditions on the functional properties of the resultant proteins was also investigated. The isolates were also prepared by ultrafiltration and diafiltration (Pompei and Lucisano, 1976). Recently, Chango *et al.* (1993)

proposed a new process to precipitate and simultaneously debitter lupin proteins by coagulating in an aqueous medium.

The functional properties of lupin isolates prepared under different conditions were investigated (Oomah and Busmuk, 1983; King *et al.*, 1985). Lupin protein isolates exhibited better solubility than soy isolate and similar emulsification capacity. Swelling and gelation were inferior but modification of extraction processes improved these properties. Foaming capacity could also be improved by adding salt or some carbohydrates (Sathe *et al.*, 1982).

The potential of lupins as a protein source for human consumption, and its nutritional evaluations, have been extensively investigated (Egana *et al.*, 1992). Various applications have been studied including lupin sprouts, fermented foods from lupin seeds such as tempeh and soy sauce (Hung *et al.*, 1993), tofu supplemented with lupin extracts (Hung *et al.*, 1986), breads and noodles supplemented with lupin proteins or flours (Hung and Nithianandan, 1993; Ballester *et al.*, 1984). Romana *et al.* (1983) studied the quality of lupin protein in children foods.

2.2.6 SAFFLOWER

World production of safflower (*Carthamus tinctoria L.*) has increased with the increasing production of vegetable oil. By 1985, safflower seed and oil production were 1.2 and 0.4 million tonnes, respectively (FAO, 1985). Defatted meal, a by-product of the oil extraction has been considered as a potential source of vegetable protein for human consumption. Mexico and India are the two major producers of safflower in the world. (Betschart, 1975, 1979)

Safflower proteins are low in lysine, threonine, isoleucine and leucine but contain adequate amounts of other essential amino acids (Betschart and Sawnders, 1978; Paredez-Lopez and Ordorica-Falomir, 1986). The PER values for all safflower protein isolates are lower than those of casein. Also, the phenolic glucosides associated with the bitter taste and cathartic activity are undesirable factors, thus preventing the safflower meal from being utilised as a food product (Lyon *et al.*, 1982).

Safflower was originally grown for the dyestuff carthamin and recently for its excellent polyunsaturated oil (80% linoleic acid). As an oilseed, safflower seed contains only 14-18% protein. However, defatted safflower meal could contain up to 40% protein and has been used as the raw material for flours, protein concentrate and isolates (Paredez-Lopez and Ordorica-Falomir, 1986).

Safflower proteins were prepared from the seed or defatted meal by alkaline extraction and acid precipitation with 80% protein recovery (Arntfield *et al.*, 1985; Betschart and Saunders, 1978). Also, micellization isolates could be prepared by a micellization technique comprising a dilution of previously ultrafiltered protein concentrate extracts from a neutral salt solution (Paredes-Lopez and Ordorica-Falomir, 1986).

Compared with soybean protein, safflower protein isolate (SPI) exhibits favourable solubility and foaming properties (Paredes-Lopez and Ordorica-Falomir, 1986). Extraction conditions such as the precipitation pH, neutralisation and precipitation could change the composition and quality of SPI. For example, SPI precipitated at pH 6 was more soluble at acidic pH values and performed better in wheat flour breads than did SPI precipitated at pH 5 or isolates produced by a micellization technique. These isolates were more soluble than those obtained from isoelectric precipitation (Paredes-Lopez and Ordorica-Falomir, 1986). Various treatments for oil extraction showed only minor effects on the functional properties of the resulting protein isolates.

The potential use of safflower protein for human food has been reviewed (Paredes-Lopez *et al.*, 1991). Safflower protein could be compared with soy protein in baked products and beverages (Ranhotra *et al.*, 1974; Bertchart *et al.*, 1979). Food use of safflower protein is so far limited. For wider application, the bitter flavour and harmful cathartic activity must be removed (Palter *et al.*, 1972).

2.2.7 FABABEAN

The world production of faba bean or field bean (*Vicia faba L.*) from 1989 to 1991 was estimated at 4.2×10^6 tonnes per year and accounted for 7.3% of the world production of major food and feed legumes (Oram, 1992). This old crop has recently attracted more interest since the seeds have high protein levels.

Genetic and phenotypic factors greatly affect the level of protein which varies from 26-39% in the seed. Compared with soy bean protein, faba bean protein has lower sulphur amino acid and a deficiency of tryptophan (Bhatty, 1974). Faba bean is still mainly used for animal feeding but its application for human foods has been extensively investigated.

Faba bean protein concentrates and isolates have been prepared by either wet or dry processes. The wet processes employed by several investigators (Gueguen, 1991; Flink and Christiansen, 1973; Vose *et al.*, 1976; Gueguen, 1980) consisted of alkali extraction and acid precipitation steps. The process gave a recovery yield of 65% total protein, with

highly purified (90-95%) protein isolates. This process was further modified by a salt extraction micellization technique (Arntfield *et al.*, 1985), using ultrafiltration to recover the extracted proteins instead of acidification (Olsen and Anderson, 1978; Berot *et al.*, 1987). Other factors affecting the yield and quality of the extracted proteins, such as extraction pH, solvent and ionic strength, have been examined (Ismond *et al.*, 1986; Gueguen 1980).

Faba bean flour was fractionated into a starch and protein rich fraction by pin milling and air classification (Vose *et al.*, 1976). This dry process produced two protein rich fractions containing 69 and 50% protein, respectively.

Functional properties of native and denatured faba bean proteins, and the influence of the processing parameters on functionality, have been reported (Schwenke *et al.*, 1981; Schwenke *et al.*, 1983; Summer *et al.*, 1981; Bau *et al.*, 1979). Some functional properties of faba bean proteins were improved by acetylation, succinylation or enzymatic treatments (Schmandke *et al.*, 1981; Ludwig and Ludwig, 1985). Combinations of enzymatic and mechanical treatment could also improve some functional properties of faba bean proteins (Behnke *et al.*, 1982). Digestibility of native and modified faba bean protein has also been examined (Krause *et al.*, 1984).

A review of the food applications of faba bean proteins by Simpson (1983) summarises the direct use of faba bean for human consumption (Simpson, 1983). Faba bean proteins were incorporated into noodles, breads (Youssef and Bushuk, 1986) but major applications related to meat products such as sausage (Muschiolik *et al.*, 1982), fried hamburgers and frankfurter type sausages (Muschiolik *et al.*, 1982). Faba bean proteins have been used to prepare special bread in USA (Lareo and Bresani, 1982), Egypt (Abdel-Hamid *et al.*, 1986) and in infant formulae (Begrat, 1981).

2.2.8 FIELD PEA

The total world production of field pea or garden pea (*Pisum sativum L.*) in 1991 was 1.7×10^7 tonnes and accounted for 29% of major food and feed legumes (Oram, 1992). Field pea is a high yield crop (1.8 tonne/ha). The yield increased about 59% over the last ten years and yields up to 4-5.5 tonnes/ha have been reported (Sumner *et al.*, 1979; FAO, 1990). The seed has a high protein content (26-35%) with an excellent lysine level. To date, the major use of field pea has been limited to animal feed, but more recently, the potential utilisation for human consumption has attracted much interest (Klein and Raidh, 1986).

Field pea protein concentrates have been prepared either by wet methods or by air classification. Wet methods included wet milling, ultrafiltration, diafiltration or enzymatic coagulation. The isolated protein exhibited excellent properties for meat sausages (Madsen and Buchbjerg, 1987). Sumner *et al.* (1981) produced sodium proteinate and isoelectric proteins from field peas by alkali extraction and precipitation at the isoelectric point, respectively. Different drying processes such as freeze, spray and drum drying affected the chemical composition and functional properties of the products.

Sosulski and McCurdy (1987) fractionated field pea flour to produce protein isolates by pin milling and air classification. These isolates were compared with those isolates from acid and alkaline wet extraction. The air classified field pea proteins exhibited excellent whippability and foam stability compared with soybean proteins, indicating their potential use in meat emulsions, beverages and bakery products.

The major obstacles for human consumption are the high level of tannin (Wassimi *et al.*, 1988) found in some varieties of field pea and the off flavours encountered in some field pea products.

2.2.9 SINGLE CELL PROTEIN

Recently, proteins produced by microorganisms such as yeast, fungi, bacteria and algae have attracted considerable interest. Proteins of microbial origin are called by the generic name of Single Cell Protein (SCP) which could be compared well with other conventional proteins such as egg, milk or soybean (Guzman-Juarez, 1983; Lipinski and Litchfield, 1974). Compared with the production of other proteins, SCP production is more economical because microorganisms can grow in many cheap substrates including by products from oil refining processes or from other waste products. Climatic factors and land availability have no great effect on SCP production (Tuse, 1984).

Among single cell proteins, yeast protein production has been well investigated (Guzman-Juarez, 1983). Yeast contains about 45-49% protein which is rich in lysine but low in cystine and methionine. Consequently, yeast proteins have lower nutritional quality than casein and egg proteins.

Typically, yeast proteins were concentrated by alkali extraction of protein from disrupted yeast cell slurries and isoelectric precipitation by heat and acid (Vananuvat and Kinsella, 1975a; Achor *et al.*, 1981) Functional properties of yeast protein have been extensively investigated. Solubility, foaming ability, emulsifying capacity of yeast proteins are inferior to

those of soybean proteins (Schachtel, 1981; Vanavuvat and Kinsella, 1975b) but these functional properties of yeast proteins could be improved by extrusion, spinning, plasmolysis and chemical modification (Huang and Rha, 1978; Hayakawa and Nomura, 1977; Vanavuvat and Kinsella, 1975b).

Bacteria is another source of single cell protein. Bacterial proteins have been extensively reviewed (Schlingmann *et al.*, 1984). Bioproducts of bacterial origin have compositions similar to yeast or plant proteins. Bacterial proteins from methane and methanol sources have an exceptionally high level of protein (60-80%).

Compared with other protein sources, bacterial proteins could be produced with a minimum of process steps. A typical process involves an alkali treatment of ruptured bacterial cells to solubilise proteins at pH 9-12, followed by isoelectric precipitation of the solubilised proteins at pH 4.5. A process developed by Hoechst, A.G., uses anhydrous ammonia as a lipid extraction solvent, without alkaline treatment, to produce better bacterial proteins (Waslien *et al.*, 1970).

2.3 CHICKPEA

The chickpea (*Cicer arietinum L.*) also called Bengal gram, boot, channa chola, hyokkomame or kadale, belongs to the plant family *Leguminous*. The seeds of this grain legume have been used for centuries for human consumption and also for animal feed (Pushpamma and Geervani, 1987).

2.3.1 CHICKPEA PRODUCTION

2.3.1.1 World Production

The total world production of grain legumes in 1991 was 58,342 million tonnes. The cool season legumes (faba bean, chickpea, lentil, pea, lupin and vetch) accounted for 56.9% or 33.2×10^6 tonnes while the warm season legumes (mainly bean, cowpea and pigeon pea) accounted for 43.1% or 25.1×10^6 tonnes. The total world production of chickpea was 7.1×10^6 tonnes, accounted for 12.1% of total grain legumes. The six major chickpea producing countries (India, Turkey, Pakistan, China, Mexico and Australia) produced 6.5×10^6 tonnes sharing 84% of the total world production. The annual growth rate over the period between 1980 to 1991 of chickpea yield and production was 1.79%, although there was a large gap in

the chickpea yield between developing (0.72 tonne / ha) and developed countries (1.00 tonne / ha).

2.3.1.2 Australia Production

Chickpea is a relatively new pulse crop in Australia. Initially, chickpea was mainly produced in the tropical and subtropical climates of northern Australia. Success in plant breeding for better yield and climate adaptability contributed to a marked increase in production of both Desi and Kabuli types in southern states. Prior to 1985, Australian chickpea production was not recorded by FAO but within 7 years the production increased more than 200% from 6.3 x 10⁴ tonnes in 1986/87 to average 1.95 x 10⁵ tonnes for 1991-1993 seasons (Fig 2.6).

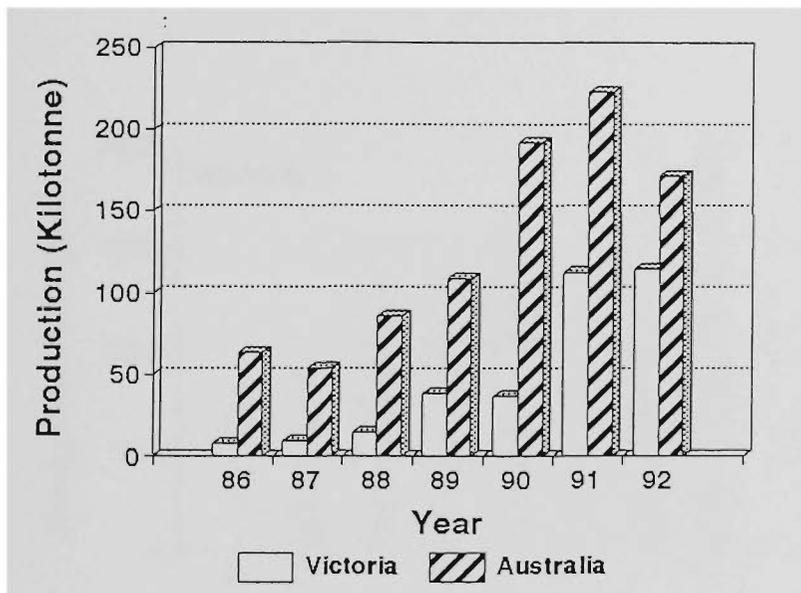


Fig 2.6 Chickpea production in Australia and in the State of Victoria from 1986-1992.

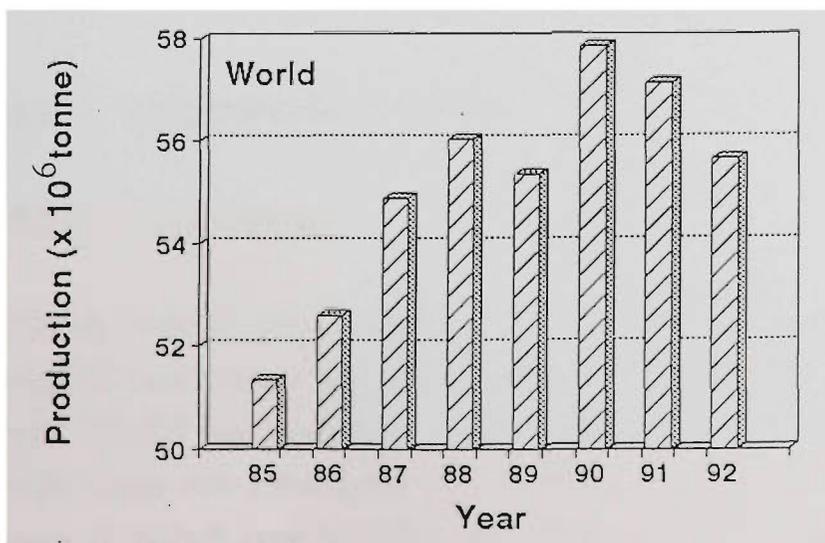


Fig 2.7 World production of grain legumes.

The increased production of chickpea reflects a strategically important change in Australian agricultural production. Falling market values of traditional commodities such as coarse and cereal grains in the 1970's prompted Australian farmers to consider oil seeds and grain legumes as economic alternatives. Grain legumes also improved the soil by adding inexpensive nitrogen through cereal-pulse rotation systems. Moreover many varieties can be grown with limited water supplies on poor quality land. Consequently, while the world production of grain legumes only increased by 8% over 7 years (1985-92) (Fig 2.7), production of Australian grain legumes, over the same period, increased by almost 150% (Fig 2.8). Within Australia, chickpea production increased rapidly in Victoria, perhaps due to the suitable climate and soil type. In 1992, Victoria produced 115,000 tonnes, accounted for 69% of total Australian chickpea production and this crop became an important new grain legume in the state of Victoria (ABARE, 1993).

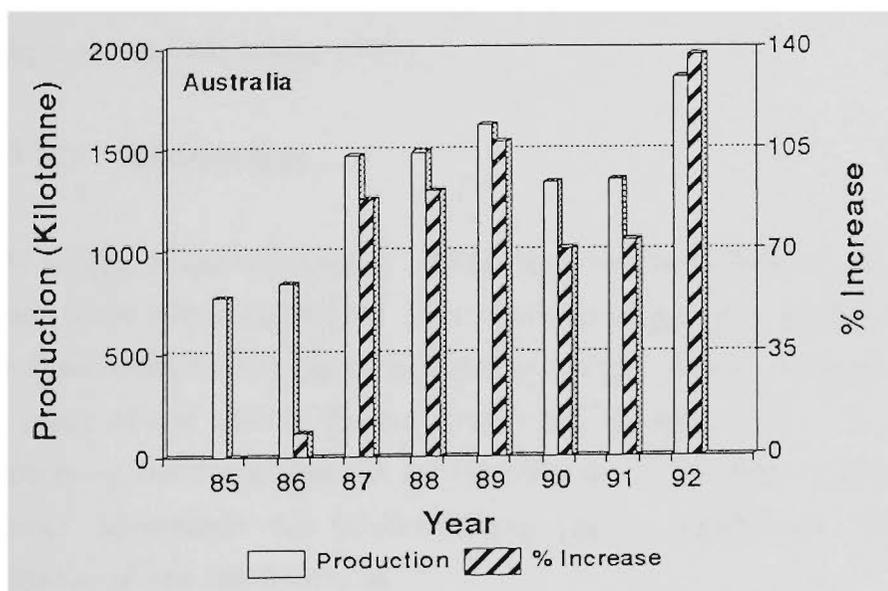


Fig 2.8 Grain legumes production in Australia.

2.3.2 AGRONOMIC ASPECT

2.3.2.1 Classification

Chickpea, a self pollinated annual crop, is grouped into two types, Kabuli and Desi, based on geographic distribution and colour of the seed and flower. The seeds of Kabuli type weigh about 350-400 mg/ each and are light coloured with a smooth surface. The seeds of Desi weight about 100-150 mg are smaller, yellow to black in colour with a rough surface. The flower of Kabuli type is white while that of Desi type (Nene, 1987). The Kabuli type chickpeas account for about 10-15% of a world annual production, mainly grown in

Afghanistan, Western Asia, the Mediterranean and the Americas. The Desi type chickpeas constitutes 85% of world total production, is mainly confined to the Indian Continent, Ethiopia, Mexico and Iran (Summerfield *et al.*, 1987).

2.3.2.2 Botany

There are 39 known species from the genus *Cicer*. The cultivated *C. arietinum* is closely related to *C. pinnatifidum*. *C. echinospermum* and its hybrids have been obtained between *C. arietinum* and its two closest relatives - *C. echinospermum* and *C. reticulatum* Ladz. The International Crop Research Institute for the Semi Arid Tropics (ICRISAT, India) and the International Centre for Agriculture Research in the Dry Areas (ICARDA, Syria) collected over 14,000 and 6,000 chickpea germplasm lines, respectively, from 39 countries. A major collection of chickpea is also kept at the National Seed Storage Laboratory at Pullman, Washington, USA. In Australia, a chickpea centre is established in Horsham, Victoria. (Browner and McIntyre, 1992).

2.3.2.3 Distribution

The origin of the chickpea is unclear although both Kabuli and Desi chickpea types existed from times of earliest record. The chickpea presence in India since 2000 BC was confirmed by their Sanskrit and carbonised names (Singh, 1990). Recipes of chickpeas are also found in some of the earliest Roman cookbooks (Oram, 1992). In the past, there were several centres in Asia and eastern Mediterranean. From these centres of origin, chickpea was spread throughout the Mediterranean region, south east Asian subcontinent and later introduced into the Americas.

2.3.2.4 Growth Condition

As a subtropical crop, chickpea can grow best as a post monsoon, cool season crop in the south east Asian subcontinent, during the dry season in North Africa and during spring and summer in the Mediterranean region. The optimum growth temperature falls within the range of 18-29°C. Chickpea is a drought resistant crop (650-750 mm per annum) but can be grown successfully with a rainfall of 1000 mm per annum (Huda and Virmani, 1984). The chickpea can be adapted to a wide range of soil types. Acidic soil is favourable but some chickpea genotypes are reasonable tolerant to alkalinity and salinity (Rupela and Kumar Rao, 1984). Chickpea has been grown successfully on plains as well as at elevations of 300-1,200 m.

Chickpea is a low yield crop compared with cereal or other pulses (0.7-1.0 tonne/ha). At its optimum growth conditions, chickpea could produce up to 3-4 tonnes/ha (Kay, 1979).

2.3.3 CHICKPEA COMPOSITION

Genetic and environmental factors such as location, climate, soil type, irrigation and chemicals affect the chemical composition and particularly the protein content of chickpea seeds (Sosulski and Gadam, 1988; Singh *et al.*, 1993; Singh *et al.*, 1990; Singh *et al.*, 1988). Based on various published data, Hulse (1976) found a wide range of variations in the chemical composition of whole chickpea. Ether extracted lipid ranged from 3.9 to 6.2%, protein from 20.8 to 25.9%, soluble carbohydrate from 60 to 63% and crude fibre from 8.0 to 8.7% (Hulse, 1976). A typical composition of Australian chickpea, with a similar wide range of variations, is tabulated in Table 2.2. These figures were calculated from several investigations on Australian chickpeas (Petterson and MacKintosh, 1994). The chickpea seed coat, ranging from 6-16% of total seed weight, contains most of the crude fibre and calcium while the cotyledon (80-84% by weight) stores most of the protein (95%) and lipid (94%).

Table 2.2 Typical composition of Australian chickpea.

Variety	Desi			Kabuli		
	Composition %	Mean (%)	Range (%)	Sample No.	Mean (%)	Range (%)
Moisture	8.7	7.2-10	28	8.7	7.5-13	19
Protein	20	16-23	76	22	20-24	33
Ash	2.5	1.9-2.9	22	2.6	2.3-2.8	14
Fat	3.4	2.6-4.5	23	4.7	3.9-6.3	15
ADF	14	11-17	7	5.0	3.3-7.5	3
NDF	29	25-30	6	11	5.2-16	2

Source (Petterson and MacKintosh, 1994)

2.3.3.1 Protein content and amino acid profile

The crude protein content of chickpea seed is affected by several genetic and environmental factors, which has resulted in a considerable variation. The level ranges from 12 to 32%. Hulse (1976) found the average protein content of chickpea was about 23.3% while Chavan *et al.* (1989) reported that the calculated average of chickpea protein was 21.5%. The average protein content of Australian chickpea is about 19.6% for Desi cultivars and 21.7% for Kabuli cultivars. Irrigation and fertiliser application can improve protein content and yield

of chickpea (Singh and Ram, 1990; Singh, 1990) while salinity can depress both yield and protein content (Sharma *et al.*, 1986; Kumar *et al.*, 1983).

Amino acid profiles of the major fractions of chickpea seed are given in Table 2.3. There are some differences in terms of amino acid levels in these fractions such as lysine, threonine, leucine and isoleucine (Singh and Jambunathan, 1982). Tryptophan, valine and methionine are found to be the limiting amino acids in chickpea proteins (Rossi *et al.*, 1984)

Table 2.3 Amino acid composition of different seed fractions of chickpea (g/16 g N)^a

AMINO ACID	Albumin	Globulin	Prolamine	Glutelin
Lysine	6.2	5.0	6.7	7.9
Histidine	2.7	2.4	2.7	2.6
Arginine	10.9	4.2	10.8	10.3
Aspartic acid	12.2	9.0	11.8	10.4
Threonine	4.0	3.7	3.8	4.5
Serine	5.5	4.7	5.3	5.0
Glutamic acid	16.3	10.7	16.1	17.6
Proline	4.0	3.9	3.9	2.6
Glycine	4.1	4.3	3.9	4.6
Alanine	4.0	3.9	4.2	5.1
Cystine	1.3	1.1	1.5	1.5
Valine	5.0	5.2	4.8	5.1
Methionine	1.1	1.1	1.1	1.5
Isoleucine	4.5	3.5	4.2	4.1
Leucine	7.6	6.3	7.2	7.4
Tyrosine	2.8	2.4	2.7	3.2
Phenylalanine	5.5	4.6	5.5	4.3
Total	97.7	76.0	96.2	97.7
N recovery, %	91.5	54.4	89.1	88.5

a: Source: Singh *et al.*, 1982

2.3.3.2 Characterisation of chickpea proteins

Singh and Jambunathan (1982) fractionated chickpea proteins into four fractions: albumin, globulin, prolamine and glutelin. Globulin (56%) and glutelin (18%) were the major fractions, while albumin accounted for 12% and prolamine was a minor fraction (2-4%). Although several grain legumes have been extensively investigated, chickpea proteins have not been

adequately examined (Ganesh-Kumar and Venkataraman, 1980). Ganesh-Kumar and Venkataraman (1975) found chickpea globulin as an oligomeric high molecular weight protein with three subunits.

2.3.3.3 Nutritional values of chickpea protein and protease inhibitors

Like other grain legumes, the nutritional values of chickpea proteins can be evaluated based on the growth methods (PER, protein efficiency ratio; NPR, net protein retention; RPV, relative protein value) and nitrogen balance methods (BV, biological value; NPU, net protein utilisation, TD, true digestibility). Khan *et al.* (1979) found that chickpea proteins had higher TD, BV and NPU than mung beans and cowpea. The NPU and PER of chickpea proteins were also higher than those of faba bean, mung beans and soy beans.

Trypsin and chymotrypsin inhibitors are the two main protease inhibitors in chickpea seed proteins (Bansal *et al.*, 1988). The level of these inhibitors varied greatly with cultivars. For example, Smirnoff *et al.* (1986) found chickpea proteins had a lower trypsin inhibitor activity than lentil and soybean proteins which were in contrast with the finding of Chavan and Hejgaard (1981).

2.3.3.4 Lipid

Chickpea lipid range from 3 to 7% with triglycerides as the major fraction of the neutral lipid. Chickpea lipid contains mainly unsaturated fatty acids (67%) with linoleic and oleic acid as the two major fatty acids.

2.3.3.5 Carbohydrate, flatulence and hypocholesterolemic effect

The carbohydrate content of chickpea ranges from 52-71% with starch as a major constituent. Desi cultivars have lower level of starch than the Kabuli cultivars. In chickpea starch, amylose content (40%) is usually lower than amylopectin (60%). Amylose has an average chain length of 22 glucose units while amylopectin has a longer average chain length of about 1,800 glucose units.

The total sugar content in chickpea ranges from 5 to 10%, depending on the cultivar. Kabuli cultivars contain more sucrose, raffinose and soluble sugars than Desi cultivars (Saini and Knights, 1984). Chickpea contains more raffinose, stachyose and verbacose than many grain legumes and these carbohydrates are believed to be responsible for the flatulence encountered in human and animal (Jaya and Venkataraman, 1979). The flatulence can be reduced by several processing procedures such as soaking, fermentation and germination.

The crude fibre level in Desi cultivars is higher than that in Kabuli cultivars, perhaps due to a close association between the seed coat content and fibre level (Singh, 1984). Fibre from the chickpea seed coat was found to have a favourable hypocholesterolemic effect (Singh *et al.*, 1983).

2.3.4 CHICKPEA FOOD APPLICATIONS

2.3.4.1 Beverage

Protein isolates from ten legume species including chickpea were evaluated as the protein component in imitation milks. The isolates had a wide range of solubility, fat homogenisation, viscosity and conductivity at the same protein level. Imitation milk prepared from chickpea isolate was acceptable in terms of taste and texture (Sosulski *et al.*, 1978). Soluble solids obtained from roasted chickpeas were used as ingredients of soluble imitation coffee powder (Scanpellino *et al.*, 1983). Germinated chickpeas were used for a chocolate flavoured beverage which compared favourably with a commercial chocolate milk (Luz Fermander and Berry, 1987).

2.3.4.2 Infant Formula

Chickpea flour and proteins were used to formulate protein rich infant foods in Egypt and Bangladesh (Morcos *et al.*, 1983; Kabirullah *et al.*, 1976). Homemade weaning food mixtures, with chickpea as supplementary ingredient, met the protein quality evaluated by PER, NPR, RPU and NGI (nitrogen growth index) (Mathew and Pellett, 1986). The values of PER, NPU and NPR obtained from a chickpea formula were similar to commercial calcium caseinate or soybean based products (Sotelo *et al.*, 1987 a,b). Recently, germinated chickpea flours were blended with wheat flour to prepare nutritionally improved weaning food (Livingston *et al.*, 1993). These chickpea based formulas could be prepared at low cost but still complied with infant food specifications, set up by Codex Alimentarias Commission in terms of lysine and S-containing amino acids (Valencia *et al.*, 1988). These formulae could be used to replace cow milk for lactose intolerant children (Sotelo *et al.*, 1987b). Chickpea protein concentrate, specially designed for infant formula (Ulloa *et al.*, 1988) also obtained from an ultrafiltration process.

2.3.4.3 Meat Emulsion Extender

El-Aswad *et al.* (1980) replaced beef in meat product with soy and chickpea and reported that the chickpea fortified product was organoleptically similar to the meat product. The

emulsifying capacity and the water binding capacity of meat was not significantly affected by replacing muscle proteins with chickpea proteins up to a 50% level (Verma *et al.*, 1984 a,b). Abo Baker *et al.* (1986) found that inclusion of chickpea (up to 20%) in sausage meat emulsion increased its water holding capacity and improved tenderness.

Verma *et al.* (1985) reported that up to 30% of meat protein could be substituted with chickpea flour in skinless sausage without causing any significant loss of quality or acceptability. The process was further improved if the flour was subjected to heat treatment (Verma *et al.*, 1984). Net protein utilisation, biological value and protein efficiency ratio were unaffected by 30% meat protein replacement (Verma *et al.*, 1985).

2.3.4.4 Bread and bakery products

Protein value of wheat flour used in bread making could be improved by the addition of chickpea flour (Figuerola *et al.*, 1987). Bread supplemented with chickpea flour up to 15% level showed an increase in protein content, lysine, fibre, fat and a higher PER value (Estevez *et al.*, 1987). The effect of chickpea flour or protein isolates on wheat flour dough characteristics and baking quality have been investigated (Liu *et al.*, 1994). Noodles could be supplemented with chickpea flour to about 12% without taste and texture compromise (Hung *et al.*, 1993).

2.4 REVIEW SUMMARY

Chickpea is one of the most important grain legumes in the world in terms of production. The seeds have been used for human consumption for centuries. Many traditional foods have been made with chickpea in the Indian subcontinent, North Africa, Mediterranean, Central and Latin American and also in some European regions.

Regardless of being a relatively new crop, chickpea production in Australia increased rapidly. The crop is particularly popular in the state of Victoria with over 70% of the total Australian production. It is likely that chickpea production will be greatly expanded and the crop will have an increasingly important role in the Victorian crop system. Nutritional studies indicate that chickpea lipid, fibre and protein exhibit hypocholesterolemic effects. Chickpea proteins show several nutritional values which could be compared favourably with those of soybean and other grain legumes.

Chickpea seeds contain high levels of protein. This valuable components of chickpea could be widely used for human foods like other grain legume proteins presented in this review. However, unlike soy or some other grain legume proteins, chickpea proteins have not been subjected to extensive investigation. A novel grain legume protein must exhibit some desirable functional properties as food ingredient since in the industrial country, an economic evaluation based greatly on its functionality more than its nutritional value. As pointed by many investigators, information on the extraction and characterisation of proteins in chickpea is not adequate. In particular, the functional properties of chickpea proteins require more attention since the information on these properties is essential for any application in the processing food industry.

Most research on vegetable food proteins and their functionality as food ingredients has been focussed on soy proteins. As described in detail in this review, many aspects in the soy production and technology have also been applied for other grain legumes. Consequently, this investigation will take account of the knowledge and technology developed for soy and other legumes in order to define the functionality and potential food applications of chickpea proteins.

CHAPTER 3

PROTEIN EXTRACTION AND CHARACTERISATION

PROTEIN EXTRACTION AND CHARACTERISATION

As presented in Chapter 2, food protein concentrates and isolates, including those of oil seeds and grain legumes, could be prepared by several procedures (Fig 2.5). Although these procedures may have some unique features, they all consist of three common essential steps: solubilisation of the protein from the raw material, concentration of the solubilised proteins and finally purification of the concentrated proteins. The selection of a particular procedure depends on the composition of raw material and also on the end uses of the isolated proteins.

3.1 WATER ABSORPTION

Regardless of the end use, soaking grain legumes in water is common in enhancing protein extraction. Since soaking is a long process and soaking conditions affect nutritional qualities and physical properties of end products, the soaking process needs to be characterised for practical applications including protein extractions (Sefa-Dedeh *et al.*, 1978; Kon, 1979). Water absorption in this soaking process needs to be predictable as a function of time and temperature.

Water absorption of soybean, pigeon pea, cowpea and rice has been investigated and several rather complex models, mainly based on Fick's law of diffusion, have been suggested to describe it (Hsu, 1983; Singh and Kulshrestha, 1987; Sefa-Dedeh and Stanley, 1979; Hendrickx *et al.*, 1987). Peleg (1988) proposed a simple, empirical equation not derived from any set of physical laws or diffusion theories, to model water absorption of food materials. The model has been used to predict long range moisture absorption from experimental data obtained over a short time.

The predicted water absorption of several chickpea varieties grown in Australia was calculated using the following equation proposed by Peleg as the first step in the selection of a suitable variety for protein extraction:

$$M(t) = M_0 + t / [K_1 + K_2t] \quad [3-1]$$

where

$M(t)$ is the moisture content at time t ,

M_0 is the initial moisture content,

K_1, K_2 are constants

$$\text{and } M_{eq} = M_0 + 1/K_2 \quad [3-2]$$

where M_{eq} is the equilibrium moisture content as $t \rightarrow \infty$

Equation [1] can also be transformed to the linear relationship:

$$t/[M(t) - M_0] = K_1 + K_2 t \quad [3-3]$$

where $1/K_1$ is the initial rate of absorption. The unit of K_1 is hr/% weight and that of K_2 is the reciprocal of % weight.

Australia is a vast country. The relatively recently introduced chickpeas have been grown at several different climatic regions. The first step in this investigation is to select a variety which can be produced economically and also contain a high protein level.

Seven chickpea (*Cicer arietinum*) and three field pea (*Pisum sativum*) cultivars were studied for water absorption characteristics. All chickpeas, consisting of three Desi (small grain) cultivars (Tyson, Dooen and Amethyst), three Kabuli (large grain) cultivars (Kaniva, Macareena and Garnet) and a cultivar intermediate between Desi and Kabuli (Semsen) were grown in Kaniva, Victoria, Australia

Table 3.1.1. Chemical composition (%) of chickpeas and field peas

Grain	Cultivar	Moisture	Ash	Fat	Protein	Carbohydrate	Relative size ²
Chickpea Desi	Tyson	8.60	2.52	3.27	23.1	62.5	377
	Dooen	8.38	2.62	2.98	23.1	62.9	284
	Amethyst	8.34	2.59	3.29	23.3	62.5	391
Intermediate	Semsen	8.03	2.64	3.07	23.8	62.4	231
Kabuli	Kaniva	8.15	2.86	5.05	23.7	60.2	139
	Macareena	8.37	2.91	4.56	23.2	61.0	112
	Garnet	8.39	2.76	4.54	23.4	61.0	137
Field pea	Dun	8.85	2.71	0.98	24.1	63.4	215
	Dundale	8.73	2.63	0.88	23.9	64.0	233
	Progetta	8.52	2.55	0.97	24.0	63.0	186

¹ Carbohydrate calculated by difference ² Number of seeds in 50 g

3.1.1 MOISTURE ABSORPTION CURVES

Gross chemical compositions of the seven chickpea and three field pea cultivars (Table 3.1.1) showed no differences ($P < 0.05$) in moisture, fat, protein, and carbohydrate among the cultivars. The fit of equation [3.3] to experimental data on water absorption of the seven chickpea cultivars was demonstrated (Fig 3.1.1). Values of constants K_1 and K_2 derived from the linear fit (Table 3.1.2 and 3.1.3) showed regression coefficient ranges from 0.974 to 1.000 (Table 3.1.2) and the degree of fit was generally good. The plots of $t/[M(t) - M_0]$ vs. t also showed no apparent curvature. The capacity of the Peleg model [3.1] to predict the entire water absorption process, using limited data, is demonstrated in the characteristic moisture absorption curves for chickpea (Macareena and Dooen, Fig 3.1.2) at 3 different temperatures. The moisture absorption curves show that water content increases with increased soaking time and temperature. Similar results have been reported for soybean, peanut, pigeon pea, rice and cowpea (Singh and Kulshrestha, 1987; Engels *et al.*, 1987; Hendrickx *et al.*, 1987; Sopade and Obekpa, 1990).

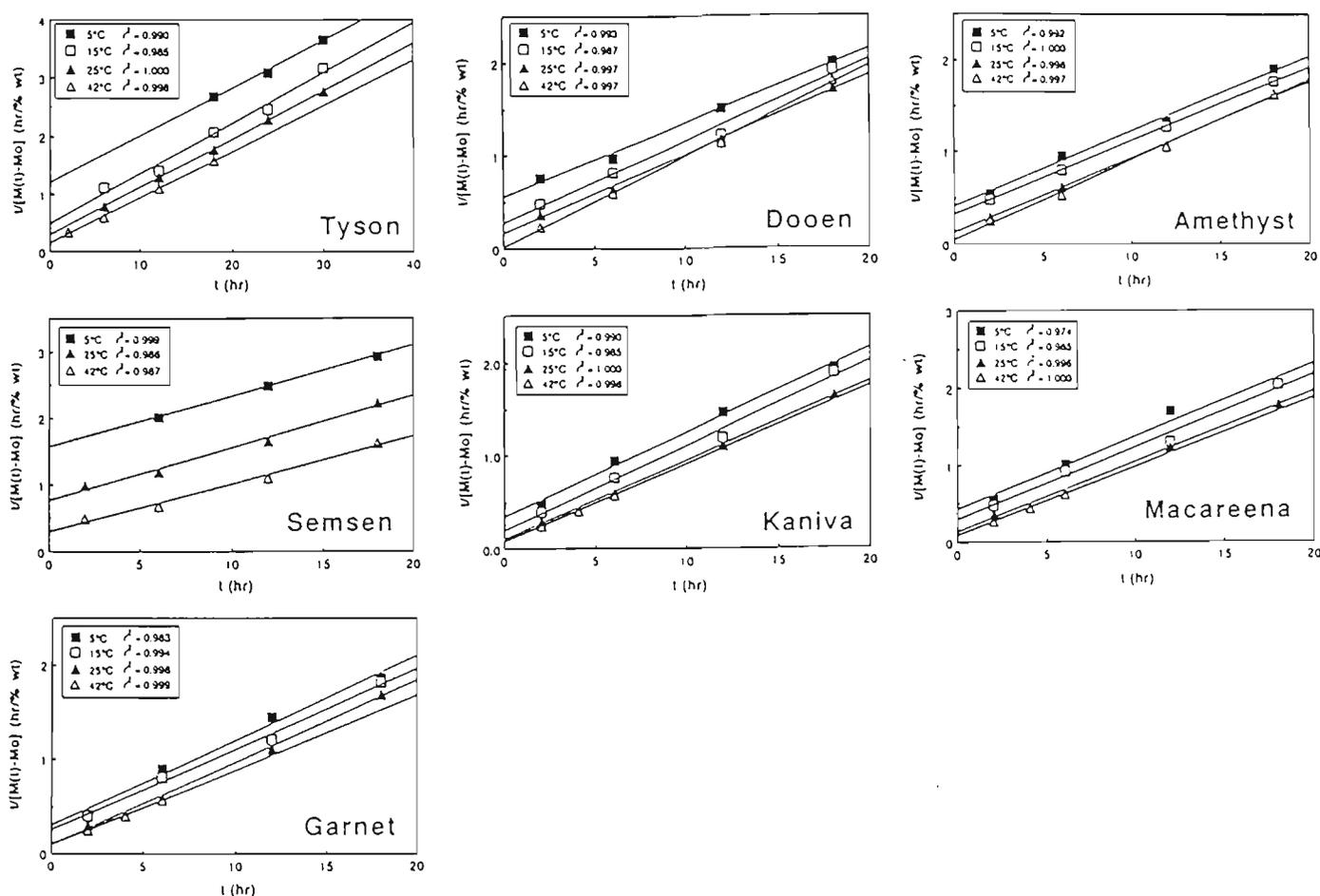


Fig 3.1.1 Fit of the linear model of Peleg's equation to observed water absorption of different cultivars.

Data for the Tyson cultivar of chickpea at 5°C were not collected at soaking times < 12 hour since weight gains at shorter times were small and Peleg indicated that the model did not apply under such conditions (Fig 3.1.1, Tyson). Generally, for the Desi type, the equation could give a fairly good fit after a rather long period of soaking (6-12 hour) and for the Kabuli type during the first 6-7 hour of soaking.

Table 3.1.2 Peleg constants (K_1 , K_3) and initial absorption rate ($1/K_1$) of chickpeas and field pea

Grain	Cultivar	°C	K_1 (hr/% wt)	$1/K_1$ (% wt/hr)	K_3 (hr/% wt°C)
Chickpea Desi	Tyson	42	0.15	6.67	1.2×10^{-2}
		25	0.30	3.38	
		15	0.49	2.06	
		5	1.19	0.84	
	Dooen	42	0.01	72.3	1.3×10^{-2}
		25	0.16	6.12	
		15	0.28	3.62	
		5	0.55	1.82	
	Amethyst	42	0.04	27.6	1.1×10^{-2}
		25	0.12	8.26	
		15	0.32	3.13	
		5	0.41	2.45	
Intermediate	Sensen	42	0.20	3.39	3.4×10^{-2}
		25	0.76	1.31	
		5	1.56	0.64	
Kabuli	Kaniva	42	0.07	14.9	0.7×10^{-2}
		25	0.10	9.91	
		15	0.19	5.15	
		5	0.34	2.94	
	Garnet	42	0.09	11.0	0.6×10^{-2}
		25	0.10	10.2	
		15	0.25	4.02	
		5	0.30	3.36	
	Macareena	42	0.08	12.6	0.8×10^{-2}
		25	0.13	7.66	
		15	0.29	3.42	
		5	0.38	2.66	
Field pea	Dun	42	0.38	2.60	4.4×10^{-2}
		25	0.66	1.50	
		15	1.74	0.57	
	Dundale	42	0.37	2.67	3.3×10^{-2}
		25	0.68	1.48	
		15	1.33	0.75	
	Progretta	42	0.10	9.94	2.4×10^{-2}
		25	0.22	4.50	
		15	0.44	2.29	

Table 3.1.3 Peleg constant K_2 and calculated equilibrium moisture content (Meq) of chickpeas and field peas

Grain	Cultivar	°C	K_2 (% wt ⁻¹)	Mean K_2 (% wt ⁻¹)	Meq (% wt)	Mean Meq (% wt)
Chickpea Desi	Tyson	42	7.9×10^{-2}	8.2×10^{-2}	13.7	13.1
		25	8.2×10^{-2}		13.0	
		15	8.6×10^{-2}		12.5	
		5	8.1×10^{-2}		13.2	
	Dooen	42	9.7×10^{-2}	8.7×10^{-2}	11.2	12.9
		25	8.5×10^{-2}		12.7	
		15	8.8×10^{-2}		12.3	
		5	8.0×10^{-2}		13.4	
	Amethyst	42	8.8×10^{-2}	8.3×10^{-2}	12.3	12.9
		25	8.2×10^{-2}		12.3	
		15	8.0×10^{-2}		13.3	
		5	8.2×10^{-2}		13.0	
Intermediate	Sensen	42	7.1×10^{-2}	7.5×10^{-2}	15.0	14.2
		25	6.8×10^{-2}		13.6	
		5	7.6×10^{-2}		14.0	
Kabuli	Kaniva	42	8.4×10^{-2}	8.8×10^{-2}	12.7	12.2
		25	8.5×10^{-2}		12.6	
		15	9.1×10^{-2}		11.8	
		5	9.1×10^{-2}		11.8	
	Macareena	42	9.0×10^{-2}	9.2×10^{-2}	12.0	11.7
		25	9.1×10^{-2}		11.8	
		15	9.4×10^{-2}		11.5	
		5	9.5×10^{-2}		10.6	
	Garnet	42	7.9×10^{-2}	8.6×10^{-2}	13.5	12.5
		25	8.7×10^{-2}		12.3	
		15	8.6×10^{-2}		12.5	
		5	9.0×10^{-2}		11.9	
Field pea	Dun	42	6.9×10^{-2}	7.1×10^{-2}	15.3	15.1
		25	8.5×10^{-2}		12.5	
		15	8.8×10^{-2}		18.1	
	Dundale	42	7.3×10^{-2}	7.6×10^{-2}	14.6	14.0
		25	8.2×10^{-2}		13.1	
		15	7.4×10^{-2}		14.4	
	Progetta	42	7.7×10^{-2}	7.1×10^{-2}	13.9	14.4
		25	7.3×10^{-2}		14.6	
		15	7.3×10^{-2}		14.6	

3.1.2 SEED SIZE AND WATER ABSORPTION RATE, WATER UPTAKE

Differences in water absorption rates and water uptake among chickpea cultivars are exemplified by Macareena and Dooen (Fig 3.1.2). Sopade and Obekpa (1990) suggested that the difference in water absorption between soybean and peanut was due to differences in fat

and protein contents. However, differences in fat, protein and carbohydrate among our chickpea cultivars were not significant (Table 3.1.1). The initial moisture content of the beans would affect the rate of absorption (Smith and Nash, 1961). In our study, the difference in initial moisture was also insignificant. Consequently, there is probably a factor other than gross fat, protein and carbohydrate that contributes to differences in water absorption of different chickpea cultivars.

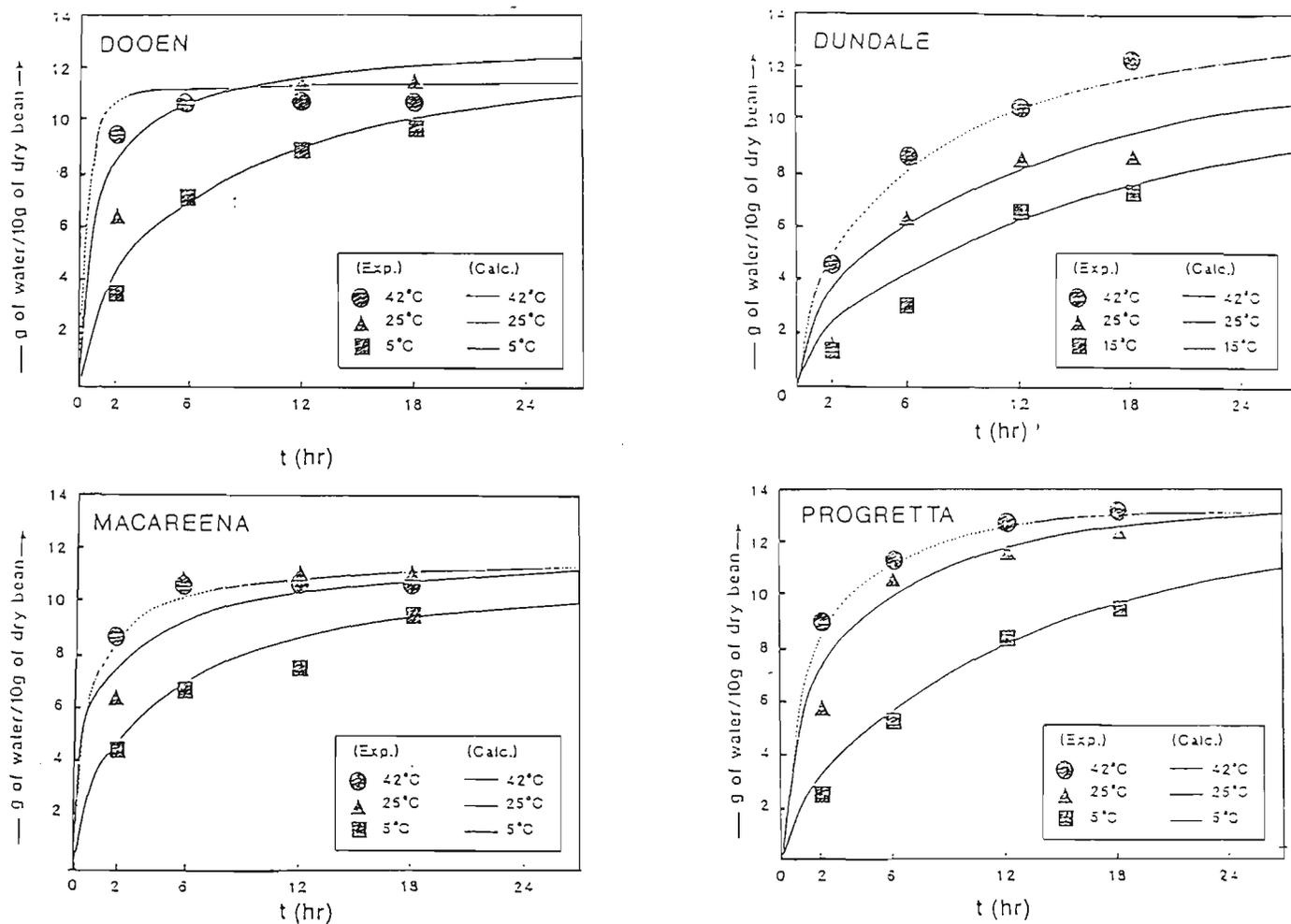


Fig 3.1.2 Experimental and predicted water absorption characteristics of two chickpea cultivars (Dooen and Macareena) and two field pea cultivars (Dundale and progetta).

Seed size should be considered because water absorption rate is influenced by surface area. The smaller the seed, the larger the relative surface area for given seed weight (Ituen *et al.*, 1985). The reciprocal of constant K_1 derived from equation [3-3] is used to present the initial absorption rate (Table 3.1.2). The results clearly indicate that the effect of temperature on initial absorption rate, increases with increasing temperature. The large seed cultivars (Kabuli type) had higher absorption rates than the smaller seed cultivars (Desi type). Average $1/K_1$ values of three Kabuli cultivars at 15 and 25°C were 4.19 and 9.25% wt/hr respectively.

while the corresponding $1/K_1$ values for the three Desi cultivars were 2.93 and 5.91% wt/hr respectively (Table 3.1.2). This finding could not be compared with other studies which focused on water absorption between different types of grain legumes rather than different cultivars. However, it suggested that seed size or the water absorption surface of the seed coat alone was not a major factor affecting water absorption, at least among chickpeas.

King and Ashton (1985) studied water absorption by whole soybeans and soybeans with seed coat partially abraded. They reported that the rate of absorption by the abraded seeds was more than that for unabraded seeds. Similar results have been reported by Singh and Kulshrestha (1987). Sopade and Obekpa (1990) reported no major difference in absorbed water between dehulled and whole peanuts. Chickpea seed coat is much thicker than that of the peanut seed which contributes only 5% of seed weight while chickpea seed coat contributes up to 16.4% (Singh *et al.*, 1981; Chavan *et al.*, 1986). In an earlier study on water absorption of soybean, Smith and Nash (1961) reported that the principal factor controlling absorption in whole bean was the seed coat. The ratio of coat thickness to seed size and, to some extent, seed coat structure were the only obvious differences between the Desi and Kabuli chickpea types. Polyacrylamide gel electrophoretic analysis did not distinguish between the two types of chickpea by protein patterns (Jambunathan and Singh, 1981). Gross chemical composition also showed no significant differences. The seed coat in Desi seeds contributed 16% of seed weight while in the Kabuli seed the average was 7% (Jambunathan and Singh, 1981). The fact that all Kabuli chickpeas had an unexpectedly higher water absorption than Desi chickpeas, suggested that the thickness of the seed coat and also the seed coat structure had critical effects on water uptake and absorption rate (Singh and Kulshrestha, 1987; Gandhi and Bourne, 1991).

3.1.3 EFFECT OF TEMPERATURE

A constant K_3 ($K_1 = K_3T + K_4$) was developed to describe the temperature effect on the initial water absorption rate by plotting K_1 against temperature (Fig 3.1.3). A straight line with K_4 as the ordinate intercept and K_3 as the gradient was obtained. This confirmed that the Peleg constant K_1 varied with temperature as reported by Sopade and Obekpa (1990). Note that at a given temperature, the lower the K_1 , the greater the amount of water absorbed.

The temperature effect on water absorption of Desi type chickpea was greater than that of the Kabuli type. All constants K_3 for the Kabuli type ($\leq 0.8 \times 10^{-2}$) were lower than those of the Desi type ($\geq 1.1 \times 10^{-2}$). Constant K_3 for the field pea ranged from $2.4 - 4.4 \times 10^{-2}$. There was a clear correlation between constant K_3 and the seed number of different cultivars (Desi/Kabuli) as well as the grain type (chickpea/field pea). Since there was no significant

difference in protein patterns or gross chemical compositions between Desi and Kabuli chickpea, this constant could serve as a characterising parameter for grain type based upon water absorption behaviour (Fig 3.1.4).

In contrast to K_1 and K_3 , constant K_2 was almost unaffected by temperature with mean values ranging from 8.2 to $9.2 \times 10^{-2} \% \text{ wt}^{-1}$ for chickpea and 7.1 to $7.6 \times 10^{-2} \% \text{ wt}^{-1}$ for field pea (Table 3.1.3). The independence of K_2 with respect to temperature, indicated that the same equilibrium moisture content would be obtained regardless of the soaking temperature. The equilibrium moisture content of each cultivar was obtained by inserting the K_2 value into equation [3-2]. The mean equilibrium moisture content of chickpea was 12.4% wt and that of field pea was 14.5% wt. Note that the Semsen cultivar with seed size nearer that of field pea than Desi or Kabuli chickpeas also had both K_2 value ($7.5 \times 10^{-2} \% \text{ wt}^{-1}$), and equilibrium moisture content (14.2% wt) closer to those of field peas.

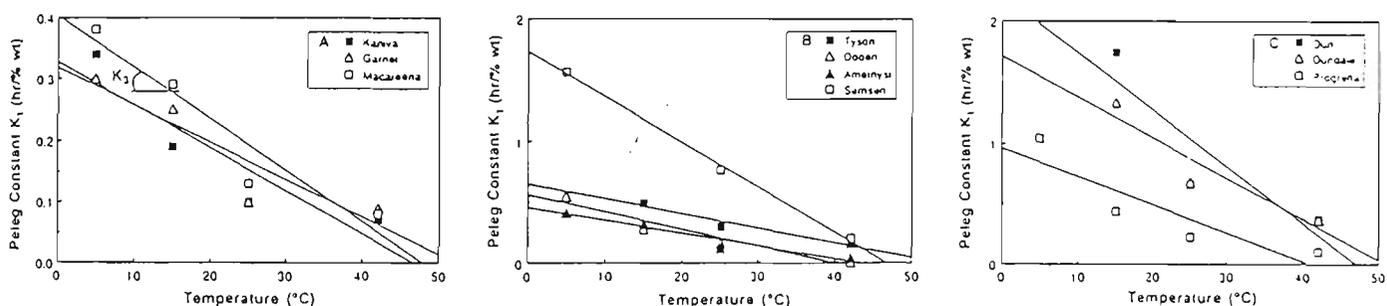


Fig 3.1.3 Effect of temperature on the Peleg constant K_1 of chickpeas and field peas; (a) Kabuli type; (b) Desi type; (c) Field pea.

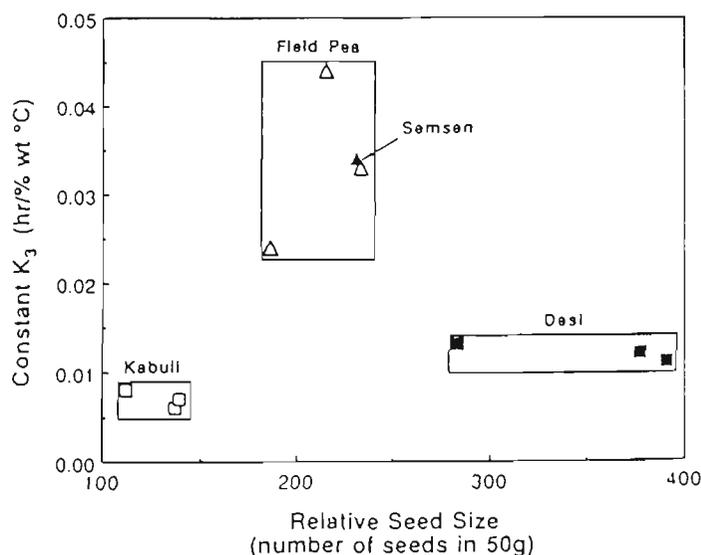


Fig 3.1.4 Correlation between seed size of chickpea or field pea and constant K_3 .

3.1.4 ' SUMMARY OF WATER FINDINGS AND SELECTION OF THE REPRESENTATIVE SAMPLE

Moisture absorption in seven chickpea cultivars and three field pea cultivars was investigated at 5, 15, 25 and/or 42°C using the Peleg model ($M(t) = M_0 + t/[K_1 + K_2t]$). The Peleg constant K_1 varied with temperature. At a given temperature, the lower the K_1 , the more water was absorbed. The Peleg constant K_2 was almost unaffected by temperature and could be used to predict the equilibrium water absorption. A constant K_3 expressing the temperature effect on water absorption ($K_1 = K_3T + K_4$) was developed to distinguish two types of chickpea - Desi and Kabuli. All chickpeas had similar composition and initial moisture. The difference in water absorption rate was probably due to thickness and structure of the seed coat. The Peleg model could be used to predict water absorption in chickpea and field pea.

The water absorption of chickpea and field pea cultivars could be described by using the Peleg model. The initial moisture absorption rate ($1/K_1$) and constant K_3 were influenced by the soaking temperature. Constant K_2 was almost unaffected by temperature. Using only short term soaking data, the Peleg model could provide a useful approach to calculating the equilibrium moisture content of chickpea and field pea at any soaking temperature.

The results revealed that all seven chickpea varieties, investigated in this section have a similar chemical composition and also have a rather constant level of proteins (Table 3.1.1). The selection of a representative variety had to be based on other factors other than solely chemical composition or the protein level. Since the Kaniva is a very popular chickpea variety in Victoria, consequently, the seed of this cultivar was selected for this investigation.

3.2 EXTRACTION

3.2.1 PRELIMINARY EXTRACTION PROCEDURE

Preliminary experiments were conducted to determine the optimum conditions for extraction, concentration and purification of chickpea proteins based on a wet method, widely employed to extract legume proteins.

Defatted chickpea grits with different sizes, prepared from the Kaniva variety, grown in Horsham, Victoria were used as raw materials. The grits were subjected to protein extraction

over a pH 2-12 range and the soluble nitrogen in the extracts at each pH was measured to establish a solubility profile.

The extractability of two chickpea grits over a pH 2-12 range is illustrated in Fig 3.2.1. A typical U shape, observed in many grain legume extractions, was obtained with both grits. Like soybean and other grain legume proteins, extractability of chickpea protein is a function of extraction pH, bearing a common characteristic (Deshpande and Cheryan, 1984). More proteins (64-73%) were extracted at high pHs (pH>8) or low pHs (pH<3). The protein extractability was low (5-7%) around pH 4 and the lowest extraction yield (<1%) was found at pH 4.4 when the extracted protein reached its isoelectric point (pI).

The extractability of a protein is dictated by its solubility which is ultimately an interaction between the protein and the solvent. When extracted in water, the extractability involves two types of interactions: protein-water and protein-protein. As shown in Fig 3.2.1, chickpea proteins were more soluble at pH>8 and at pH<3. At these pHs, the net charge of protein molecules was either negative or positive which enhances the repulsive forces between protein molecules. This creates unfavourable conditions for protein aggregation but favours either protein solubilisation or protein stability in dispersion. Proteins were more soluble at high alkaline pHs (75%) than at low acidic pHs (65%). Carbarano *et al.* (1993) found that some insoluble protein aggregates, bounded by hydrophobic interactions or by hydrogen bonds, could be resolved into soluble components. Likewise, Prakash and Narasinga Rao (1986) also found that the alkali peptidisation at high alkaline pH was predominantly responsible for the increase in oil seed protein solubility. These factors may contribute to the higher extraction yields at pHs>9.

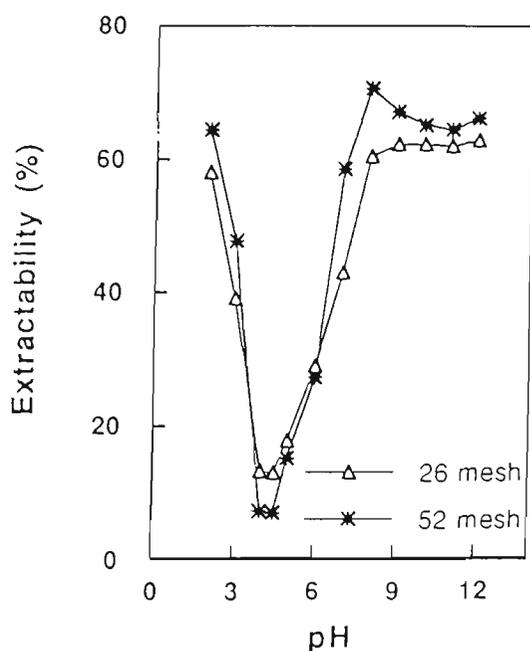


Fig 3.2.1 The protein extractability of two chickpea grits as a function of extraction pH.

In contrast, proteins were much less soluble in a region close to pH 4. In this region the net charge of proteins approaches neutrality and the repulsive forces between proteins are replaced by attractive forces, creating favourable conditions for protein aggregates. This common phenomenon is encountered with many food proteins.

The results confirmed that chickpea protein behaviour in aqueous medium was similar to those of soybean, faba bean, peanut, lupin and other seed proteins. Thus, an extraction procedure could be devised in which chickpea proteins could be effectively extracted at either low acidic pHs or high alkaline pHs and could be effectively concentrated at pH 4.

3.2.2 FACTORS AFFECTING EXTRACTION YIELD

Chickpea proteins, like most proteins in foods, are complex and heterogeneous mixtures. Therefore, their extractability is remarkably influenced by the environmental conditions, extraction pH, temperature, duration and extraction medium (Lindsay *et al.*, 1977; Tu, 1978; Ibrahim *et al.*, 1986). The composition, the size of extraction particles and other pre-treatments also affect the extraction yield (Pokharkar and Suresh, 1991; Ibrahim *et al.*, 1986). Extractability could be simply defined in terms of the amount of protein from the raw material solubilised into the extraction medium under given conditions (Kinsella, 1984). Based on results of the preliminary experiments, certain factors affecting the extraction results were investigated. These factors were extraction pH, temperature (15, 35 and or 50°C), extraction medium (water and 1M NaCl solution), time of extraction (45 and 90 minutes) and particle sizes (14, 26 and 52 mesh). The effects of some combined factors were also evaluated. The extractions were conducted at three specific pHs: pH 2, pH 9 and pH 7. Extraction at the first two pHs was expected to give a high yield of protein and pH 7, being neutral, is the most commonly encountered pH in the food industry. Extraction at pHs > 9 may give a higher yield but the products obtained may have lower nutritional values (Gould and MacGregor, 1977; Ayamard *et al.*, 1978; Lawrence and Jelen, 1982)

3.2.2.1 Extraction in water

3.2.2.1.1 Effect of pH

The effect of extraction pH on the extraction yield at three temperatures (15, 35 and 50°C) with different size chickpea grits is given in Fig 3.2.2 a, b and c. Since extractability is a function of pH, different yields were obtained at different pHs. Regardless of the extraction conditions, the highest yields were obtained at pH 9 and the lowest yields were obtained at pH 7. The difference in yields obtained at a given condition, indicating that extractability was

also influenced by factors other than pH. The results confirmed the preliminary observations (Fig 3.2.1) and could be explained by the protein net charge and protein-protein interactions.

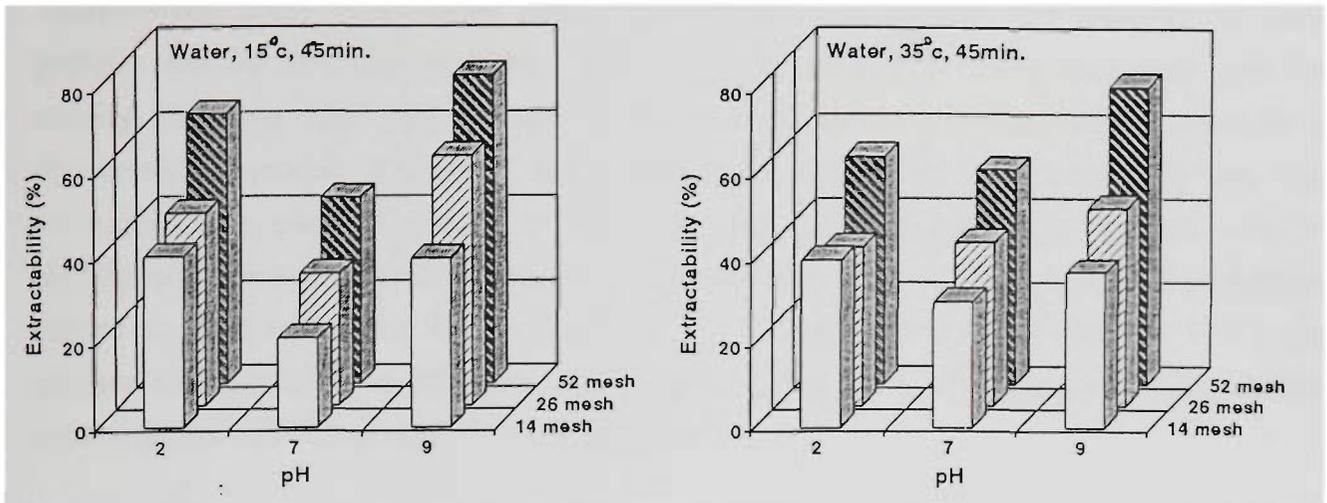


Fig 3.2.2 a-b. The effect of extraction pH on the protein yield of three chickpea grits at 15 and 35°C.

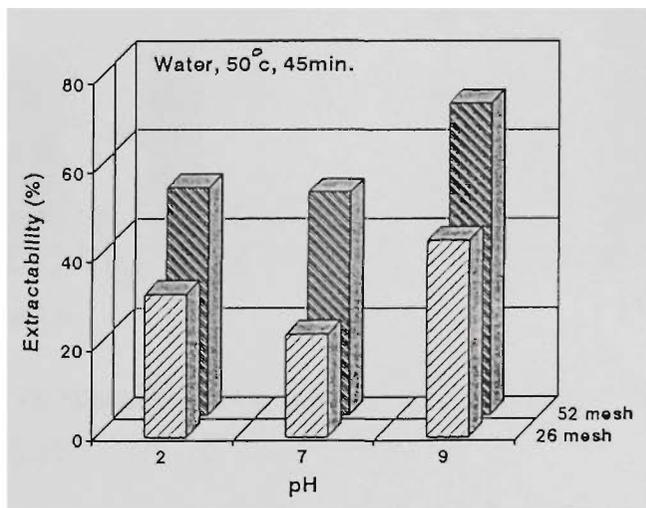


Fig 3.2.2 c. Effect of extraction pH on the protein yield of two chickpea grits (26 and 52 mesh) at 50°C.

3.2.2.1.2 Effect of temperature

The effect of extraction temperature on extraction yield of chickpea protein at 15, 35 and 50°C is given in Fig 3.2.3 a, b and c, respectively. At a similar extraction condition, temperature alone showed insignificant effect on protein yield but when combined with other factors such as particle size and extraction pH, some minor effects were observed. For example, more proteins were obtained at pH 7 from 52 mesh grit at 35°C and 50°C than at 15°C but at pH 2

less proteins were extracted at 50 and 35°C than at 15°C. At all three pHs, proteins from 26 mesh grits were less extractable at 50°C than at either 35°C or 15°C.

Temperature could considerably affect the extractability because the solubility of some proteins can be thermally changed. Increasing the temperature may destabilise proteins, causing unfolding which may promote protein-protein interactions, thus leading to an ease in the formation protein aggregates and precipitation. Increasing the temperature may also strengthen hydrophobic interactions since the water is forced into closer contact with the hydrophobic core of the protein. However, the extraction temperature showed no significant effects as reported earlier for soy (4-40°C) and lupin (20-80°C) (Manrique, 1977) and moderate effects in this investigation. Consequently there is no advantages to extract chickpea protein at low (15°C) or high (50°C) temperature.

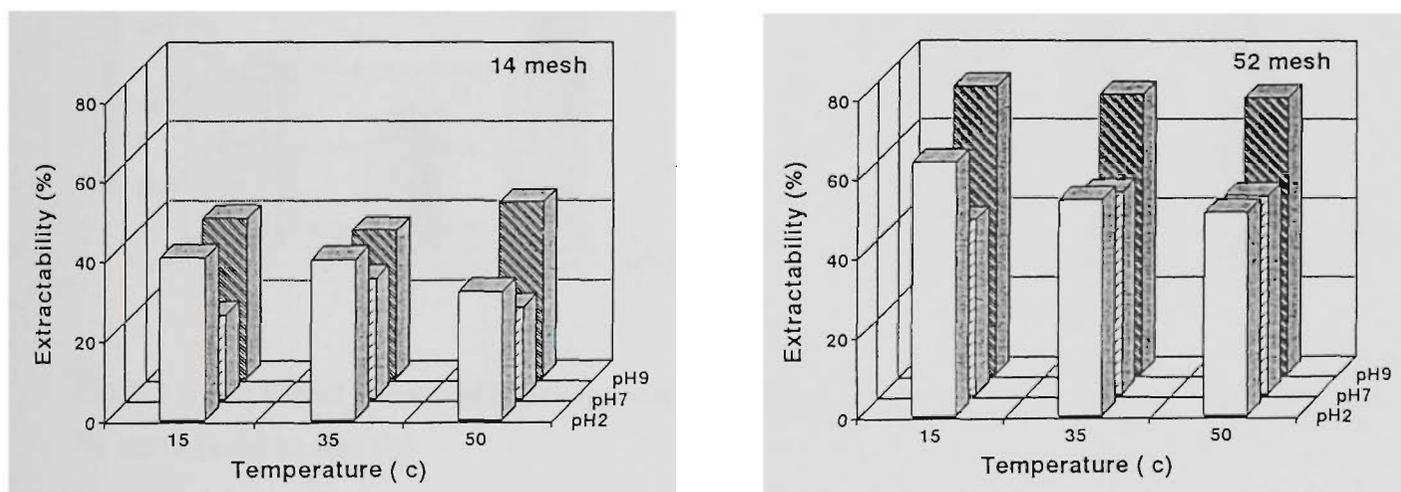


Fig 3.2.3 The effect of extraction temperature on the protein yield of two chickpea grits (14 and 52 mesh) at pH 2, pH 7 and pH 9.

3.2.2.1.3 Effect of grit particle size

The effect of particle size on the extractability yield is given in Fig 3.2.2 a, b and c. In all investigation conditions, high yields were always obtained with grits having small particle size (52>26>14 mesh). To solubilize the protein from grits, the first step must be the penetration of solvent into the particle which depends on the contacting surface area. Another factor is the distance the solubilised particles have to pass into the solution and also the matrix in which the proteins are trapped. Smaller particles would have larger interacting surface area, less distance for solubilised proteins to travel into the solution and perhaps contain a loosened matrix. These factors may contribute to the high yield obtained with small particle grits.

3.2.2.1.4 Effect of extraction time

The effect of extraction time, under different conditions of grit particle size (52 and 26 mesh) and extraction temperature (15 and 35°C) are given in Fig 3.2.4 a-b and c-d. For the 52 mesh grits, at pH 2 and pH 9 and at both temperatures of 15°C and 35°C, the extractable proteins remained almost constant after 45 minutes of extraction. There is no advantage in prolonging the extraction time to more than 45 minutes. However more proteins could be extracted at pH 7 with extended extraction time (90 min). For larger grits, the protein extractability was improved under similar conditions when the extraction time was extended to 90 minutes. These results reflected the combined effects of particle size, extraction pH and time.

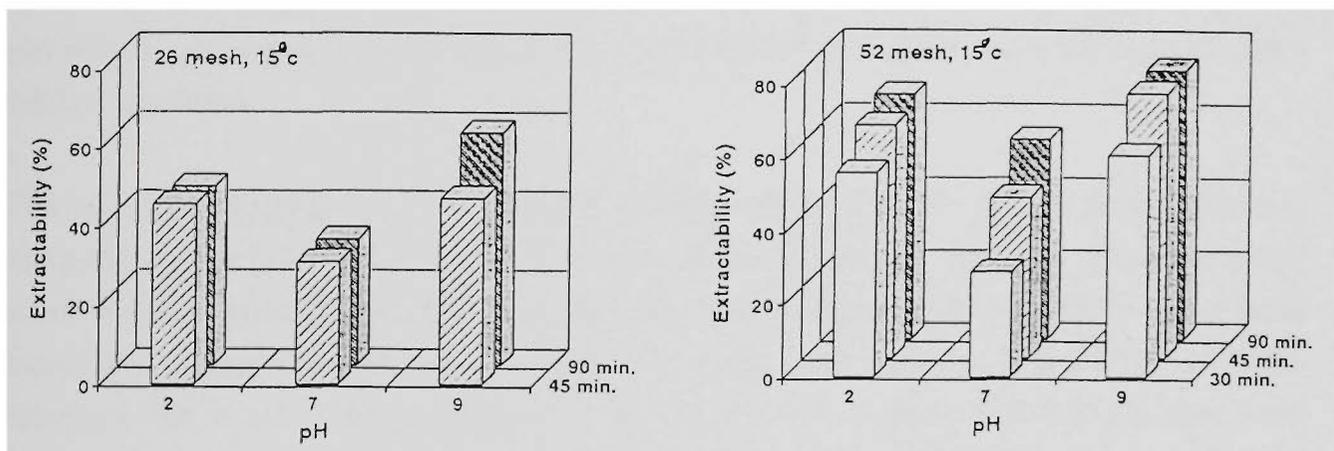


Fig 3.2.4 a-b Effect of extraction time on chickpea protein yield at 15°C with 26 mesh (a) and 52 mesh particle size (b).

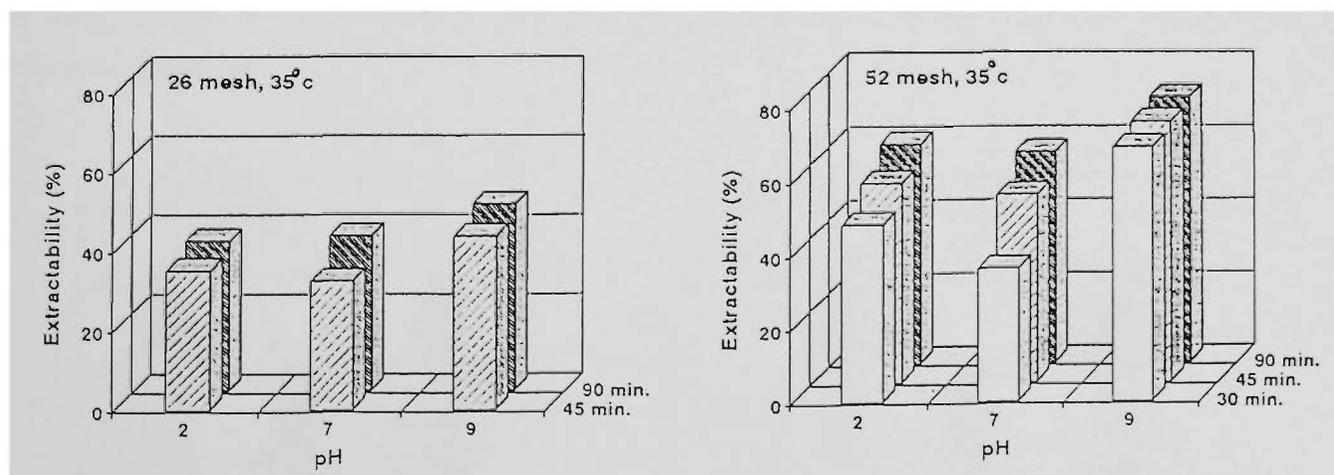


Fig 3.2.4.c-d Effect of extraction time on chickpea protein yield at 35°C with 26 mesh (c) and 52 mesh particle size (d).

3.2.2.2 Extraction in salt solution

3.2.2.2.1 Effect of pH and particle size

Salt can influence protein solubility by affecting the balance between charge frequency and hydrophobicity. The solubility can be increased (salting-in) or decreased (salting-out) in the presence of salt ions. The reaction is influenced by a three way interaction: water-salt, salt-protein and protein-water. Salt solution (0.5-2M) is, therefore, a widely used medium for protein extraction. Chickpea protein from grit of two different sizes (52 and 26 mesh) was extracted at three pHs (pH 2, pH 7 and pH 9) and two temperatures (15°C and 35°C) with 0.1M NaCl solution. The results are presented in Fig 3.2.5a-b. The effect of extraction pH was found to be similar to those in water extraction, more protein was extracted at pHs>8 and pHs<3. However the extraction yield in salt medium was lower than that in water under similar conditions.

The low extraction yield could be a result of many different factors. In a protein dispersion, inorganic anions tend to bind to protein more strongly than do inorganic cations in a salt solution due to their smaller radii. Consequently, the binding of chloride anions to the protein molecules would increase their net negative charge and hence increase their solubility. However the fixed, charged sides ready for ions to bind selectively and strongly were relatively small; an increase in solubility may only be obtained at low NaCl concentrations (<0.1M). When the salt concentration increases, all of the charged sites are filled, the excess chloride anions will compete with protein for the free water in the system and a salting out effect will be observed. Megen-Van (1974) found that the solubility of proteins appeared to be independent of salt concentrations under certain low salt contents but decreased at high salt concentrations.

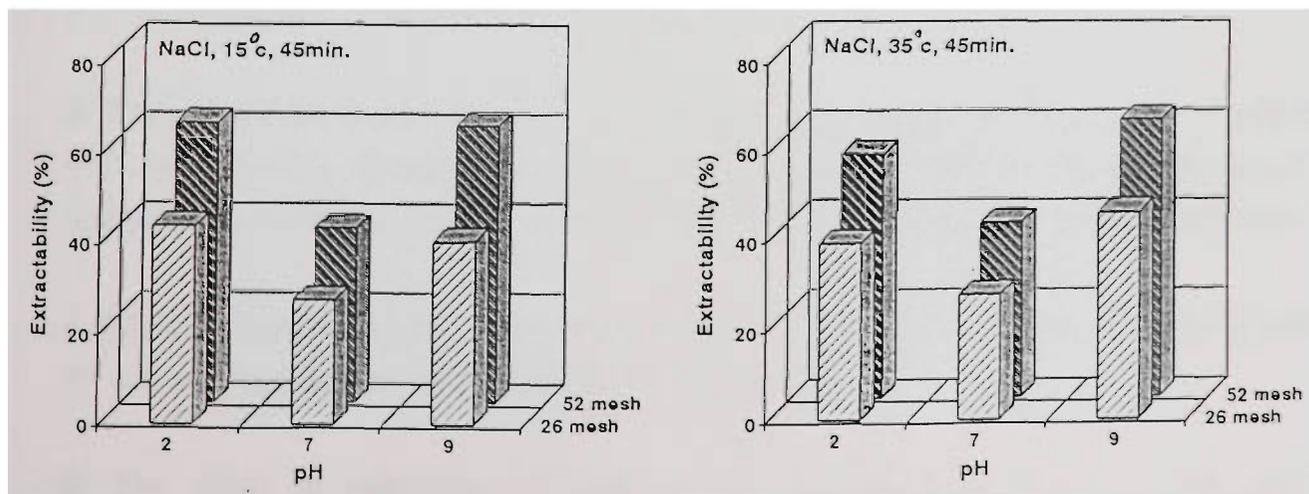


Fig 3.2.5 a-b Effect of extraction pH, temperature (15°C-35°C) and particle size (26-52 mesh) on the extractability of chickpea proteins in salt solution (0.1M NaCl).

Particle size again showed a significant effect on extraction yield. A smaller grit size gave better yields at all extraction pHs and temperature. Yield increased about 50% at all three extraction pHs and at both 15°C and 35°C when the grit particle size decreased from 26 mesh to 52 mesh.

3.2.2.2.2 Effect of extraction temperature and extraction time

Prolonging extraction time from 45 minutes to 90 minutes in salt solutions only increased the extraction yield by less than 10%. The increase in yield due to extraction time did not show a uniform pattern in terms of extraction pH or temperature. Interestingly, more proteins were extracted at pH 2 at lower temperature (15°C) than at higher temperature (35°C). In contrast less proteins were extracted at pH 7 and pH 9 at the same temperatures. There is no significant advantage in increasing the extraction time.

3.2.3 STATISTICAL ANALYSIS

The results presented in this section indicate that the extractability of protein is greatly influenced by several factors. It is difficult to describe the effect of a single factor on the yield since the extraction process is always multifactorial. Thus, the combined effect of two or more factors was examined, using factorial design, by constructing groupings to include every combination of all factors at all the levels (Table 3.2.1). The result of forty eight combinations were analysed with a factorial analysis of variance. The results are presented in Table 3.2.2. The main effect shown by the factorial analysis are as follows:

- a. The particle size effect was the most significant ($F_{(1,47)} = 920.52$, $p < 0.001$). When the particle size reduced from 52 mesh to 26 mesh, extraction yield increased from 38.5% to 57.5%, an increase of approximate 49%.
- b. The pH and the solvent effects were the second most significant ($F_{(2,47)} = 272.7$ and $F_{(1,47)} = 71.58$, $p < 0.001$). Extraction yield at pH 2, pH 7 and pH 9 were 49.7%, 38.4% and 56.1%, respectively. Extraction yield in water (50.7%) was higher than in salt solutions (45.4%).
- c. The duration of extraction was not significant ($F_{(91,47)} = 5.08$, $p < 0.05$). Extraction yield for 45 min and 90 min were 47.3% and 48.7%, respectively.
- d. The effect of extraction temperature was not significant ($F_{(1,47)} = 2.08$, $p = 0.17$). Extraction yield at 15°C and 35°C were 48.5% and 47.6%, respectively.

The interactions between pH and temperatures, particle size and solvent, pH and particle size, pH and solvent, particle size and time had significant effects on extraction yield (Table 3.2.2).

a. Effect of pH and extraction medium.

The combined effect of pH and extraction medium was most significant at pH 7. Extraction yield in water was 25%, 6% and 8% higher than that in salt solutions at pH 7, pH 2 and pH 9, respectively.

b. Effect of pH and extraction temperature.

The combined effect of pH and extraction temperature varied with different extraction media.

Table 3.2.1 The results of protein extractability (% of total protein) under all different conditions investigated..

pH	Extraction Solution		Water				0.1M NaCl			
	Temperature (°C)	Time (minute)	15		35		15		35	
			45	90	45	90	45	90	45	90
2	Particle	26	45.5	45.3	37.8	35.1	43.3	39.8	39.0	38.2
	Size (mesh)	52	63.8	66.8	54.1	59.6	61.7	60.7	54.3	50.0
7	Particle	26	31.1	32.0	38.9	32.5	27.3	26.9	27.5	29.0
	Size (mesh)	52	44.0	54.8	50.9	57.3	38.3	40.3	38.8	44.1
9	Particle	26	40.1	47.1	47.0	43.7	40.2	44.0	46.1	47.1
	Size (mesh)	52	72.7	73.7	70.6	72.1	61.1	63.1	61.7	66.6

3.2.4 CONCLUSIONS

Extractability of chickpea protein was influenced by several factors.

(i) Of those investigated, extraction pH and particle size of the grits were the most significant factors. The highest yield was obtained at pH>9 or pH<3.

(ii) When the particle size was reduced from 26 to 52 mesh, the yield was significantly increased

Table 3.2.2 Statistical analysis of the combined effects of several extraction parameters on the extraction yield of chickpea protein.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
pH	2	572	1286	72.7	<.001
Medium (Med.)	1	337	338	71.6	<.001
Temperature (Temp.)	1	9.81	9.81	2.08	0.177
Particle size (P.size)	1	4341	4341	920	<.001
Time	1	23.9	23.9	5.08	0.046
pH, Med.	2	75.9	37.9	8.04	0.007
pH, Temp.	2	254	127	26.9	<.001
Med., Temp.	1	3.47	3.47	0.74	0.410
pH, P.size	2	127	63.3	13.4	0.001
Med., P.size	1	108	108	22.9	<.001
Temp., P.size	1	8.42	8.42	1.78	0.209
pH, Time	2	22.4	11.2	2.37	0.139
Med., Time	1	3.58	3.57	0.76	0.403
Temp., Time	1	5.81	5.81	1.23	0.291
P.size, Time	1	33.8	33.8	7.17	0.021
pH, Med., Temp.	2	22.4	11.2	2.38	0.138
pH, Med., P.size	2	15.4	7.68	1.63	0.240
pH, Temp., P.size	2	9.03	4.52	0.96	0.414
Med., Temp., P.size	1	2.04	2.04	0.43	0.524
pH, Med., Time	2	13.6	6.81	1.44	0.277
pH, Temp., Time	2	2.73	1.36	0.29	0.754
Med., Temp., Time	1	14.4	14.4	3.06	0.108
pH, P.size, Time	2	25.3	12.6	2.68	0.112
Med., P.size, Time	1	13.5	13.5	2.87	0.118
Temp., P.size, Time	1	8.08	8.08	1.71	0.217
Residual	11	51.9	4.72		
Total	47	8106			

(iii) There was no significant advantage in increasing the extraction time to more than 45 minutes in a single extraction process or extracting the protein at low (15°C) or high (35°C) temperature.

(iv) Salt solution decreased the extraction yield compared to the use of water.

3.3 CHARACTERISATION OF WATER, ACIDIC AND ALKALI EXTRACTED PROTEIN

The results shown in Table 3.2.1 indicate that the extraction conditions greatly influence the extraction yield. The yield achieved is the combined result of several interactions between the proteins in the seed and the conditions employed. The extraction conditions not only influence the yield but also the characteristics of the isolated proteins. A protein can be characterised by its gel filtration and electrophoretic profile or by its response to proteolytic enzymes. Its amino acid profile will provide useful information in relation to its interactions with other food components or its influence in biochemical reactions. Based on potential applications in the food industry and also on the results obtained previously, the proteins extracted at pH 2 (CPI-1), at pH 7 (CPI-2) and at pH 9 (CPI-3) were selected for detailed characterisation.

3.3.1 AMINO ACID COMPOSITION

The amino acid profile of chickpea proteins isolated at pH 2 (CPI-1), pH 7 (CPI-2) and pH 9 (CPI-3) is given in Table 3.3.1.

There is no difference in the amount of essential amino acids in CPI-1, CPI-2 and CPI-3 which accounted for about 44.4%, 44.3% and 43.8% of total amino acids, respectively. These are higher than those reported for chickpea protein isolated by either isoelectric precipitation (39.6%) or by micellization (38.2%) (Paredes Lopez *et al.*, 1991) but lower than those reported for chickpea albumin (47.3%) and glutelin (46.3%) (Singh and Jambunathan, 1982). Sulfur containing amino acids and aromatic amino acids accounted for 2.8% and 9.7% for CPI-1; 2.7% and 9.4% for CPI-2; 2.8% and 9.0% for CPI-3, respectively. For essential amino acids, leucine is the highest amino acid found in all three isolates. For non essential amino acids, glutamic acid, aspartic acid and arginine accounted for 37.2%, 37.8% and 39.0% of the total amino acids.

The data indicate that the amino acid profile of these three proteins are rather similar. They all provide sufficiently the amounts of essential amino acids required for children and adults as recommended by FAO/WHO (WHO, 1985) (Table 3.3.2).

Table 3.3.1 Amino acid profile of chickpea (Garnet) proteins (g/100g protein) extracted at different pHs.

Protein	CPI-1	CPI-2	CPI-3
Extraction pH	2	7	9
Essential amino acid			
Histidine	2.66	2.57	2.55
Isoleucine	5.05	4.98	4.85
Leucine	8.15	8.18	8.32
Lysine	6.35	6.55	6.94
Methionine	1.41	1.41	1.38
Cystine and Cysteine	1.22	1.14	1.38
Phenylalanine	6.50	6.38	6.26
Tyrosine	3.05	2.95	2.64
Threonine	4.28	4.08	3.81
Tryptophan	0.80	0.86	0.72
Valine	4.99	5.16	4.92
Non-essential amino acid			
Aspartic acid	12.0	11.8	12.0
Serine	5.69	5.89	5.82
Glutamic acid	16.2	16.7	17.7
Proline	4.67	3.81	3.87
Glycine	3.76	3.87	3.58
Alanine	4.28	4.35	3.90
Arginine	8.96	9.31	9.34
EAA / TAA (%)	44.4	44.3	43.8
Protein Recovery (%)	92.3	87.3	91.6

Table 3.3.2 Essential amino acids required for children and adults as recommended by FAO/WHO

Amino Acid	FAO/WHO/UNU		
	Reference protein		
Essential amino acid	Infant	Child	Adult
Histidine	2.6	1.9	1.6
Isoleucine	4.6	2.8	1.3
Leucine	9.3	6.6	1.9
Lysine	6.6	5.8	1.6
Methionine	4.2	2.5	1.7
Cystine and Cysteine			
Phenylalanine	7.2	6.3	1.9
Tyrosine			
Threonine	4.3	3.4	0.9
Tryptophan			
Valine	5.5	3.5	1.3

3.3.2 GEL FILTRATION

Fractionation of the three isolates by using Sephadex G-200 failed to give clear resolving patterns. Alternatively, these protein isolates were subjected to Sepharose B6 gel filtration. CPI-2 and CPI-3 showed very similar resolving patterns by Sepharose with four fractions (Fig 3.3.1). In contrast, only three fractions were found with CPI-1 gel filtration pattern (Fig 3.3.1). Using a standard curve of proteins of known molecular weight, the apparent molecular weights of the four fractions of CPI-2 were estimated as about 600 kD, 105 kD, 22.4 kD and 3.5 kD while those of CPI-3 were 631 kD, 112 kD, 28.2 kD and 4.5 kD. Molecular weights of three fractions of CPI-1 were estimated of about 631 kD, 40 kD and 4.7 kD, respectively.

All four fractions of CPI-2 share a similar proportion of the total eluted protein while the major fractions of CPI-3 are those having molecular weight of 112 kD and 28.2 kD. The major fraction of CPI-1 was found of having molecular weight of 40 kD.

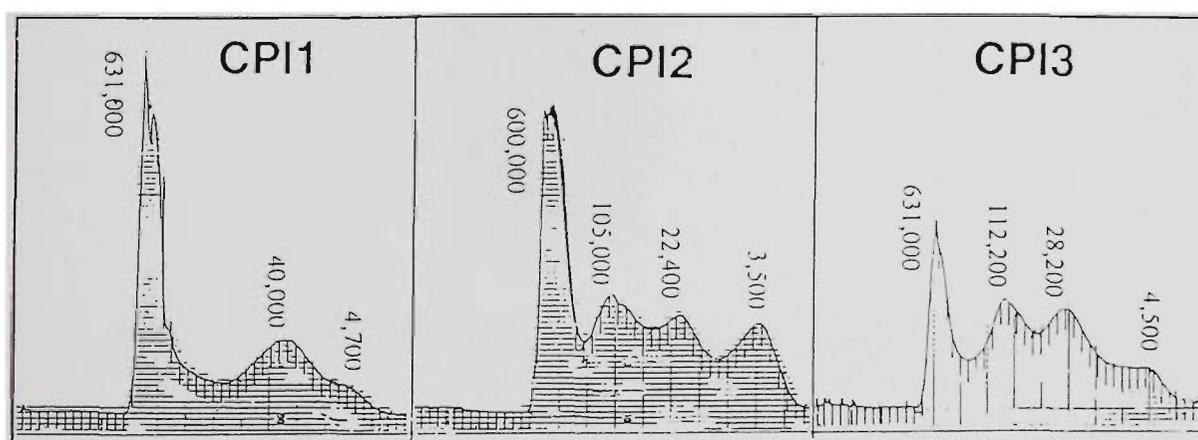


Fig 3.3.1 The gel filtration patterns of chickpea protein isolates CPI-1, CPI-2 and CPI-3 with estimated molecular weights of each fraction (kD).

3.3.3 GEL ELECTROPHORESIS

The SDS-PAGE patterns of CPI-1, CPI-2 and CPI-3 are given in Fig 3.3.2.

The electrophoretic profile of CPI-1 showed seven bands with four major bands having Rf values of 0.24, 0.50, 0.54 and 0.81, respectively. The electrophoretic patterns of CPI-2 and CPI-3 also showed seven bands with four major bands having Rf values of 0.23, 0.49, 0.55 and 0.80, respectively. The patterns revealed some structural difference between proteins isolated at different pHs. The electrophoretic pattern of CPI-2 is strikingly similar to that of

CPI-3, reflecting a similarity observed with their gel filtration pattern. The electrophoretic pattern of CPI-1 is quite different from those of CPI-2 and CPI-3, not in terms of band number but in band intensity. This indicated that both CPI-2 and CPI-3 were constituted with subunits having high molecular weights while CPI-1 was largely built up with low molecular weight subunits.

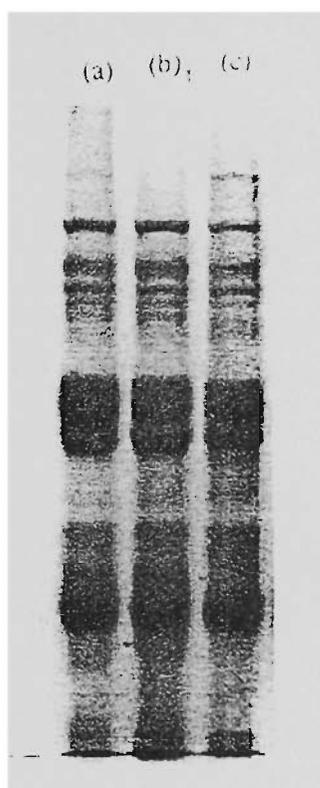


Fig 3.3.2 SDS-PAGE patterns of protein isolates: (a) CPI-3; (b) CPI-2 and (c) CPI-1.

3.3.4 DENSITOMETRIC SCANNING PROFILES

The effect of extraction conditions on the isolated protein structures was illustrated clearly by using densitometric scanning of the SDS-PAGE pattern of the proteins examined. The densitometric scanning profiles of CPI-1, CPI-2 and CPI-3 are shown in Fig 3.3.3.

The CPI-1 pattern consists of 7 bands. Calculated from a standard curve of proteins of known molecular weight, the estimated molecular weights of these subunits were 85.1, 70.8, 66, 47.9, 42.7, 32.4 and 18.4 kD. The CPI-2 and CPI-3 pattern also consist of 7 bands with corresponding molecular weights similar to those found in CPI-1. Although these proteins showed similarities in terms of subunit's number and its corresponding molecular weight, the densitometric patterns revealed a high proportion of subunits having high molecular weight (62.7-64.6 kD) in CPI-2 and CPI-3 while CPI-1 showed a high proportion of subunits having low molecular weight (18.4 kD).

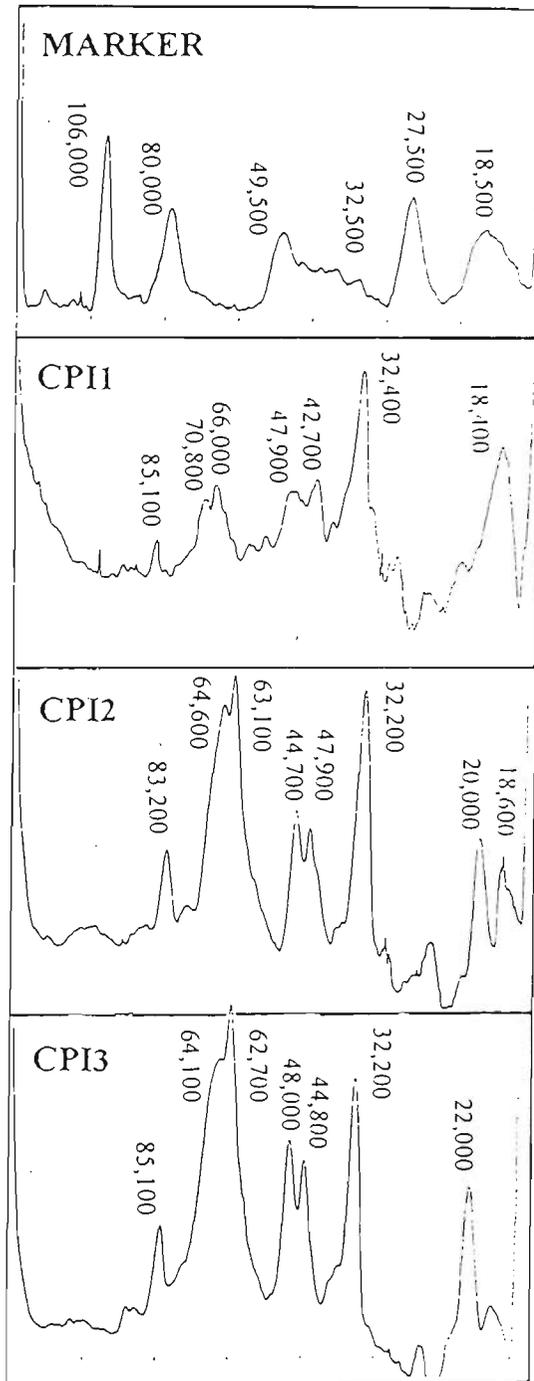


Fig 3.3.3 Densitometric scanning profiles of CPI-1, CPI-2 and CPI-3 showing the estimated molecular weight of the major subunits.

3.3.5 *IN VITRO* DIGESTIBILITY

Amino acid analysis failed to show any significant differences between chickpea proteins extracted under different conditions. Their SDS-PAGE profiles also exhibited very similar

3.3.5 *IN VITRO* DIGESTIBILITY

Amino acid analysis failed to show any significant differences between chickpea proteins extracted under different conditions. Their SDS-PAGE profiles also exhibited very similar patterns. The obvious differences that exist among these three proteins were only recognised with their densitometric scanning profiles. These profiles suggested that the proteins extracted at neutral and alkaline pH (CPI-2 and CPI-3) were predominantly constituted of subunits having high molecular weight in a similar proportion while the protein extracted at acidic pH (CPI-1) were made with a higher proportion of low molecular weight subunits. The difference in their composition and structure, undetected clearly by their SDS-PAGE profile, could be recognised by their interactions with other components or in their *in vitro* enzymatic hydrolysis.

3.3.5.1 Gel filtration profile of chickpea hydrolysates by papain and trypsin

The effect of different extraction conditions on the protein structure could be illustrated by analysing their *in vitro* digestibility profile. The hydrolysates obtained from each of the three proteins by trypsin and papain at several digestion times were subjected to Sepharose filtration. Their resolving patterns are given in Fig 3.3.4 and Fig 3.3.5.

a. Trypsin

As shown in Fig 3.3.4, fractions with low molecular weight (4.70 kD-40 kD) of the CPI-1 were hydrolysed very rapidly within 30 minutes of incubation. The fraction having molecular weight of 40 kD was almost completely broken down to 3.2 - 5.7 kD fraction but the fraction with a molecular weight of 630 kD remained almost intact. This fraction was gradually degraded after 3 hours but a small part of this fraction still remained after 24 hours of incubation. Similar observations were also obtained with the CPI-2 and CPI-3. The only noted difference was the more susceptibility to enzymatic hydrolysis of the fraction with highest molecular weight (630 kD) of these two proteins. This fraction was almost completely degraded after 0.5 hour of digestion in the case of CPI-2 and 3 hours in the case of CPI-3.

b. Papain

Fractions with low molecular weight (4.7 kD-44 kD) of the CPI-1 were hydrolysed more slowly with papain than with trypsin within 30 minutes of incubation. Like trypsin, papain has also failed to completely degrade the fraction with the highest molecular weight (630 kD) of this protein isolate after 24 hours of incubation. In contrast, CPI-2 and CPI-3 were more susceptible to papain than CPI-1. The first fraction of both proteins (630 kD) was almost

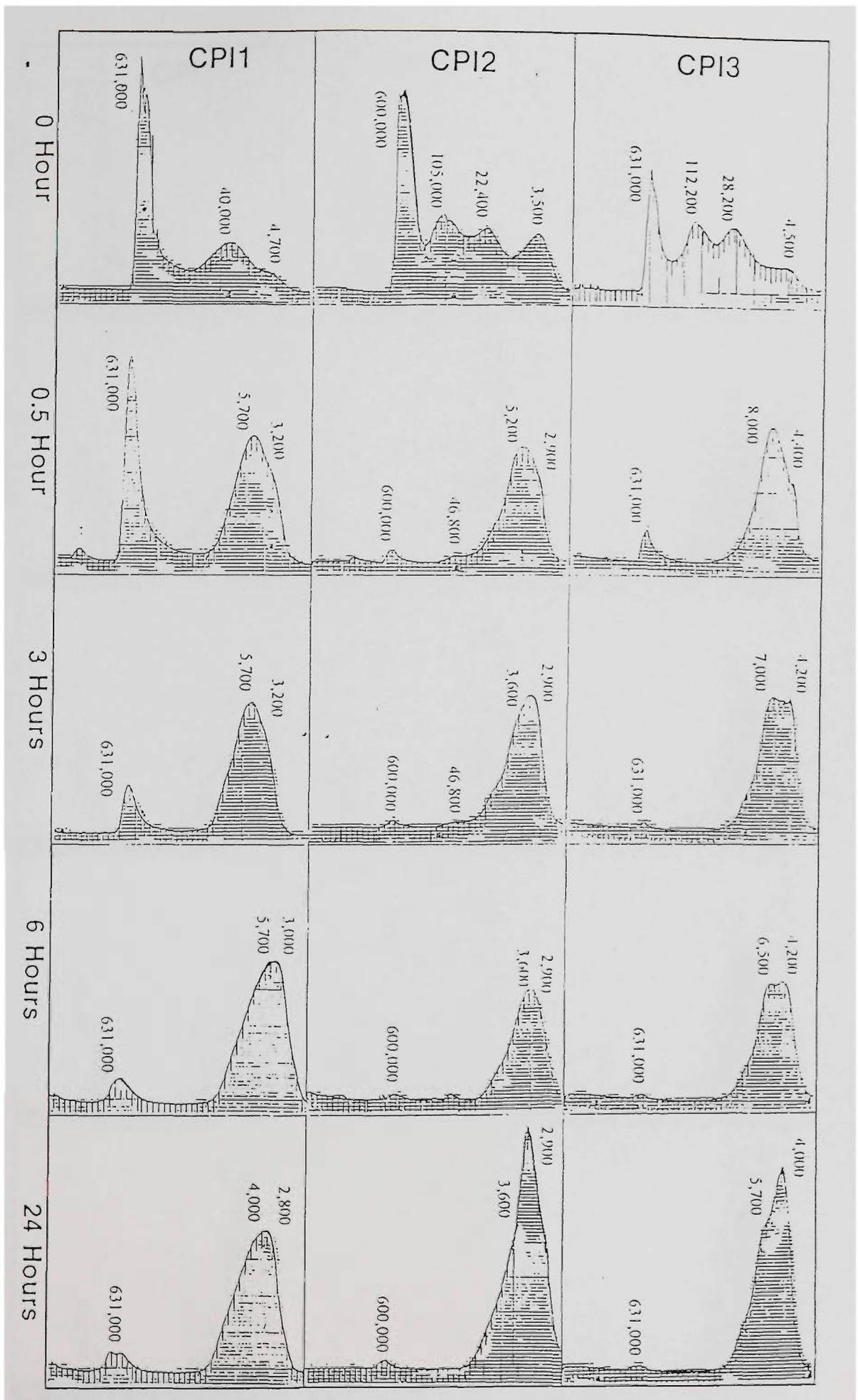


Fig 3.3.4 Sepharose filtration pattern of the hydrolysate obtained from each of the three proteins CPI-1, CPI-2 and CPI-3 by trypsin after 0, 0.5, 3, 6 and 24 h of digestion. Estimated molecular weights of the major fractions are shown (kD).

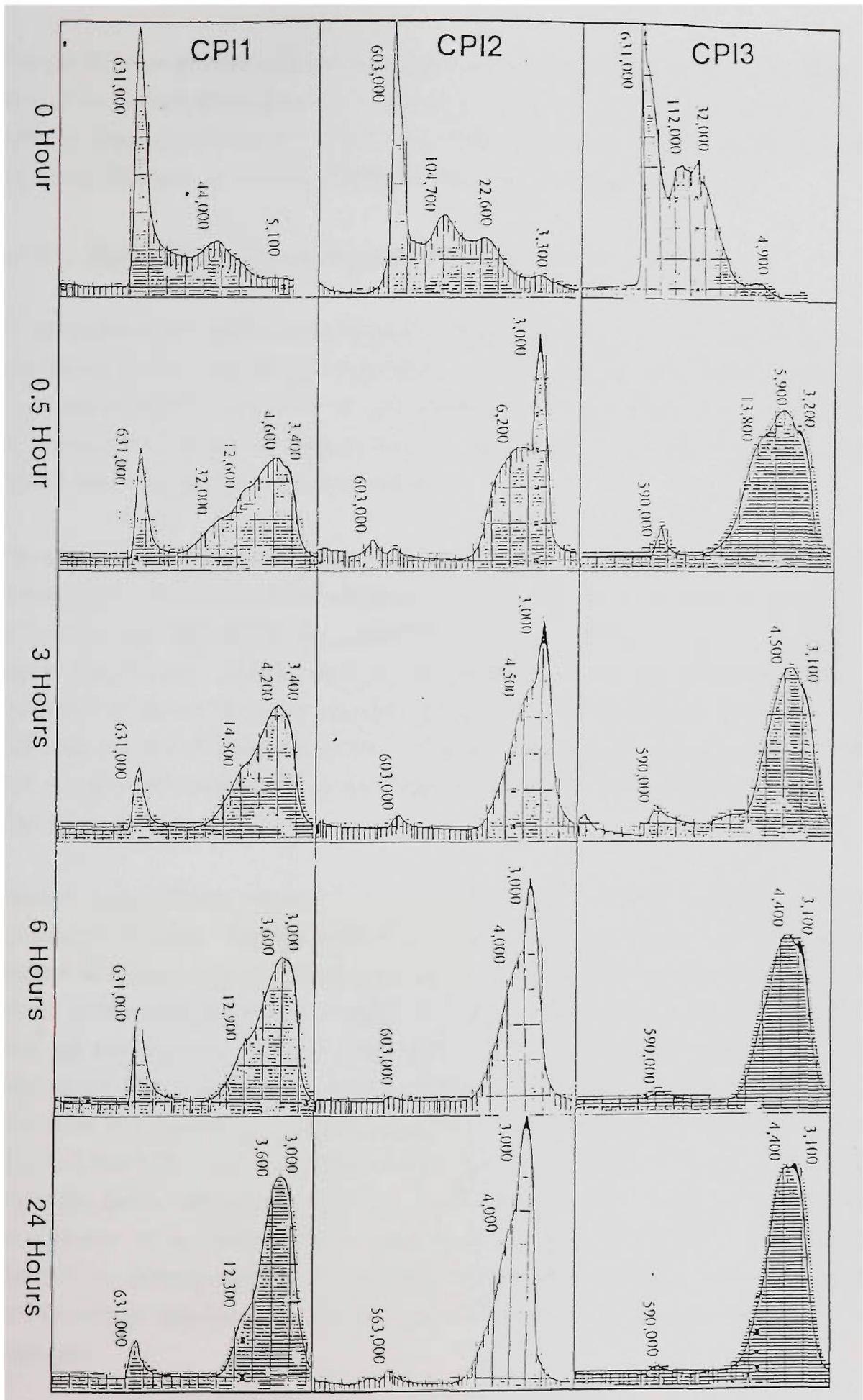


Fig 3.3.5 Sephadex filtration pattern of the hydrolysate obtained from each of the three proteins CPI-1, CPI-2 and CPI-3 by papain after 0, 0.5, 3, 6 and 24 h of digestion. Estimated molecular weights of the major fractions are shown (kD).

The gel filtration profiles of three chickpea proteins hydrolysed by papain and trypsin over 24 hour of incubation showed a close similarity between CPI-2 and CPI-3 but they were rather different when comparing with CPI-1. The difference in their filtration profile reflected their structural difference as a result of different processing conditions (Fig 3.3.5).

3.3.5.2 Hydrolysis by papain, trypsin and pepsin. Relative activity

Three proteins were further characterised by examining their *in vitro* digestibility by papain, trypsin and pepsin. The *in vitro* digestibility of these proteins over 24 hour of incubation is expressed as the relative activity of each enzyme. The extend of hydrolysis is calculated from the measurable amount of small proteins or larger peptides soluble in trichloroacetic acid (TCA) after a given time of protein hydrolysis.

The similarity in amino acid composition and perhaps also in the structure of the proteins extracted at neutral (CPI-2) and alkaline pH (CPI-3) was again reflected by a close similarity in their *in vitro* digestibility by trypsin (Fig 3.3.6b), pepsin (Fig 3.3.6c) and particularly, papain (Fig 3.3.6a). Neither of these two proteins was as very susceptible to enzymatic hydrolysis by any of the three enzymes as was the protein extracted at acidic pH (CPI-1). Although the rate of hydrolysis by each of these proteolytic enzymes was very similar at the first 4-6 hour of reaction, CPI-1 was degraded much more than CPI-2 and CPI-3 after 24 hour of incubation.

Proteins with different molecular weights and subunits undergo hydrolysis differently by proteolytic enzymes. Romeo and Ryan (1978) found that *in vitro* hydrolysis of globulin isolated from bean was much lower than that of bovine serum albumin since the molecular weight of bean globulin was much higher than that of bovine serum albumin. As indicated by their gel filtration and electrophoretic patterns, chickpea proteins isolated at neutral and alkaline pH (CPI-2 and CPI-3) were constituted of subunits with higher molecular weights than those of the acidic extracted protein (CPI-1). Consequently, the higher molecular weight of CPI-2 and CPI-3 may contribute to their lower digestibility. The size of a protein is an important factor affecting its hydrolysis by enzymes but its structural configuration, the arrangement of its peptide bonds and its amino acid composition, also influence its digestibility. In some circumstances, albumin such as the CPI-2 could form larger proteins by protein-protein interaction, thus creating some structural constraints leading to a lower hydrolysis.

On the other hand, the relative activity of the three enzymes used to hydrolyse chickpea proteins, followed the order of papain, pepsin and trypsin. The difference in relative activity can be attributed to the selectivity of each enzyme, the peptide linkages and amino acid

composition of each protein. Although the amino acid compositions of three proteins in this investigation are rather similar, there are some differences in their levels of aromatic amino acids. Although being relatively non specific enzymes, pepsin and trypsin still preferentially hydrolyse amino acid residues containing aromatic acids (Bhatty, 1988). A higher level of aromatic acids found in the CPI-1 could be a factor contributing to its higher hydrolysis than that of either CPI-2 or CPI-3. Both pepsin and trypsin are relatively non specific proteolytic enzymes but the former is normally less specific than the latter. Trypsin hydrolyses only lysyl and arginyl residues of aromatic amino acids (Kakade, 1974). This relatively high specificity of trypsin could contribute to a lower hydrolysis of chickpea proteins treated by this enzyme (Fig 3.3.6 a,b,c).

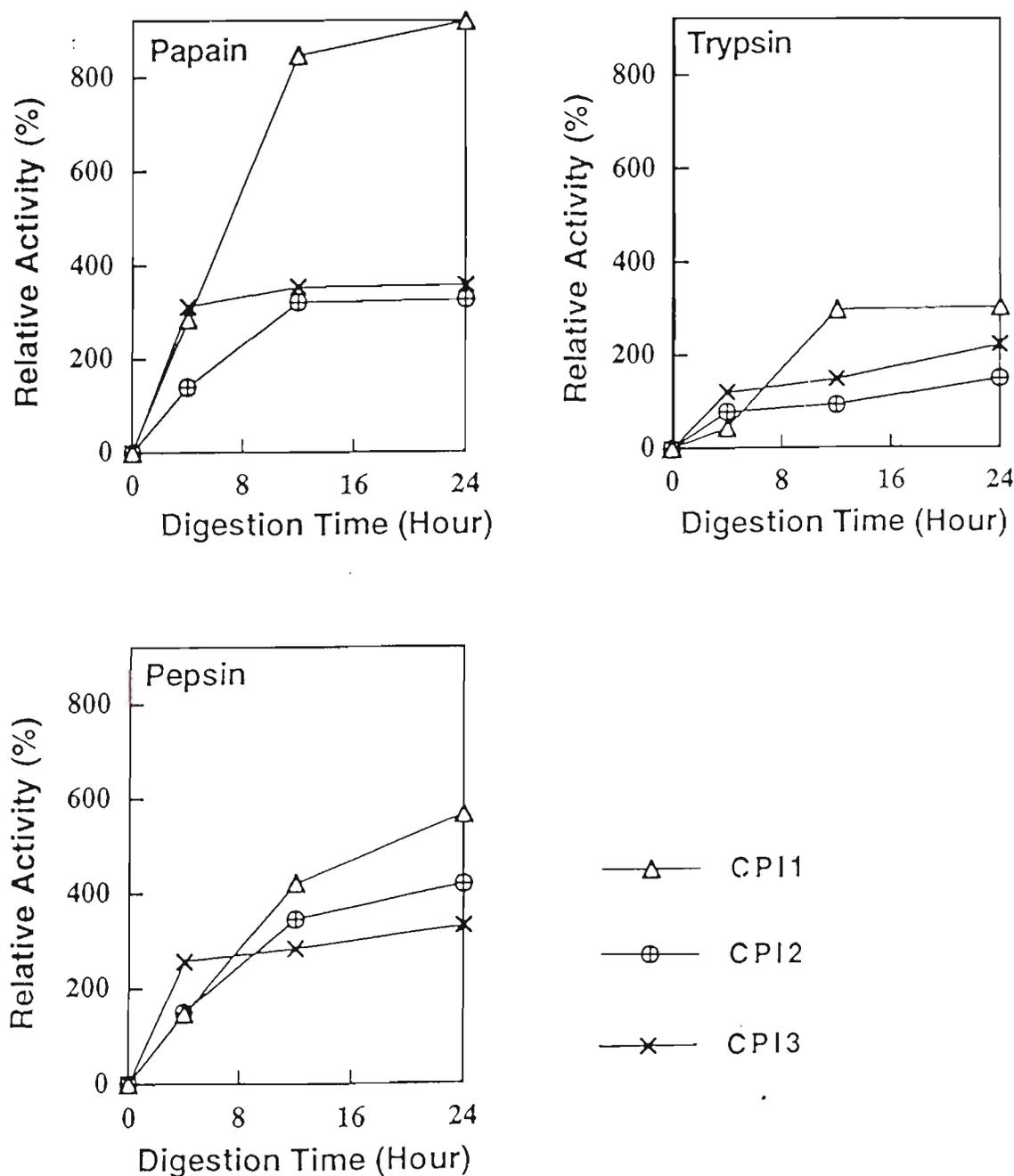


Fig 3.3.6 a, b, c *In vitro* digestibility of chickpea protein isolates by papain (a), trypsin (b) and pepsin (c).

The investigation proved that extraction of chickpea seed proteins under different conditions would not only give different extraction yields but also yield different types of protein. A high yield was obtained at extraction pH 2 and pH 9. Gel filtration patterns, electrophoretic and densitometric analysis together with enzymatic *in vitro* digestion studies indicated that water extracted proteins (CPI-2) are characteristically more similar to those extracted at alkaline pH (CPI-3) than proteins extracted at acidic pH (CPI-1).

3.4 EXTRACTION AND CHARACTERISATION OF CHICKPEA ALBUMIN AND GLOBULIN

Like other legume proteins, chickpea albumin is a rich source of essential amino acids (Singh *et al.*, 1981). The sulfur containing amino acids, tryptophan, threonine and lysine are all higher in albumin than in globulin (Bhatty, 1982). Albumin was also found to be readily hydrolysed by proteolytic enzymes (Murray and Roxburgh, 1984). Regardless of these desirable nutritional values, only a few investigations of this protein fraction have been reported. This may be due to the fact that albumin was generally accepted as only a minor fraction while globulin and glutelin were considered as major protein fractions. Singh *et al.* (1981) reported that the albumin fraction extracted from chickpea with NaCl solutions accounted for only 12% of the total protein. Similar results were also reported by Bhatty (1982) and Singh *et al.* (1988). Schroeder *et al.* (1988) found an albumin/globulin ratio of 0.23 for Hyprosok, a high yield chickpea variety.

As described in Section 3.2 a major water soluble protein fraction (48%) was obtained in our search for economical means of quantitatively extracting chickpea proteins. The different results obtained in this study compared with those reported by other investigators suggests that the yield of this protein fraction might vary considerably, depending on extraction conditions and the physico chemical procedures used (Bhatty, 1988). Because of its high nutritional values and the potential applications in food uses, the albumin fraction warrants further investigation.

3.4.1 PROTEIN EXTRACTABILITY

The yield of the albumin and globulin fractions of three chickpea varieties, extracted by three procedures is summarised in Table 3.4.1. The protein yields varied with the procedure employed and with varieties, accounting for 43-77% of protein in the defatted flours. More proteins were extracted by using either NaCl (Method B) or K₂SO₄ solution (Method A),

adjusted to pH 7 (61.9 to 77.2%) than with water (43 to 49%) (Method C). There was no significant difference in the amount of protein extracted by these two different salt solutions. Regardless of the procedure employed, the highest yield of proteins was extracted from the Dooen variety (49.9-77.2%), followed by the Garnet (43.4 to 72 %) and Kaniva (48.8 to 61.9%) variety.

Although the extractability of K_2SO_4 solutions was similar to that of NaCl solutions, more globulin was separated from the albumin / globulin extracts by the K_2SO_4 divalent salt. Using the K_2SO_4 system, up to 72% of the total protein in the albumin / globulin extract of the Dooen variety was separated as globulin. In contrast, smaller portions of the total protein was isolated as globulin (10.7%-67%) with NaCl solutions. The water extracts (Method C), after dialysis and separation, gave low precipitated globulin fractions (5.2 to 21%) and the soluble proteins, after freeze drying, gave major albumin fractions ranging from 22.4 to 44.7% of the total protein. Mineral contents in grain legumes is usually higher than in cereals. Consequently, salts in the chickpea flours converted the distilled water which was used as extraction medium, to a dilute saline solution. This could explain why not only albumin but also globulin was extracted with water.

Table 3.4.1 The yield of total albumin/globulin, albumin and globulin (% of the total protein) fractions under different extraction conditions.

Extraction medium	Method A			Method B			Method C		
	K_2SO_4 , pH 7			NaCl, pH 7 ¹			H ₂ O		
Protein	Total	Alb	Glob	Total	Alb	Glob	Total	Alb	Glob
Kaniva	72.0	28.5	43.5	67.2	60.3	07.2	43.4	22.4	21.0
Dooen	77.2	21.7	55.5	77.5	66.2	11.3	49.9	44.7	05.2
Garnet	61.9	30.4	31.5	63.9	21.6	42.3	48.8	32.2	16.7

1. Adjusted to pH 7 with K_2HPO_4 solution

The total amounts of albumin and globulin extracted with K_2SO_4 and NaCl solution in this study was similar to those reported in other investigations (Singh *et al.*, 1981; Schoeder *et al.*, 1988; Singh *et al.*, 1988). A ratio 1:4 for albumin / globulin was normally found not only with chickpea but also with other grain legumes such as black gram (Padhye and Salunkhe, 1979), pea (Grant *et al.*, 1976), lentil and faba bean (Bhatty, 1988). In contrast, Murray and

Roxburgh (1984) reported that albumin accounted for 38% of chickpea embryo protein. In this study, the ratio of albumin to globulin varied greatly. Wide variations between albumin and globulin fraction of different or within grain legume species, have been reported (Bhatty, 1982). Under certain conditions, albumin was separated as a major fraction of the total albumin - globulin extract. The large variation observed in this investigation indicates that the isolation of chickpea albumin is very dependant on extraction conditions, particularly, the nature of extraction medium.

The obtained albumin proteins (water soluble proteins) presented in Table 3.4.1 were subjected to detailed characterisation by their amino acid profiles and other procedures in order to confirm that although extracted under different conditions, they are indeed similar or identical.

3.4.2 AMINO ACID PROFILE

Table 3.4.2.a Amino acid profile of chickpea (Kaniva) albumin (A1, B1 and C1) and globulin (A2, B2) proteins (g/100g protein)

Protein	72A1 ¹	72B1 ²	72C1 ³	72A2 ¹	72B2 ²
Essential amino acid					
Histidine	2.57	2.89	2.60	2.79	2.82
Isoleucine	4.58	4.58	4.45	4.72	4.72
Leucine	7.30	7.62	7.16	7.40	7.17
Lysine	8.79	7.40	8.93	5.61	5.19
Methionine	2.40	1.80	2.51	1.30	1.93
Cystine and Cysteine	2.55	2.08	2.86	1.25	1.11
Phenylalanine	4.99	5.99	4.63	6.89	6.78
Tyrosine	3.70	3.36	3.73	2.64	2.75
Threonine	4.94	4.17	4.63	3.24	3.28
Tryptophan	0.71	0.72	0.79	0.64	0.86
Valine	5.17	4.95	5.28	5.03	5.00
Non-essential amino acid					
Aspartic acid	11.5	11.1	11.5	11.6	12.0
Serine	5.19	5.36	5.76	6.27	6.04
Glutamic acid	14.8	15.7	14.2	17.2	16.7
Proline	3.92	4.69	4.04	5.28	5.32
Glycine	4.50	4.12	4.28	3.66	3.83
Alanine	4.72	4.49	4.43	4.18	4.41
Arginine	7.76	8.81	9.65	10.3	10.2
EAA / TAA (%)	47.7	45.7	47.4	41.5	41.5
Protein Recovery (%)	76.6	68.0	84.2	85.4	88.5

1. Method A, extracted with K₂SO₄; 2. Method B, extracted with NaCl; 3. Method C, extracted with water

Table 3.4.2.b Amino acid profile of chickpea (Dooen) albumin (62A1, 62B1, 62C1) and globulin (62A2) proteins (g/100g protein)

Protein	62A1 ¹	62B1 ²	62C1 ³	62A2 ¹
Essential amino acid				
Histidine	2.72	2.79	2.69	2.68
Isoleucine	4.88	5.04	5.02	4.78
Leucine	7.56	7.58	7.79	7.60
Lysine	8.07	6.61	8.83	5.57
Methionine	2.25	1.40	2.13	0.89
Cystine and Cysteine	2.76	1.67	2.28	0.86
Phenylalanine	5.13	6.68	4.91	6.99
Tyrosine	3.47	3.07	3.69	2.70
Threonine	4.73	3.89	4.91	3.14
Tryptophan	0.62	0.75	0.86	0.61
Valine	5.33	5.39	5.51	5.06
Non-essential amino acid				
Aspartic acid	11.0	10.6	11.1	11.6
Serine	5.50	5.98	5.28	6.15
Glutamic acid	14.8	16.1	14.6	17.7
Proline	4.71	4.90	4.17	4.83
Glycine	4.22	3.95	4.47	3.78
Alanine	4.42	4.49	4.85	4.26
Arginine	7.69	9.62	7.44	10.3
EAA / TAA (%)	47.6	44.6	48.4	41.4
Protein Recovery (%)	96.2	91.0	99.5	97.2

1. Method A, extracted with K₂SO₄

2. Method B, extracted with NaCl

3. Method C, extracted with water

The amino acid profiles of nine albumin and three globulin fractions, extracted by three procedures from Kaniva, Dooen and Garnet variety are given in Table 3.4.2 a, b and c.

All six albumin fractions of Kaniva (72A1, 72C1), Dooen (62A1, 62C1) and Garnet (24A1, 24C1) extracted either with K₂SO₄ solutions or with water showed similar amounts of essential amino acids (46.4%-48.4%, mean=47.38%). Compared with the albumin fractions isolated from NaCl solutions or the globulins, these six albumins had higher contents of lysine (8.07-8.93%, mean=8.59%), methionine (2.06-2.51%, mean=2.24%), and threonine (4.63-4.94%, mean=4.80%) and lower contents of glutamic acid (14.2-14.9 % mean=14.7). In contrast, the common features of globulin fractions isolated from Dooen (62A2) and Kaniva (72A2, 72B2) were a constant but lower amount of essential amino acids (41.5, 41.5 and 41.4%), and a higher levels of phenylalanine (6.78-6.89%) and glutamic acid (16.7-17.7%).

In summary, albumin fractions have higher levels of the total essential amino acids and particularly lysine, methionine and threonine than globulin fractions. The globulin fractions contain higher levels of phenylalanine and glutamic acid than the albumin fractions.

The albumin fractions of Kaniva, Dooen and Garnet varieties (72B1, 62B1, 24B1) extracted with NaCl solutions had a lower amount of total essential amino acids (mean=44.73%), particularly, lysine, methionine and threonine (mean=6.99, 1.72 and 4.03%, respectively) than those of the six albumin fractions (72A1, 72C1, 62A1, 62C1, 24A1 and 24C1) extracted with water or with K₂SO₄ solutions. However, the amounts of phenylalanine and glutamic acid are higher than those of the other six albumin fractions. Even though the fractionation was conducted under identical conditions, it appeared to be more difficult to fractionate globulin fraction from a mixture of albumin and globulin, extracted with NaCl solution than with the K₂SO₄ solution. This led to some degree of globulin contamination in all three albumin fractions extracted with NaCl solutions. The contamination is reflected in the amino acid profile of all NaCl extracted albumin fractions (72B1, 62B1 and 24B1) which generally had higher levels of phenylalanine, glutamic acid and arginine than the K₂SO₄ extracted fractions.

Table 3.4.2 c Amino acid profile of chickpea (Garnet) albumin (24A1, 24B1, 24C1) proteins (g/100g protein)

Protein	24A1¹	24B1²	24C1³
Essential amino acid			
Histidine	2.82	2.95	2.71
Isoleucine	4.62	4.83	4.70
Leucine	7.20	7.27	7.30
Lysine	8.11	6.92	8.85
Methionine	2.06	1.25	2.13
Cystine and Cysteine	2.37	1.39	2.20
Phenylalanine	4.93	6.34	4.67
Tyrosine	3.32	3.12	3.47
Threonine	4.78	4.04	4.82
Tryptophan	0.74	0.72	0.81
Valine	5.38	5.14	5.37
Non-essential amino acid			
Aspartic acid	11.1	11.2	11.0
Serine	5.48	5.97	5.31
Glutamic acid	14.9	16.0	14.8
Proline	4.42	5.09	4.27
Glycine	4.19	3.93	4.21
Alanine	4.52	4.39	4.67
Arginine	8.83	9.62	8.99
EAA / TAA (%)	46.4	43.9	46.8
Protein Recovery (%)	99.3	88.3	93.2

1. Method A, extracted with K₂SO₄
2. Method B, extracted with NaCl
3. Method C, extracted with water

3.4.3 GEL FILTRATION

All albumin fractions of Kaniva, Dooen and Garnet varieties isolated by the three procedures were subjected to gel filtration on Sephadex G-200 and Sepharose B-6. Sephadex G-200 produced very poor resolving patterns. In contrast, the Sepharose B-6 filtration showed a similar resolving pattern for all the nine albumin fractions (Fig 3.4.1) with four clear fractions. The chromatogram of these albumins was similar but the amount of protein in each individual fraction was different. Based on a protein standard curve of proteins of known molecular weight, five peaks in the albumin chromatogram were estimated at approximately 665 kD, 68 kD, 46 kD, 19 kD and 13 kD, respectively.

The protein eluded at the void volume (665 kD) could be an agglomerate of several proteins and accounted for only a small amount (15%) of the total eluded protein. The major fractions were the ones having molecular weight of 46-68 kD and of 13-19 kD, respectively. The proportion of these two fractions varied with the procedure employed and with the varieties investigated. For example, Kaniva protein extracted with water or with K_2SO_4 showed an approximately equal amount of proteins between these two fractions (Fig 3.4.1). When extracted with NaCl, the amount of protein in first fraction (68 kD) was higher than the second fraction (13 kD and 46 kD). This reflected some degree of globulin's contamination in the albumin extracted with NaCl solution. This observation was supported by the gel filtration pattern of Dooen and Garnet albumin fractions extracted with K_2SO_4 , which showed an approximately equal amount of protein in the first and second fraction.

The results suggests that chickpea albumin consisted of four distinct protein fractions with estimated molecular weights of 68, 46, 19 and 13 kD, respectively. The gel filtration chromatogram of chickpea globulin showed four fractions having estimated molecular weights of 74 kD, 44 kD, 20 kD and 10 kD, respectively.

3.4.4 GEL ELECTROPHORESIS

The SDS-PAGE patterns of the albumin and globulin fraction of seven chickpea varieties extracted with water, K_2SO_4 and NaCl solutions are given in Fig 3.4.2.

There was no difference in SDS-PAGE patterns (subunits) of all the albumins extracted under different conditions, except their intensity. The electrophoretic profiles of all albumin fractions were characterised by seven distinct subunits. These subunits were identified by the R_f values of 0.23, 0.27, 0.38, 0.55, 0.64, 0.87 and 0.93. For the albumin fractions extracted with NaCl, an increase in intensity of subunits with R_f values of 0.55-0.64 and 0.87-0.93 was

observed. This only difference in the electrophoretic profile of all of the albumin fractions confirmed a globulin contamination in the albumin fractions extracted with NaCl solution.

The electrophoretic profile of all globulin fractions was similar but it was clearly different to that of the albumin. The globulin fraction was also characterised by seven distinct subunits, having Rf values of 0.35, 0.41, 0.47, 0.53, 0.73, 0.77 and 0.85, respectively. The globulin electrophoretic pattern was different with that of albumin, both in terms of intensity and mobility. Compared with those of albumins, the electrophoretic characteristics of globulin show a smaller number of bands with faster band mobility and a higher intensity of the lower molecular weight subunits.

The SDS-PAGE pattern failed to distinguish the cultivar difference but the albumin and globulin fraction of chickpea proteins could be identified by their clearly different electrophoretic pattern. In an attempt to characterise several protein fractions of chickpea, Singh *et al.* (1981) also found little difference between the SDS-PAGE profiles of Kabuli and Desi varieties.

3.4.5 DENSITOMETRIC SCANNING PROFILES

The electrophoretic characteristics of chickpea albumins and globulins were examined more closely by densitometric scanning of their SDS-PAGE patterns. Densitometric scanning profiles of nine albumin and globulin fractions of Kaniva, Dooen and Garnet, extracted by the three procedures are given in Fig 3.4.3.

The densitometric scanning profiles of six albumin fractions (Kaniva, Dooen and Garnet) extracted with either K_2SO_4 or water were almost identical, as characterised by six distinct peaks. The molecular weight of these subunits were estimated to be 96, 84, 65, 38.5, 32.5 and 20 kD, respectively. The albumin fraction from each of three chickpea varieties extracted with NaCl solutions also showed the six distinct subunits as observed previously. There were a few extra peaks found in the ranges of 18-26 kD and also at 41-46 kD. These subunits indicated some degree of globulin contamination.

The densitometric scanning profile of all nine chickpea globulin fractions showed similar patterns. They were generally characterised by five distinct subunits with molecular weights estimated to be 69, 42, 40, 24.5 and 22 kD, respectively. The profiles of the globulins extracted with NaCl solution did not reveal individual peaks as sharp as those extracted with either K_2SO_4 or water. There was also a reduction of some subunits with molecular weight of about 65 kD. This indicated that not all albumin was separated from the globulin when

extracted with NaCl solution, forming some differences in the densitometric profile of globulins extracted under different conditions.

The present observation indicates that chickpea albumin contains some subunits having higher molecular weight than those found in chickpea globulin. Similarly, Schroeder (1988) reported that the densitometric profile of the albumin fraction of Hyprosola and its parent-two high yield chickpea varieties-showed some subunits with molecular weight (92 kD) higher than those of globulin (75 kD).

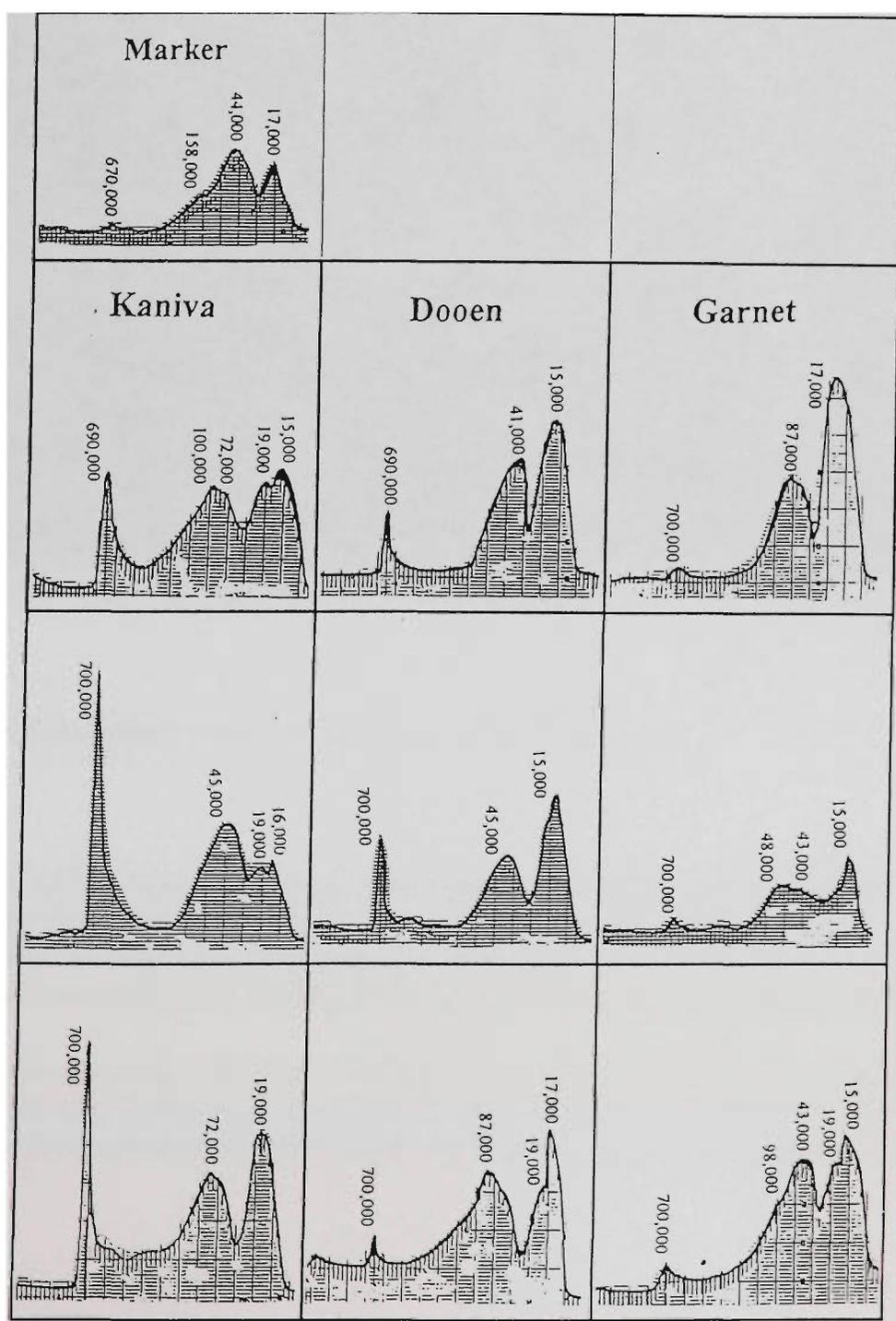


Fig 3.4.1 The gel filtration patterns of chickpea protein albumin fractions extracted with three different extraction media a) K_2SO_4 , pH 7, b) NaCl, pH 7 and c) H_2O .

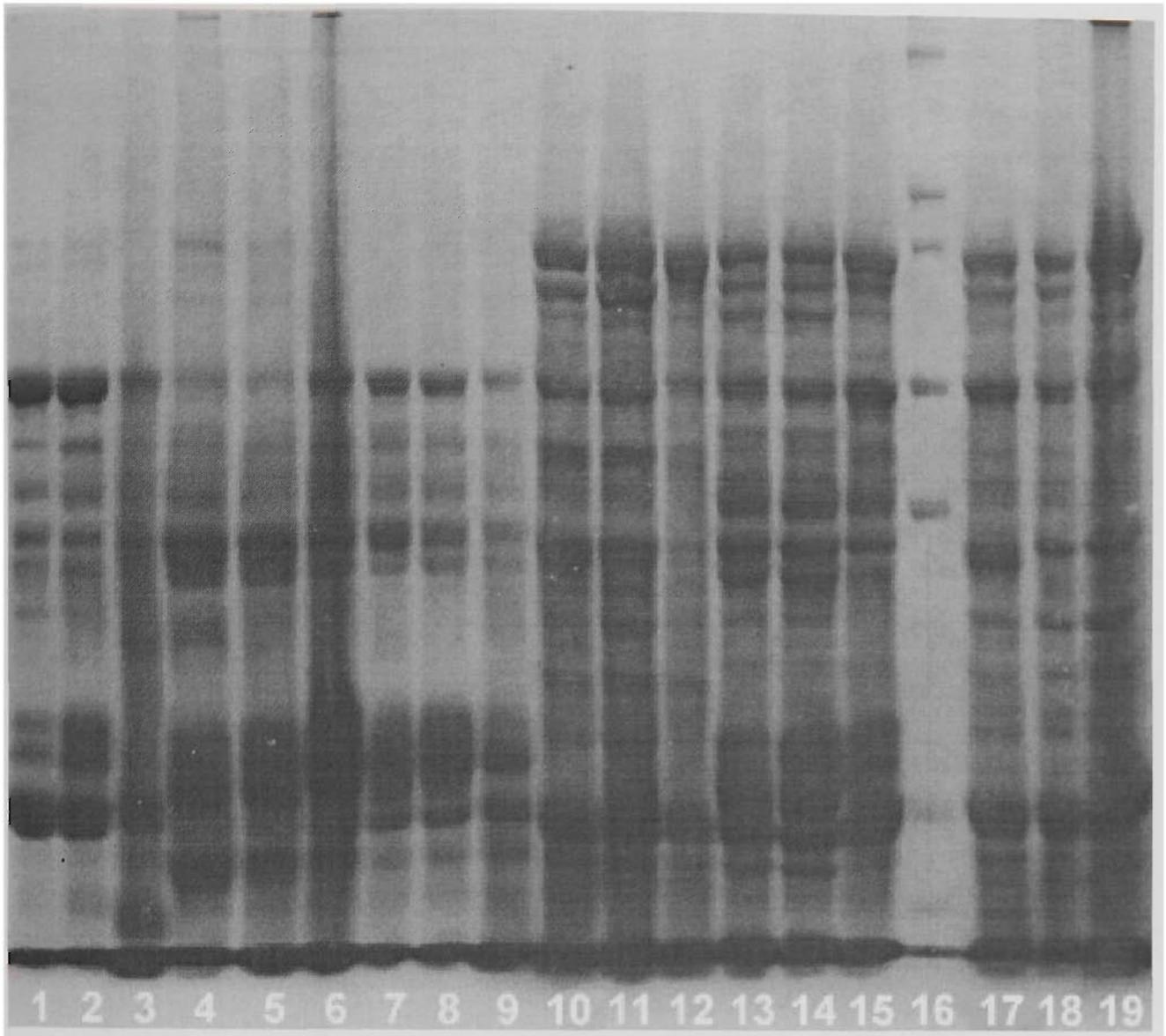


Fig 3.4.2 SDS-PAGE patterns of the albumin and globulin fractions.

- 1 2 3 "Globulins" extracted with water solution from Garnet, Dooen and Kaniva variety.
 Protein precipitated from water extracted proteins after being dialysed
- 4 5 6 Globulin extracted with NaCl solutions from Garnet, Dooen and Kaniva variety.
- 7 8 9 Globulin extracted with K₂SO₄ solutions from Garnet, Dooen and Kaniva variety.
- 10 11 12 Albumin extracted with water solution from Garnet, Dooen and Kaniva variety.
- 13 14 15 Albumin extracted with NaCl solutions from Garnet, Dooen and Kaniva variety.
- 17 18 19 Albumin extracted with K₂SO₄ solutions from Garnet, Dooen and Kaniva variety.

16 Markers

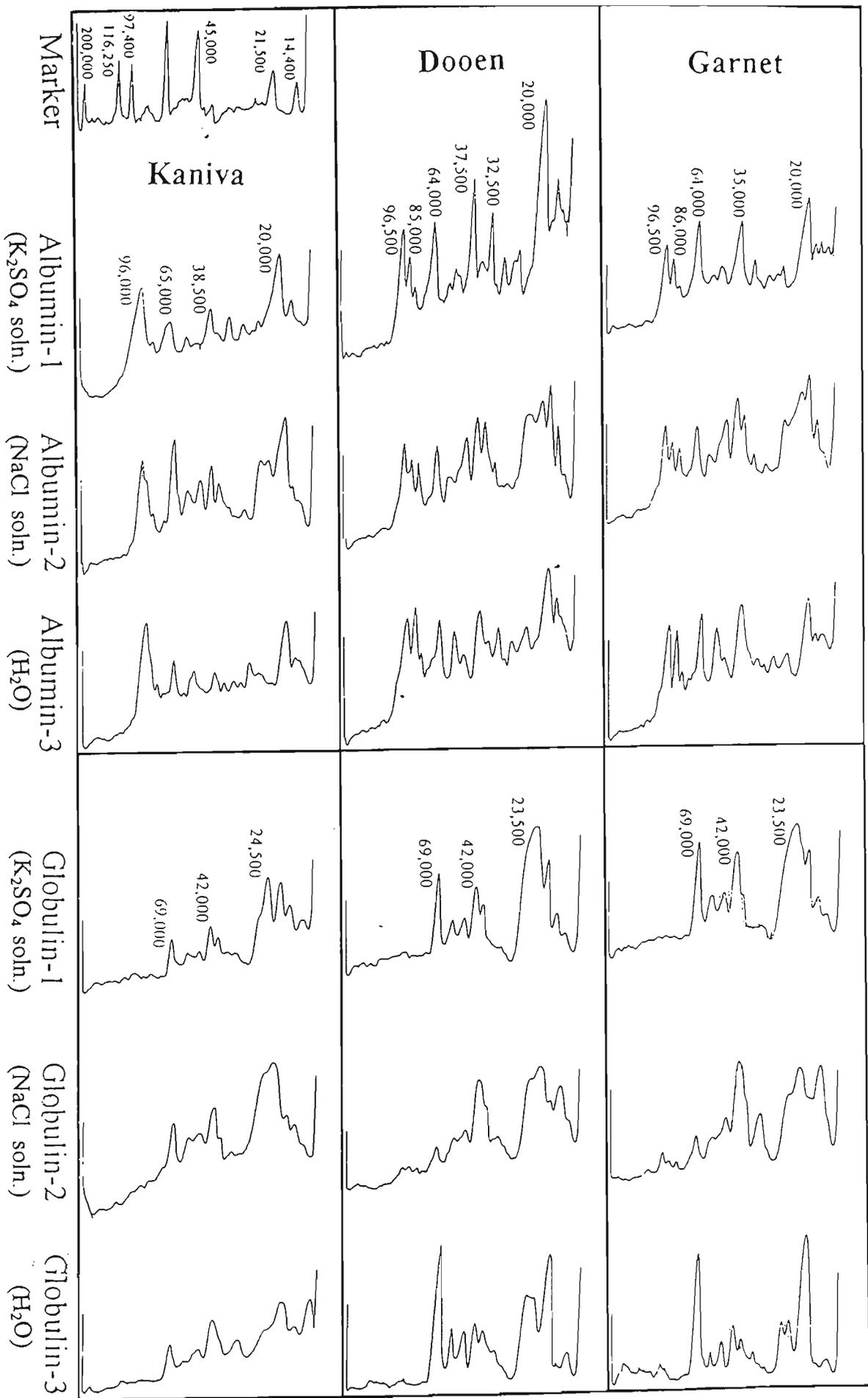


Fig 3.4.3 Densitometric scanning profiles of chickpea albumins and globulins.

3.4.6 *IN VITRO* DIGESTIBILITY

1. Albumin

The *in vitro* digestibility of Kaniva albumin by papain, trypsin, pepsin and chymotrypsin over 24 h of digestion is given in Fig 3.4.4 a-d.

The *in vitro* digestibility of all three extracted albumins by each of four proteolytic enzymes is very similar. There were only few minor differences in their digestibility behaviours of these albumins. Chymotrypsin showed a slightly higher activity towards the albumin than pepsin and trypsin but there was no significant difference in the *in vitro* digestibility of albumin fractions by pepsin and trypsin. However, the relative activity of papain towards the albumin was higher than those of the other three enzymes. After 18 hour of digestion, the NaCl extracted albumin was hydrolysed more than that extracted with either K₂SO₄ or water. An increase in relative activity of papain found with this albumin was perhaps due to the hydrolysis of some globulin which failed to be removed from the albumin fraction.

Regardless of their different extraction conditions, the similarity in the *in vitro* digestibility of the three extracted albumins by four enzymes suggests that these albumins all have a similar structure.

2. Globulin

The *in vitro* digestibility by four enzymes of chickpea globulins extracted with three procedures is shown in Fig 3.4.5 a-d.

The common feature of all extracted globulin fractions was that they were more susceptible to proteolytic enzymes than their corresponding albumin fractions. The protein hydrolysing capacity of four enzymes on globulin fraction was, in decreasing order, pepsin, papain, chymotrypsin and trypsin. Pepsin was found to be the most reactive enzyme, its relative activity increased about 12 fold within 4 h of digestion while the relative activity of trypsin increased only 4 fold after 24 h of digestion. The different rates and degrees of hydrolysis of chickpea globulin by pepsin and trypsin reflected the enzyme specificities. Being a non specific enzyme, pepsin can catalyse the hydrolysis of a wide range of substrates while trypsin, being more specific, can only hydrolyse lysyl and arginyl residues (Kakade, 1974).

The results indicated that chickpea globulins were more susceptible to proteolytic hydrolysis than albumins. Similar observation were obtained with great Northern bean albumins and globulins hydrolysed by trypsin and chymotrypsin (Sathe *et al.*, 1981; Romero and Ryan,

1978). Bhatti (1982) also found that pea globulin was more susceptible to pepsin hydrolysis than pea albumin. Although all globulin fractions showed a higher digestibility than their corresponding albumins, the globulin fraction obtained by NaCl extraction exhibited the highest digestibility, whereas the globulin extracted with K_2SO_4 solutions showed the lowest digestibility. This suggested that salt extraction may not only affect the extraction yield but also the structure of the isolated proteins. This aspect requires further investigation before any conclusion could be made.

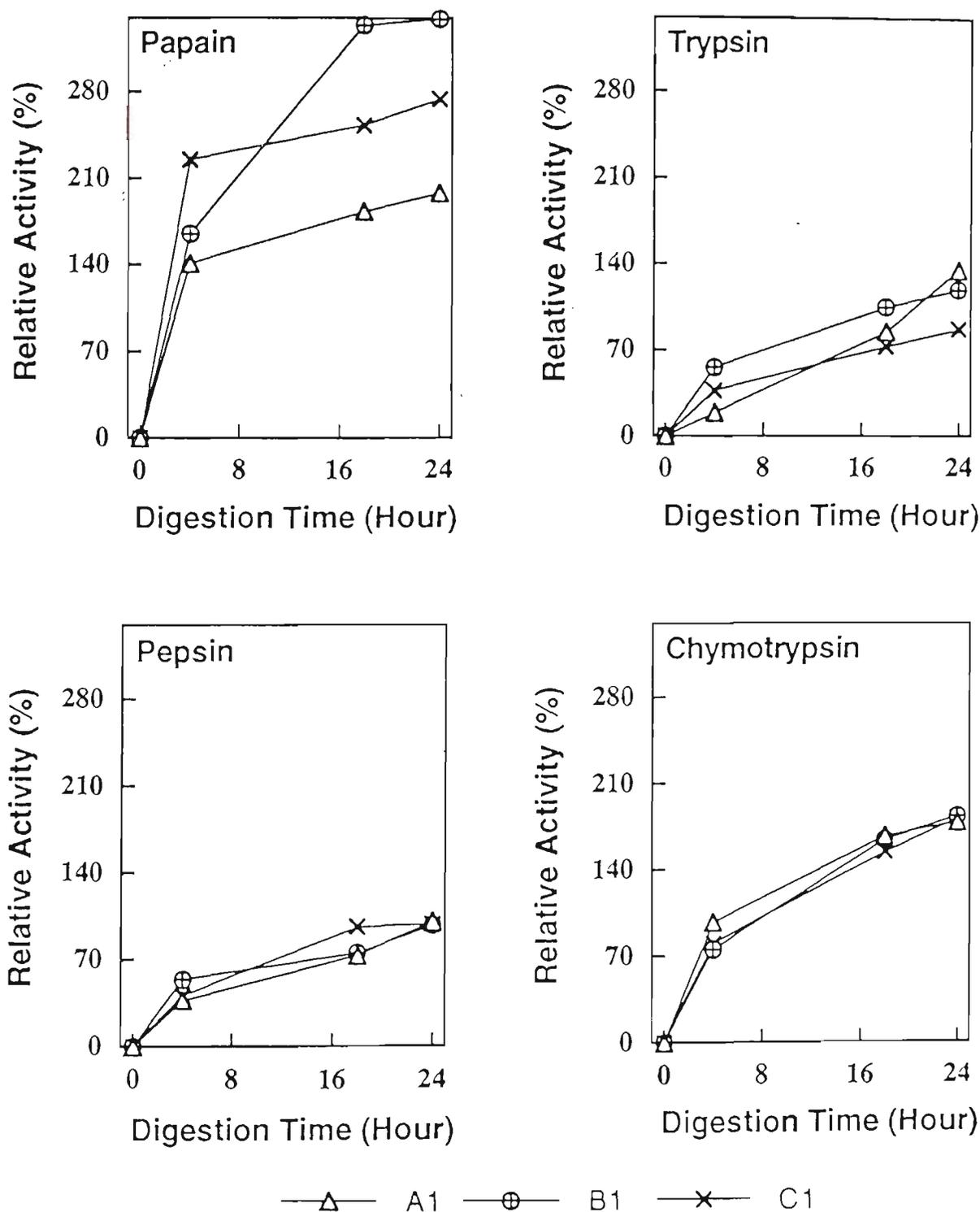
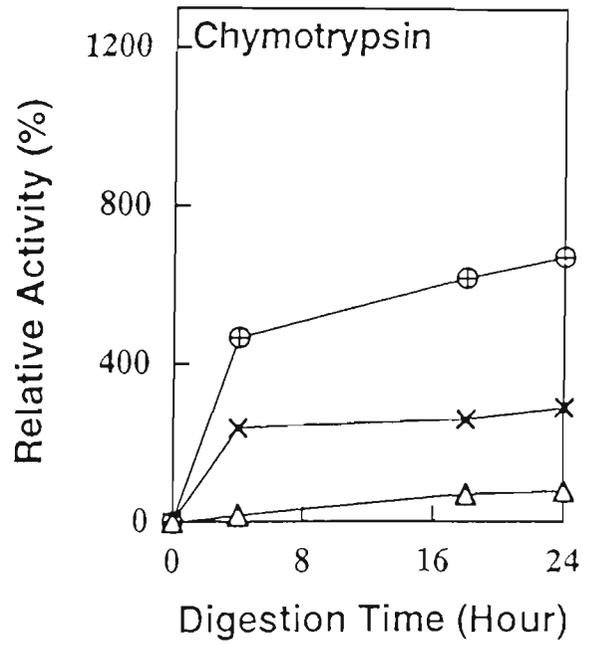
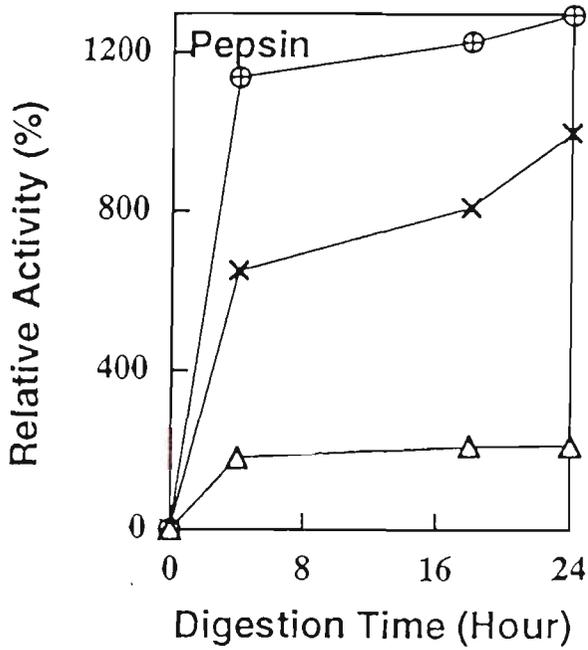
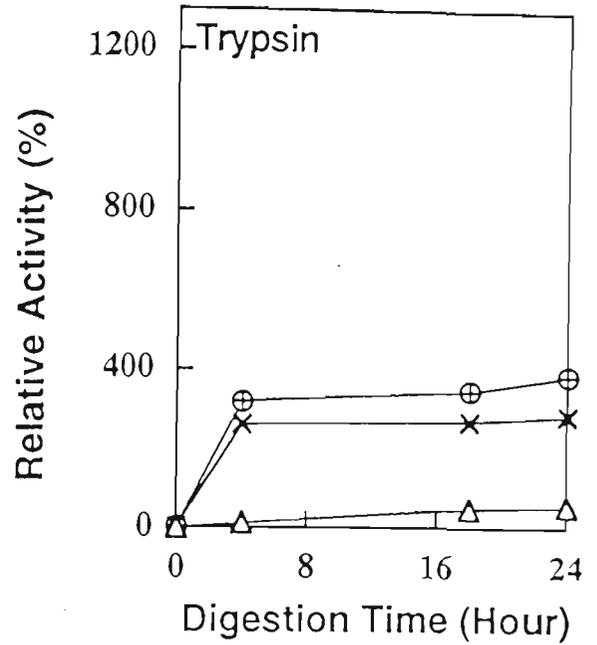
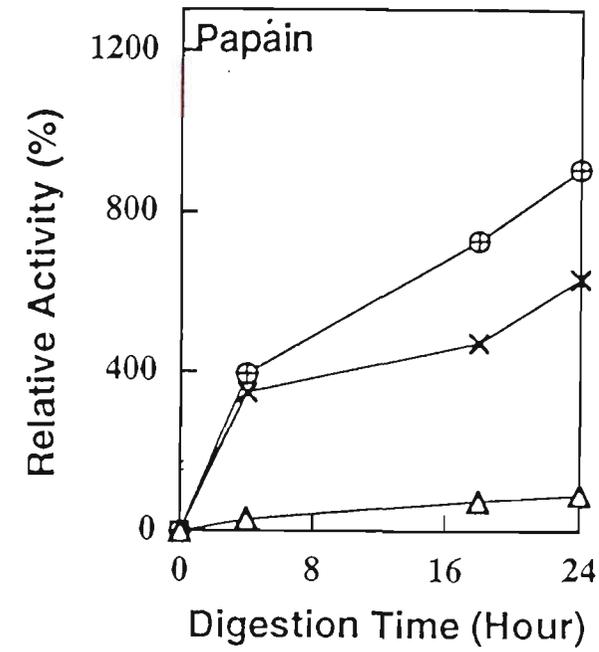


Fig. 3.4.4 a-d *In vitro* digestibility of chickpea protein albumin fractions by papain (a), trypsin (b), pepsin (c) and chymotrypsin (d).



—△— A2 —⊕— B2 —×— C2

Fig. 3.4.5 a-d *In vitro* digestibility of chickpea protein globulin fractions by papain (a), trypsin (b), pepsin (c) and chymotrypsin (d).

3.5 ' CONCLUSIONS

The results of the present study suggest that the relative amount of albumin and globulin in chickpea depend on extraction conditions. The extractable yields of the two fractions vary with extraction medium and also with different variety. Depending on the procedure employed, the albumin can be a major protein fraction. This casts doubt on the perception that the albumin fraction of chickpea only accounts for a minor proportion of the total proteins. Fractionation with K_2SO_4 solution produced highly purified albumins.

The structural differences of Kabuli and Desi chickpea albumins and globulins were clearly identified by their corresponding distinct electrophoretic patterns. The albumins are comprised of higher molecular weight subunits than those present in the globulin. The level of essential amino acids in albumin is higher than that in globulin.

Regardless of the similar structure found among globulins prepared under different conditions, the effect of processing conditions on some of their functionality was detected by their different *in vitro* digestibility.

CHAPTER 4

THE FUNCTIONAL PROPERTIES OF CHICKPEA PROTEINS

THE FUNCTIONAL PROPERTIES OF CHICKPEA PROTEINS

Grain legumes and oil seeds have been studied as an economically viable source of proteins for human consumption. As presented in previous chapters, chickpea (*Cicer arietinum*) is one of the most protein rich crops, ranked as the world's fifth most important grain legume, in terms of production. In addition, the nutritional quality of chickpea proteins can be compared favourably with soybean and other grain legumes such as faba bean and mung bean (Williams and Singh, 1987; Mohamed *et al.*, 1989). However, the effective utilisation of a protein, particularly in processed food, depends not only on its nutritional value but also on its behaviour in complex food systems. The behaviour of a food protein reflects its functional properties. Unlike soybean proteins, the physicochemical and functional properties of chickpea proteins have not been extensively investigated. As emphasised by Chavan and Kadam (1989), more information on the functionality of chickpea proteins is required for its successful application in the food industry.

Functional properties of proteins encompass any physicochemical properties which affect their processing conditions and behaviour in food systems. These include solubility, water and fat absorption, emulsifying and foaming properties, whipping and rheological properties (Kinsella, 1976; Schchtel, 1981). These in turn are affected by intrinsic and environmental factors such as protein composition and conformation, or, by the food system in which the protein is incorporated (Pour El, 1981; Acton and Saffle, 1970). Processing conditions also influence the functionality of proteins (Lopez de Ogara *et al.*, 1992).

Functional properties of major protein rich grain legumes or oil seeds such as soybean, lupin, rapeseed, sunflower and peanut have been extensively investigated (Ayres and Davenport, 1977; Sosulski *et al.*, 1976; Schwenke and Rauschal, 1983; King *et al.*, 1985; Kabirullah and Wills, 1982; Mieth *et al.*, 1983; McWatters and Holmes, 1979). Certain functional properties of chickpea flour have been examined during their incorporation in imitation milk or dried powder (Bencini, 1986; Abdel-Aal-El *et al.*, 1986; Batistuti *et al.*, 1991). Recently, Paredes Lopez *et al.* (1991) investigated only some physicochemical and functional properties of the chickpea proteins isolated by isoelectric precipitation or by micellisation.

The objectives of this chapter are to obtain more extensive information on the functional properties of chickpea proteins and to identify how these properties are influenced by different extraction conditions. The proteins extracted at pH 2 (CPI-1), pH 7 (CPI-2) and

pH 9 (CPI-3) were characterised by several procedures as described in chapter 3 and subjected to several functional property tests. A commercial soy protein (SPI) was employed as a reference sample. Most of the functional property tests used in this investigation were reported in other studies but some were either modified or developed whenever the reported assays failed to give a satisfactory performance. The most common functional tests conducted in this study are: solubility, water and oil adsorption, emulsifying capacity and stability, foam forming capacity and stability. The rheological properties of these proteins were also investigated and presented in a separate chapter.

4.1 FUNCTIONAL PROPERTIES OF CHICKPEA PROTEINS

4.1.1 SOLUBILITY

Solubility of chickpea protein isolates extracted at pH 2, pH 7 and pH 9 (CPI-1, CPI-2 and CPI-3) and soy protein (SPI) over a range of pH 2 - pH 10 is given in Fig 4.1.1. The typical U shape of solubility curves for grain legume proteins was obtained with all chickpea and soybean protein isolates. All chickpea isolates showed a maximum nitrogen solubility index (NSI) at high alkaline and acidic pHs (pH 2 and pH 10, respectively) and a minimum nitrogen solubility index at pH 4 - pH 6. The solubility curves of these isolates were similar in character but clearly differed in detail. The effect of extracting conditions on the solubilities of chickpea protein isolates was reflected by the difference in their NSI at different solubilised pHs. CPI-3 showed a sharp minimum NSI at pH 4.2 - pH 4.8, similar to that of soy protein while those of CPI-1 and CPI-2 were broader. The amino acids in CPI-3 might have had more side chain groups capable of contributing charged groups at the isoelectric point (Carbonaro *et al.*, 1993), which made it more sensitive to environmental (eg pH) change (McWatters and Holmes, 1979).

The chickpea protein extracted at pH 2 had a low solubility at all extraction pHs while the alkaline extracted chickpea protein had a higher solubility, particularly at pH 2 and pH 10. A factor affecting the solubility of a protein is the amino acid composition. Perhaps the high solubility of CPI-3 was partly due to its amino acid composition. Duke (1981) found that a high content in polar amino acids, with opposite charge in grain legume proteins, could be involved in the association dissociation phenomena of protein subunits which are at the basis of the solubility properties of legume proteins. CPI-3 had more ionic amino acid groups such as lysine, arginine and glutamic acid than both CPI-1 and CPI-2 (Table 3.2.1). These ionic groups were expected to contribute significantly to protein solubility in water. The chickpea protein extracted at pH 7 had a lower solubility at strong alkali and acidic pHs than CPI-3,

but a higher solubility at pH 7. This property should be useful for liquid foods and beverage industries. CPI-1 had lower but CPI-2 and CPI-3 had higher solubilities over a wider range of pHs when compared with soy protein isolate (SPI).

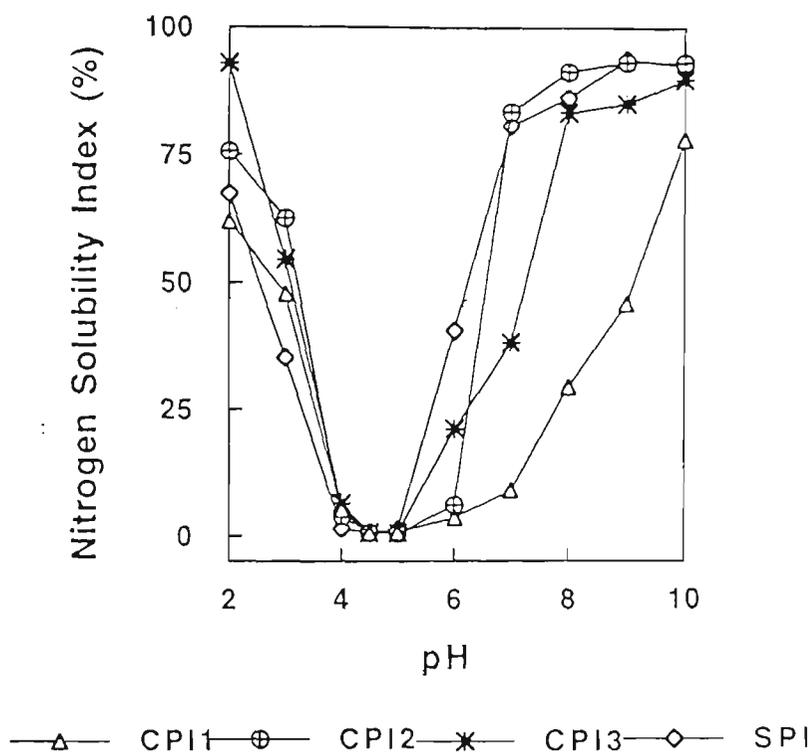


Fig 4.1.1 Solubility of chickpea proteins isolated at pH 2, pH 7 and pH 9 and of soy protein isolate.

4.1.2 WATER AND OIL ABSORPTION

Water and oil absorption of chickpea and soy protein isolates was presented in Fig 4.1.2. For oil absorption, the acidic chickpea isolate CPI-1 had the lowest oil absorption (0.36 ml/g) while both the alkaline extracted CPI-3 and the albumin CPI-2 had a higher oil absorption (1.2 ml/g). It is interesting to note that the acidic extracted isolate (CPI-1) had a lower solubility among the three chickpea isolates. Thus, it is unlikely there is correlation between its solubility and oil absorption.

The alkaline extracted CPI-3 had the highest (1.54 ml/g) and the acidic extracted CPI-1 had the lowest value (0.78 ml/g) for water absorption. The result suggested a correlation between the solubility of these two chickpea isolates and their water absorption. However solubility might not be the sole factor affecting the water absorption since CPI-2 had a much higher solubility than that of CPI-3 at pH 7 but its water absorption was lower than that of CPI-3. Similarly, soy isolate had a solubility, similar to that of CPI-2 but it had a very high water absorption (7.1 ml/g). Wagner and Anon (1990) found that solubility and water

absorption capacity of commercial soy protein were correlated with structural parameters but they were affected differently by different mechanisms. Denatured proteins had lower solubilities but higher water absorption capacities because the high surface hydrophobicity of denatured proteins promoted the formation of a protein matrix, stabilised by hydrophobic interactions capable of retaining a significant amount of water in their structures. Nakai and Powrie (1981) noted that proteins tend to decrease in solubility as their hydrophobicities increased.

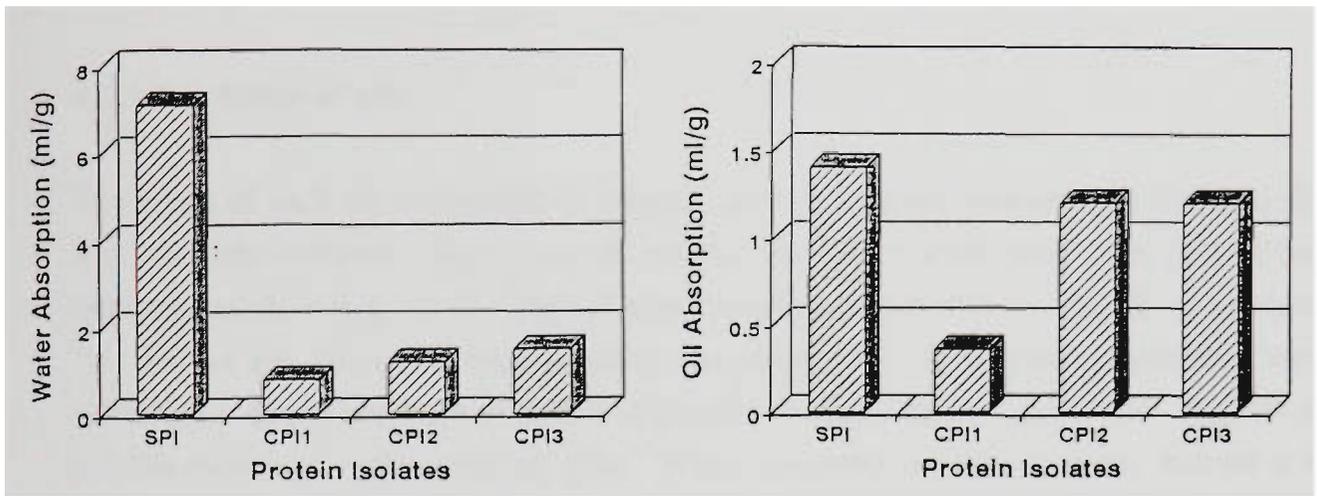


Fig 4.1.2 Water and oil absorption of chickpea and soy protein isolates.

4.1.3 EMULSION CAPACITY

4.1.3.1 Effect of protein concentration

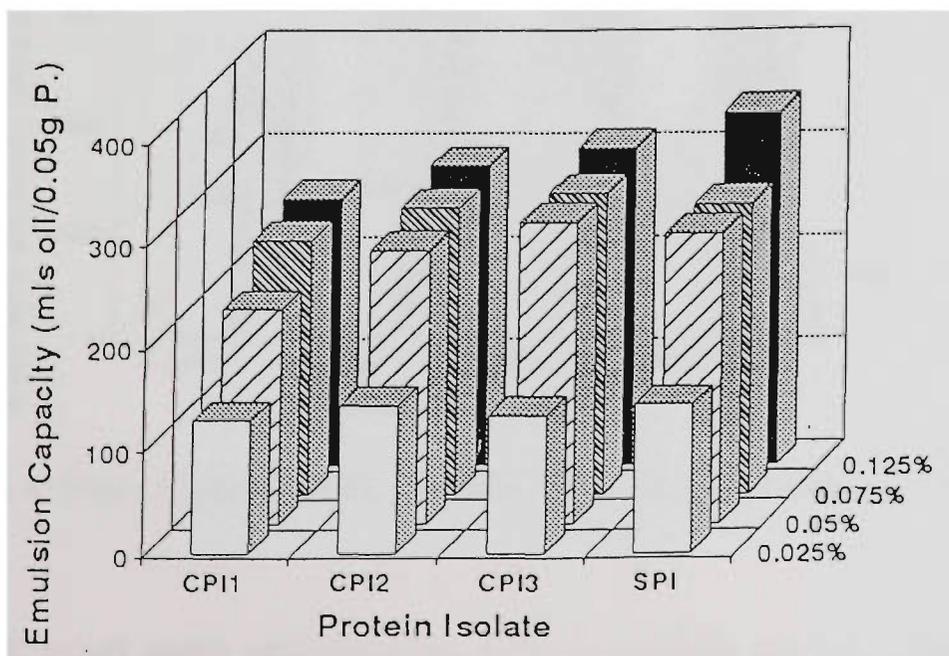


Fig 4.1.3 Effect of protein concentration on the emulsion capacity of chickpea and soy protein isolates.

The emulsion capacities of chickpea and soy protein isolates are shown in Fig 4.1.3. The EC of all protein isolates increased with an increasing protein concentration from 0.025 to 0.125% and the EC followed the order of CPI-3>CPI-2>CPI-1. At a higher concentration, the end point of EC could not be clearly recognised. The EC of CPI-3 is similar to that of soy isolate. The increased EC of grain legume proteins with increasing protein concentrations has been reported (Crenwelge *et al.*, 1974). The increasing EC tendency associated with increasing concentrations and the difference in EC among these isolates in this investigation confirmed a close relationship between EC and the corresponding solubility.

4.1.3.2 Effect of pH

The effect of each pH on the EC of chickpea protein isolates, particularly CPI-1 and CPI-3 was markedly different. The EC of all isolates was low at a pH close to pI (pH 5) but all increased at pH 9 (Fig 4.1.4). The pH also exerted a similar effect on the EC of soy isolate. The EC vs pH curves of these isolates resembled their corresponding solubility curves, indicating a strong influence of dissolved proteins on the emulsion properties. The EC of all isolates increased with increasing pHs. Water extracted chickpea protein showed a high solubility and emulsion capacity at both pH 7 and pH 9 while acidic extracted CPI-1 had a poorer solubility and emulsion capacity at these pHs (Fig 4.1.4).

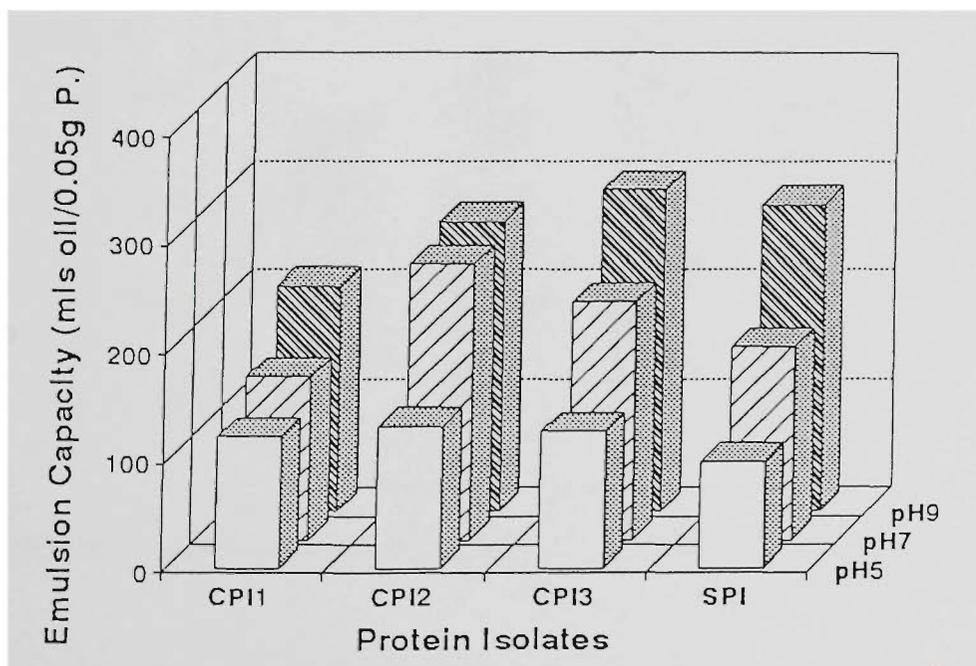


Fig 4.1.4 Effect of pH on the EC of chickpea and soy protein isolates.

The EC vs pH profile obtained in this study for chickpea and soy isolates resembled those obtained with groundnut, soy, cottonseed protein and non fat milk proteins (Ramanatham *et al.*, 1978; Crenwelge *et al.*, 1974).

4.1.3.3 Effect of temperature

The EC of CPI-1, CPI-3 and soy isolate were markedly affected by temperature. The EC of these isolates decreased progressively with increasing temperature from 15°C to 75°C (Fig 4.1.5). Thermal influence on EC of CPI-1 was moderate compared with those of CPI-2 and CP-3. The investigation of Voutsinas *et al.* (1983) on the effect of heat treatment on the EC and hydrophobicity of milk, wheat, oil seeds and grain legume proteins showed that heat treatment did not have uniform effect on different type of proteins. Pea, canola, casein were adversely affected by heat treatment while the EC of whey, bovine serum albumin, gluten were almost unaffected. Heat treatment caused protein denaturation and gradually exposed hydrophobic amino acid residues, normally buried in the interior of native protein molecules. The exposure of the hydrophobic amino acid residues made the protein more amphiphilic, capable orienting at the oil/water surface, consequently increasing its hydrophobicity (Morr, 1979) and improving its emulsifying properties. However, if some exposed hydrophobic residues participated in hydrophobic interactions, as seen in whey protein, this could lead to an decrease in hydrophobicity and emulsifying properties of the protein (Voutsinas *et al.*, 1983).

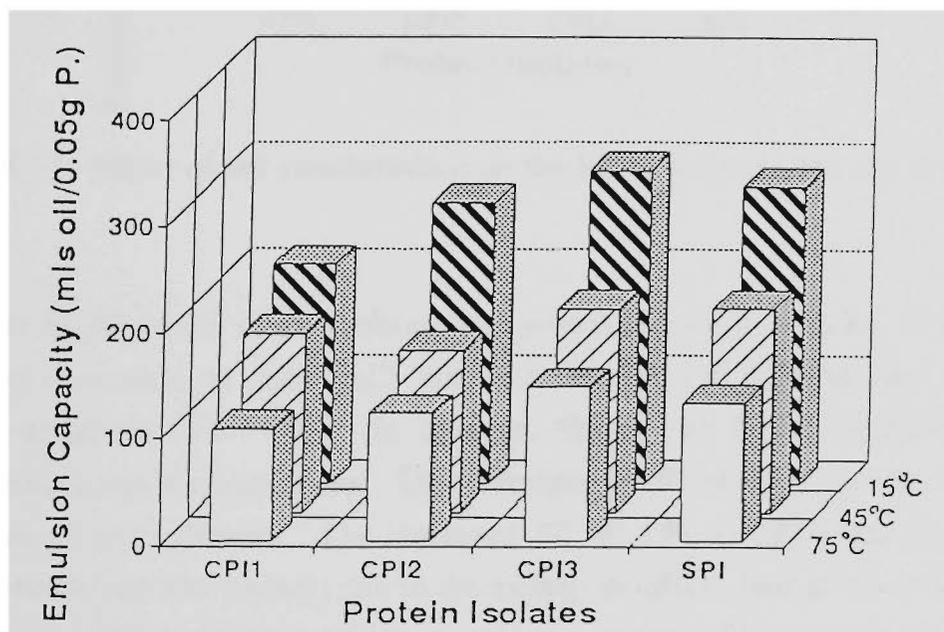


Fig 4.1.5 Effect of temperature on the EC of chickpea and soy protein isolate.

Several studies indicated that emulsifying properties and solubilities of a protein were not well correlated (Aoki *et al.*, 1980; McWatters and Holmes, 1979). Although solubility had marked effects, EC of a protein also depends much more on its hydrophobicity (Voutsinas *et al.*, 1983; Kato and Nakai, 1980). The marked effect of hydrophobicity on EC of some albumin proteins such as bovine serum albumin or ovalbumin was reported. Heating ovalbumin to 80°C for only 5 minutes, reduced its solubility up to 50%, increased its

hydrophobicity up to 50 fold and markedly improved its emulsifying properties (Voutsinas *et al.*, 1983). The difference in thermal effect on EC of these isolates reflected a difference in protein composition and conformation of these isolates. The result indicated that CPI-2 and CPI-3 are more sensitive to heat treatment than CPI-1.

4.1.3.4 Effect of salt concentration

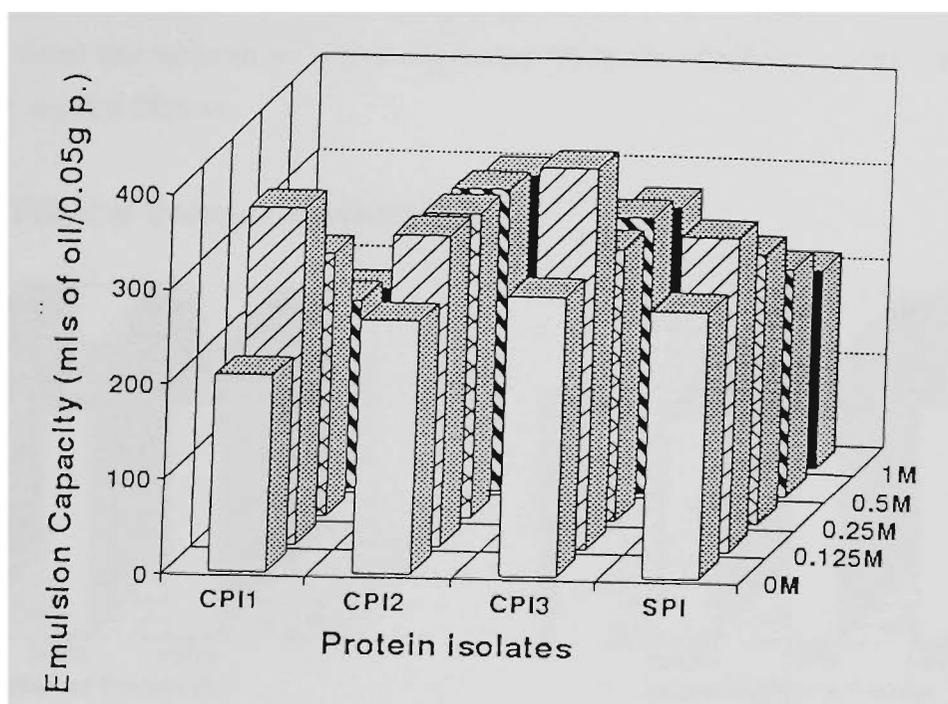


Fig 4.1.6 Effect of salt concentration on the EC of chickpea and soy protein isolates.

Effect of salt on EC of the isolates was given in Fig 4.1.6. The EC of CPI-1, CPI-3 and soy isolate increased at low NaCl concentrations (0.125-0.250M) and decreased at higher concentrations (0.5-1.0M). In contrast, the EC of CPI-2 increased progressively with increasing salt concentrations. The difference in EC of these isolates reflected the different nature of each protein. The increased EC of CPI-1, CPI-3 and soy isolate at low salt concentrations was perhaps due to the salting-in effect. Salt at low concentrations improved protein solubility of winged bean, peanut, soy protein, field pea (Dench, 1982; Ramanatham *et al.*, 1978; McWatters and Holmes, 1979; Carbonaro *et al.*, 1993). At low ionic strength, salt exerted an effect in decreasing intermolecular electrostatic interactions between oppositely charged groups, subsequently increasing the protein solubility leading to an increase of EC. In contrast, at high salt concentrations, solubility was decreased due to salting out effect, leading to a decrease in EC of CPI-1, CPI-3 and soy isolate. The salting-out effect was not observed with CPI-2 within the investigated concentrations, which may be due to its greater affinity to water. Water extracted proteins (CPI-2) could compete better with salt for water association than alkaline or acidic extracted proteins. A high

concentration of soluble proteins at 0.5-1.0M NaCl could contribute to a high EC of CPI-2 at 0.5-1.0M NaCl.

4.1.4 EMULSION STABILITY

Emulsion stability (ES) is defined as a capacity to retain an emulsion over a measured time, calculated from the volume of water separated from the emulsion. Like EC, ES was also affected by several factors.

4.1.4.1 Effect of Protein Concentration

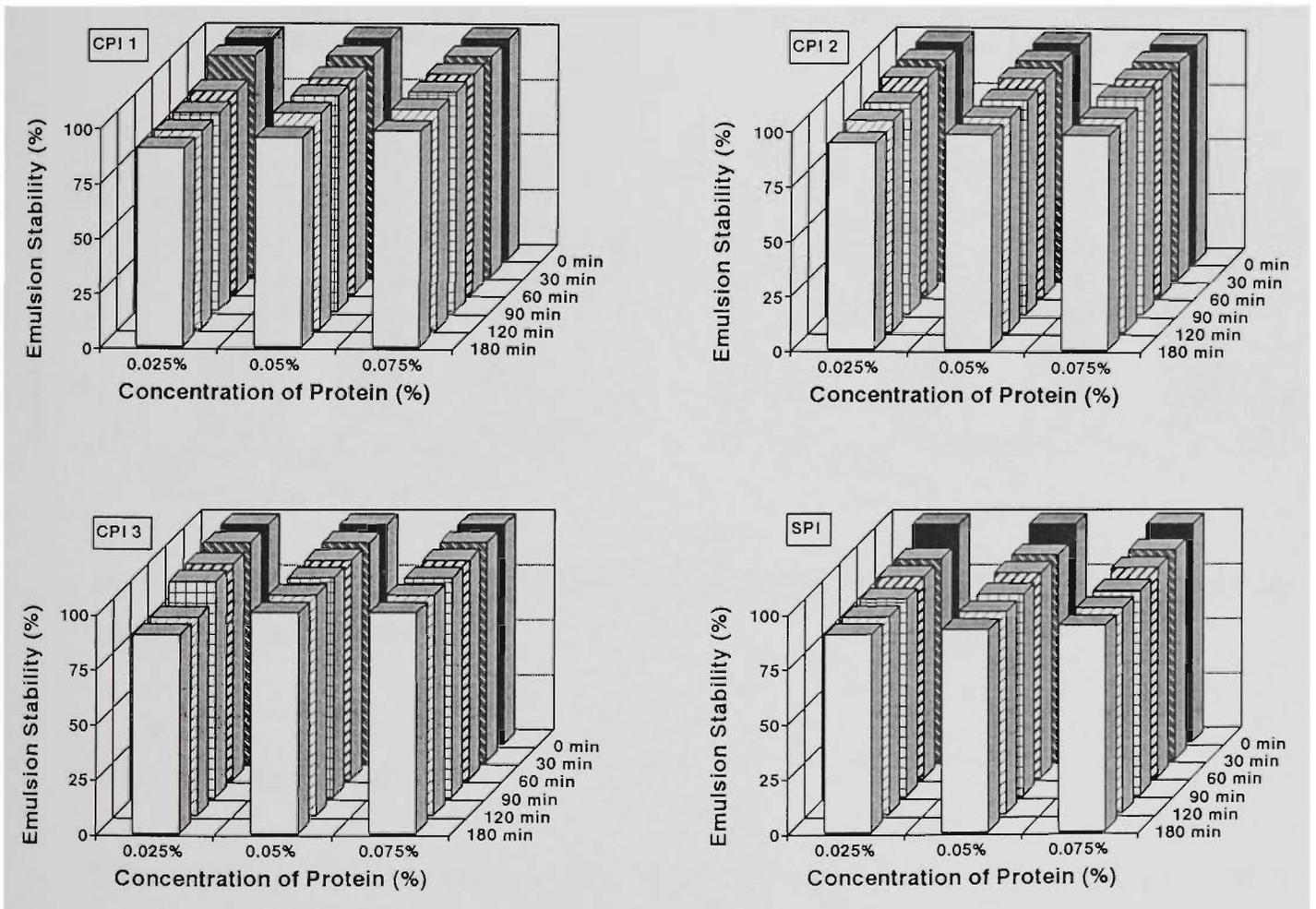


Fig 4.1.7 a-d Effect of protein concentration on the emulsion stability (ES) over 3 hours for chickpea and soy protein isolates.

The effect of protein concentration on the emulsion stability (ES) of chickpea protein isolates is not great. For short time stability (within 3 hours), there is no significant difference in emulsion stability between three chickpea protein isolates at all the concentrations. After 3 days, the CPI-1 could retain about 25% of its stability at all the concentrations of the protein.

The CPI-2 and CPI-3 could still retain about 75% its stability at all of the concentrations (Fig 4.1.7 a-d, e-h). The CPI-2 and CPI-3 show a high emulsion stability, which is higher than that of SPI. The emulsion stability of CPI-1 is similar to that of SPI.

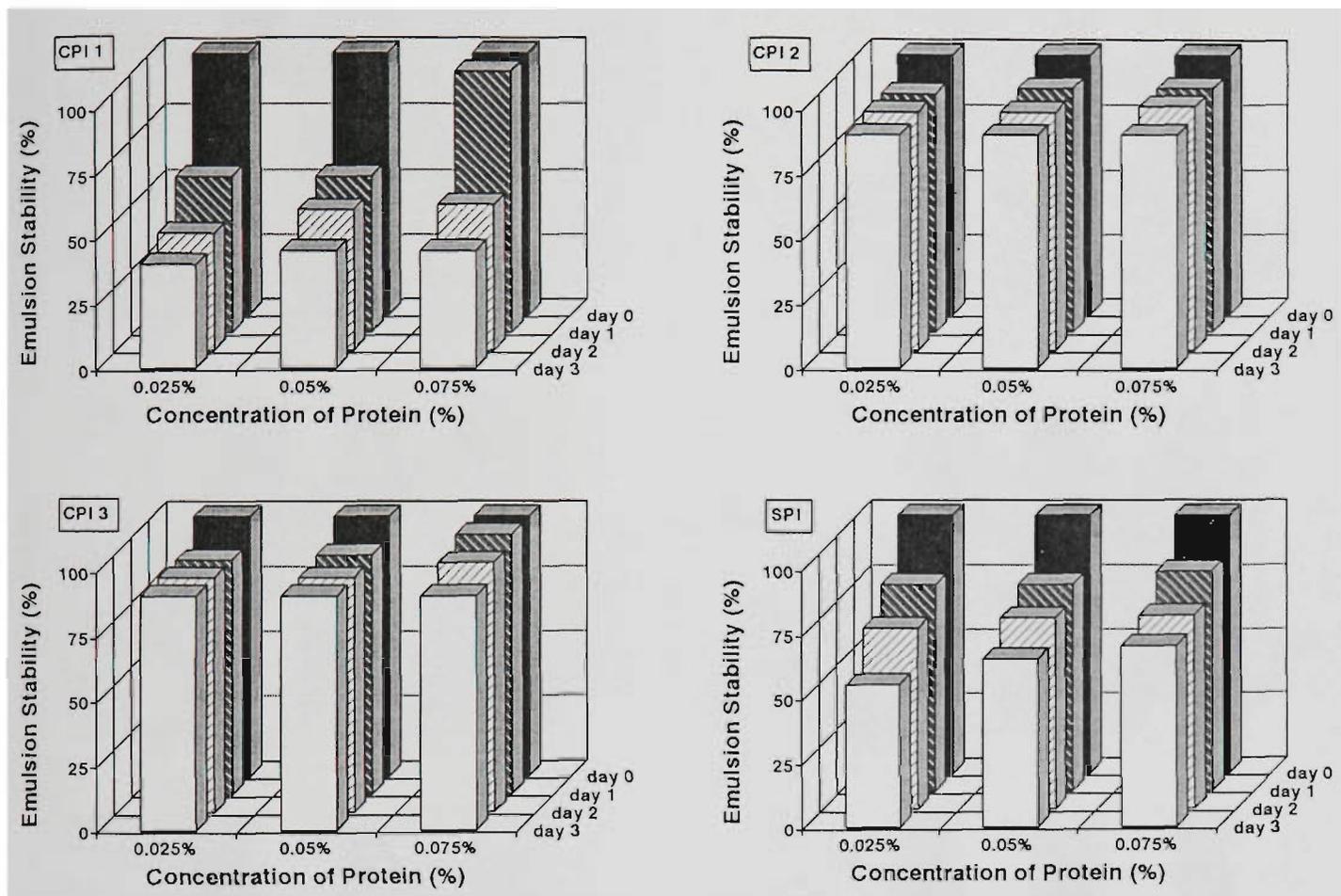


Fig 4.1.7 e-h Effect of protein concentration on the emulsion stability (ES) over 3 days for chickpea and soy protein isolates.

4.1.4.2 Effect of pH

Effect of pH on the ES of chickpea and soy proteins was given in Fig 4.1.8 a-d, e-h. At pH 5, all four isolates had a very unstable emulsion. After one day, only CPI-3 could retain less than 20% of its initial emulsion while emulsions of other proteins almost collapsed. At pH 7 all protein' emulsions were more stable. Soy protein had the most stable emulsion, retaining up to 45% of its initial emulsion after four days. The chickpea albumin CPI-2 had a relative stable emulsion, retaining 40% of its initial emulsion but totally collapsed after four days. The chickpea alkaline glutelin (CPI-3) showed a less stable emulsion, retaining a steady emulsion of 25% of its initial emulsion after two days. The chickpea acidic glutelin had a poor emulsion stability, having less than 20% of its initial emulsion within one day. At pH 9, all emulsions were more stable. Soy isolate had a more stable emulsion at pH 7 than all

chickpea proteins but at pH 9, CPI-3 had a more stable emulsion than that of soy isolate over the first two days. After one day, all proteins could retain relative stable emulsion ranging from 35-45%.

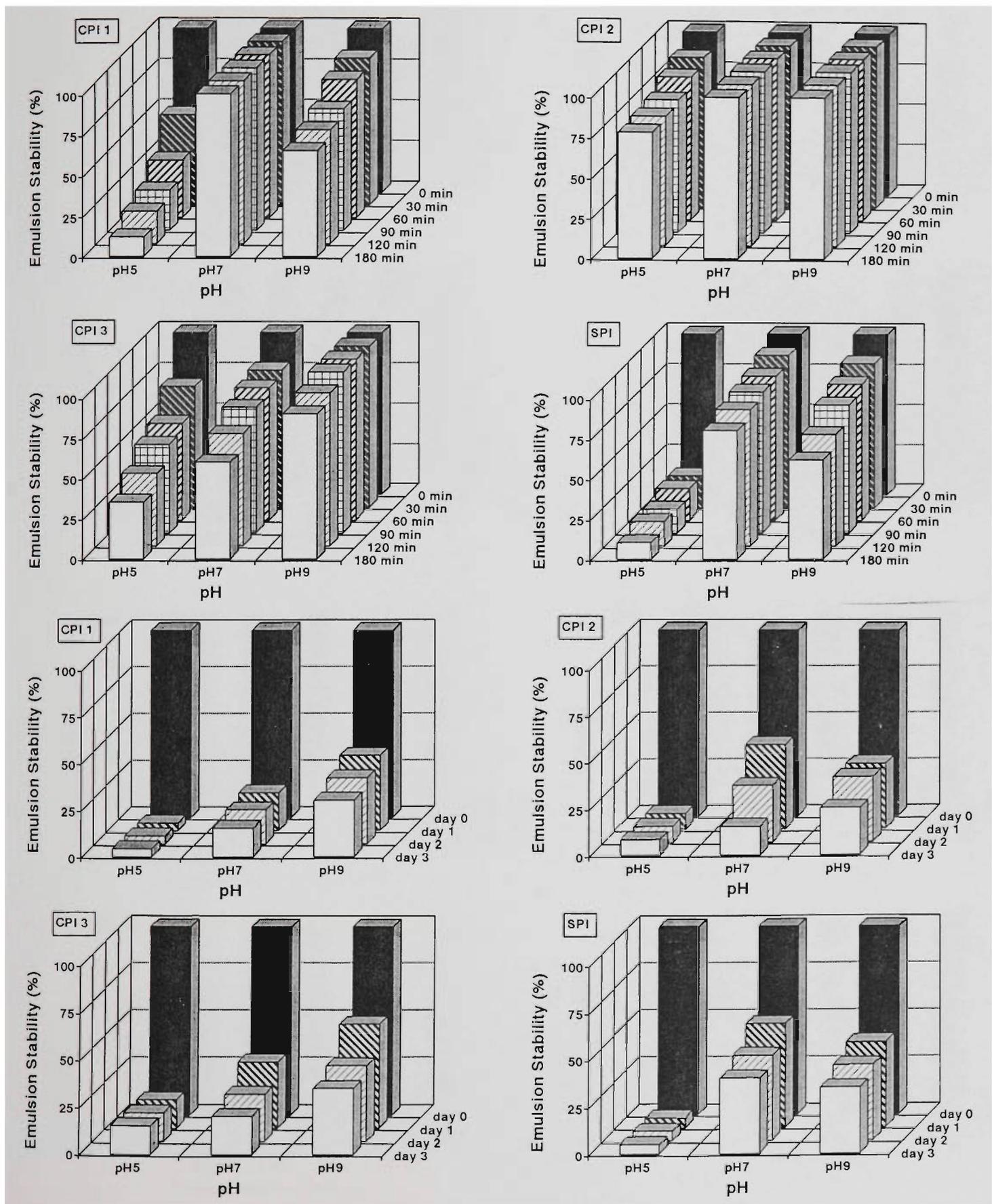


Fig 4.1.8 Effect of pH on the emulsion stability (ES) over 3 hours (a-d) and 3 days (e-h) for chickpea and soy protein isolates.

4.1.4.3 Effect of ionic strength

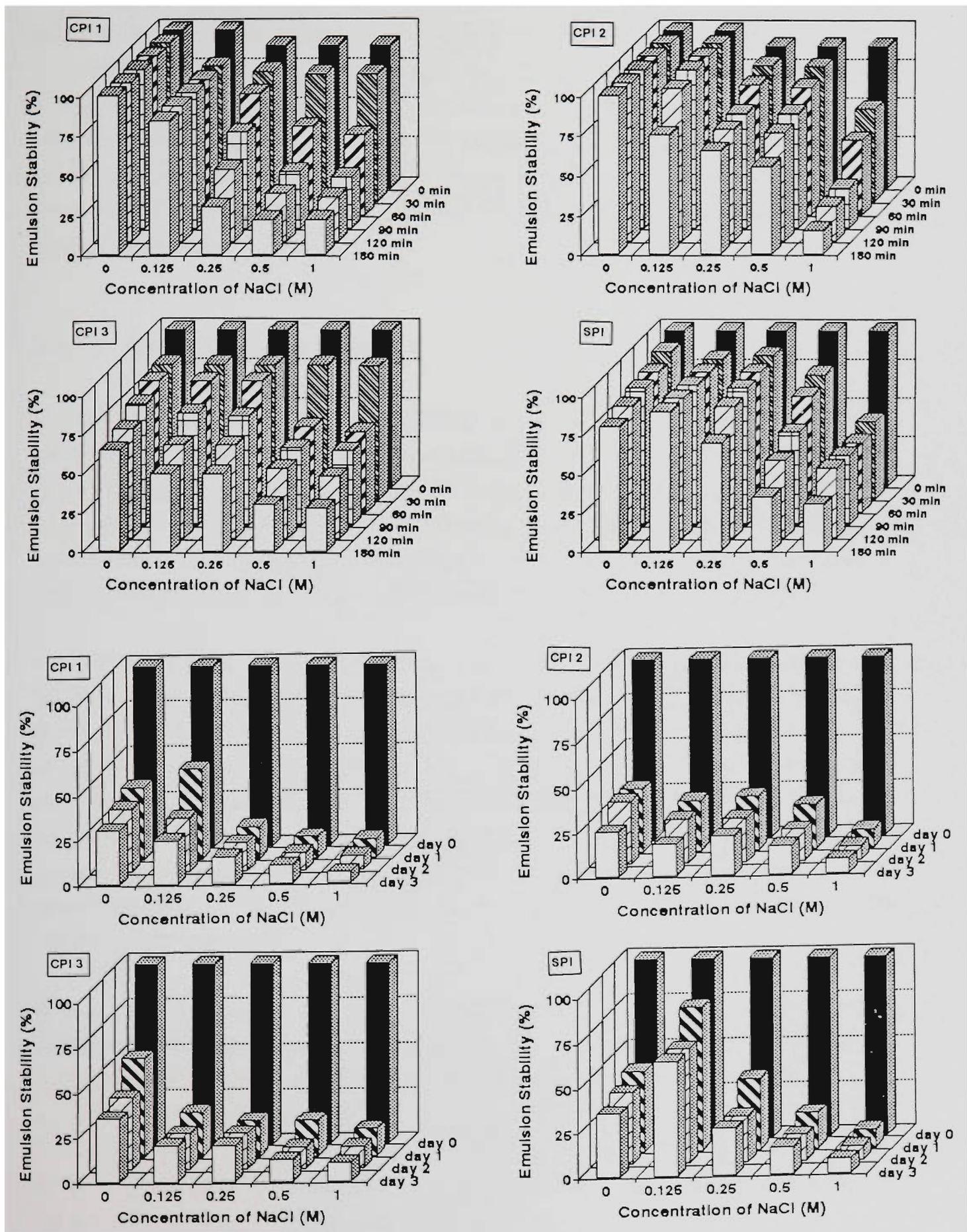


Fig 4.1.9 Effect of salt concentrations on the ES in 3 hours (a-d) and in 3 days (e-h) of chickpea and soy protein isolates.

The effect of salt concentration on the emulsion stability of 4 protein isolates in different salt concentrations over a short time (30-180 minutes) and over long time (1-3 days) was shown in Fig 4.1.9 a-d, e-h. At 0.125M NaCl, all isolates, except CPI-3, showed a stable emulsion. Soy and CPI-1 formed the most stable emulsions. At 0.25M NaCl only soy could retain its emulsion. At 1M NaCl all isolates showed an unstable emulsion with 50% of the initial emulsion collapsed within one hour. At low salt concentration, SPI could retain up to 70% of its initial emulsion over a three day period while CPI-1 could only retain about 30%. Other chickpea protein isolates were less stable. At higher concentration of NaCl, all protein isolates formed unstable emulsions.

4.1.5 FOAMING CAPACITY

Foam expansion (FE) was determined for chickpea and soy protein isolates (500E). The FE of all chickpea isolates increased with increasing protein concentration (1-5%). All chickpea protein isolates showed a higher FE than that of soy isolates at all three concentrations. At 1% concentration there was a slight difference between these isolates but at higher concentrations (3-5%), the acidic and alkaline extracted proteins CPI-1 and CPI-3 had a markedly high FE than that of CPI-2. CPI-3 had the highest FE at all concentrations.

Richert (1979) reported that FE increased with protein concentration to a maximum then decreased. The maximum varied with different types of proteins. Britten and Lavoie (1992) found the maximum or maximal foam expansion concentration for sodium caseinate was about 4% and those of whey and ovalbumin about 8%. The difference in FE of these proteins was attributed to the protein solubility. Britten and Lavoie (1992) also found that regardless of large differences in structural properties, there was an almost identical relationship between protein concentration and foam expansion of sodium caseinate, whey protein isolate and ovalbumin. It indicated that the concentration effect on FE did not seem related to the protein type.

In this study, in contrast with chickpea protein, FE of soy isolate decreased with increasing protein concentration (1-5%) under identical measuring conditions (Fig 4.1.10). Soy isolate was more soluble than CPI-1 and CPI-3 at 2% concentration, when measured at pH 6-7 but its FE was much less than those of CPI-1 and CPI-3. Also CPI-2 was more soluble than CPI-3 at pH 6 - pH 7.5, but, again FE of CPI-3 was much higher than that of CPI-2. This observation suggested that although the concentration of a soluble protein influences its FE but this effect is markedly related to the protein type.

Other environmental factors, such as pH, ionic strength and type (Phillips and Kinsella, 1990; Mohanty *et al.*, 1988; Richert, 1979) also affect FE of a protein and these effects again are closely related to protein type. The FE vs pH curve of CPI-3 (pH 2 - pH 9) resembled its solubility vs pH curve while the FE of soy isolate decreased gradually with increasing pHs (Fig 4.1.11). There was no positive correlation between soluble protein concentration and FE for soy isolate while FE of CPI-3 correlated well with its soluble protein content.

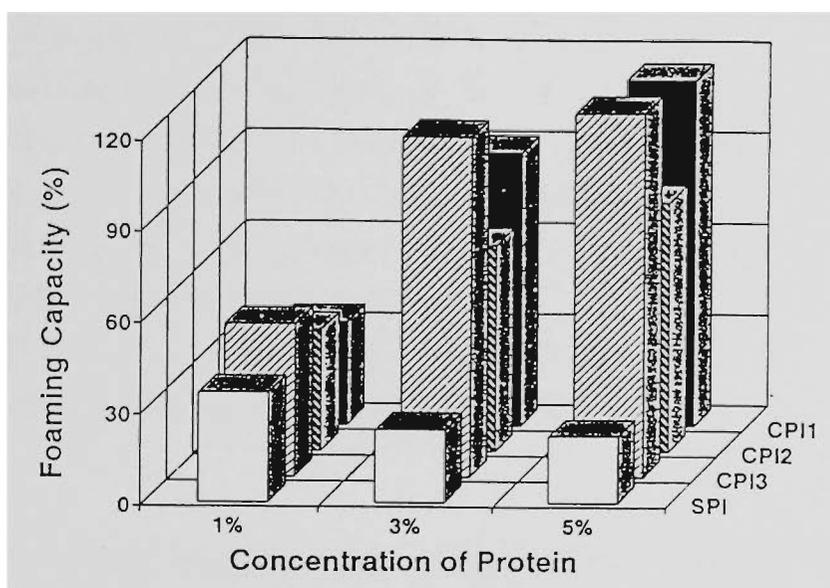


Fig 4.1.10 Foam expansion of chickpea and soy protein isolates.

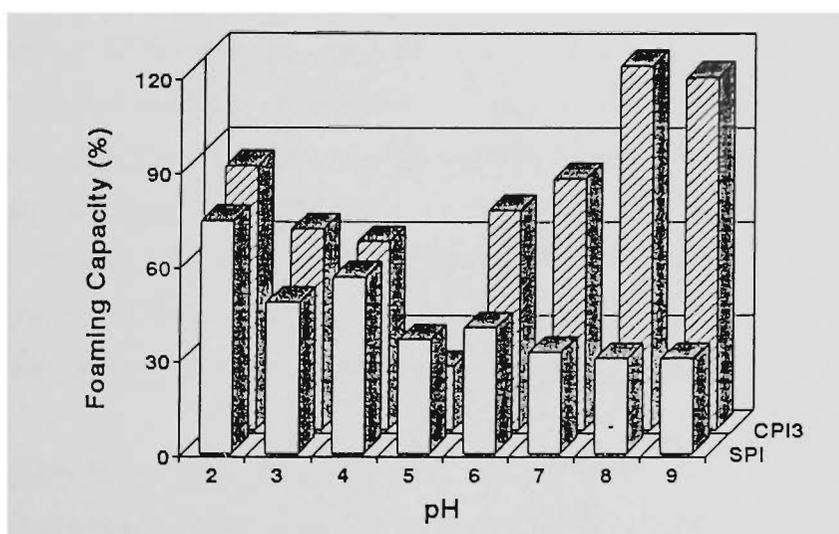


Fig 4.1.11 Effect of pH on the FE of chickpea and soy protein isolates.

4.1.6 CONCLUSION

Chickpea proteins extracted at pH 2, pH 7 and pH 9 (CPI-1, CPI-2, CPI-3, respectively) display different functional properties. Like soy protein, CPI-3 showed a shape decrease in soluble nitrogen index at the isoelectric point region (pH 4.4-4.8), while those of CPI-1 and CPI-2

were broader. CPI-3, CPI-2 were more soluble than CPI-1, over a range of pH 2-10. At certain pHs they were more soluble than soy protein isolate (SPI). CPI-3 and CPI-2 also absorbed more oil and water than CPI-1.

Emulsion capacity (EC) of three chickpea protein isolates increased with increasing protein concentration and showed a good relationship between their solubility and EC. Their pH/EC curves resembled those of their pH/solubility. Thermal effect on EC of CPI-3 and CPI-2 was greater than that of CPI-1 which indicates that CPI-3 and CPI-2 were more sensitive to heat treatment than CPI-1. Depending on the concentration, salt could decrease or increase the EC of CPI-1, CPI-3 and SPI. The salt effect was less with ES of CPI-2. All emulsions were stable at pH 9 and EC of CPI-3 was more stable than that of SPI at pH 9. With salt addition, all chickpea protein isolates were less stable than soy protein isolates. Foaming capacity (FC) of all chickpea protein isolates, particularly CPI-3, was higher than that of soy protein isolate. The difference in these functional properties reflected the difference in their composition and structures due to different processing conditions.

4.2 SOLUBILITY OF CHICKPEA PROTEINS MEASURED BY INFRARED SPECTROSCOPY

As indicated in the previous section (4.1), functional properties of chickpea proteins were affected by processing conditions. These properties, in turn, affected the potential application of these proteins in the food industry. Solubility is considered as the most important functional property of a protein either for practical application or for research interests. Most food applications are presented in aqueous or semi aqueous forms. On the other hand, solubility shows a closer link with other functional properties of a protein such as water absorption, hydration capacity and emulsifying capacity.

To examine the protein solubility, the Kjeldahl procedure is widely used to determine the nitrogen in extracts of the protein over a range of pH levels (Bradstreet, 1965). Regardless of several modifications including semi- automation, the Kjeldahl procedure is still a time consuming and rather laborious assay (Pearson and Pertz, 1976; Fosdick *et al.*, 1982; Hach *et al.*, 1987; Devani *et al.*, 1989; Barbano *et al.*, 1990).

With increasing interest in food applications of vegetable proteins, there is a need for an accurate, rapid and less laborious method to examine their solubility. Infrared spectroscopy may provide a quick, non-destructive and easy to perform alternative procedure. Goulden (1964) and other investigators (Biggs, 1979; Sjaunta and Schaar, 1984) have successfully

estimated proteins in cow milk by Infrared absorption. With its accurate results, compared to those obtained from the Kjeldahl method, instrumental Infrared estimation of milk proteins has been recognised as an official method for the dairy industry (Van de Voort, 1980). In addition, Hung *et al.* (1988) used a single cell infrared milk analyser - *the Milkoscan 104* - to estimate the protein content in soy beverage and aqueous extracts from lupin and peanut seeds. Since the Milkoscan 104 gave as accurate an estimation of protein content in these legume extracts as the Kjeldahl method, this instrument should also serve as a simple means to study the solubility of grain legume proteins.

The solubility of a protein is greatly dependent on the pH of the extraction medium since the pH influences charge and the electrostatic balance within and between protein molecules (Chapter 3 and Chapter 4, Section 1). Proteins of grain legumes are almost insoluble near their isoelectric points (pI), but their solubility increases at both lower and higher pHs, because at these pHs proteins have a net negative or positive charge.

This section evaluates the application of an infrared spectroscopic technique for a study of the solubility of the previous three chickpea proteins, field pea and soybean proteins over a wide range of pH by comparing results from both the Kjeldahl procedure and the instrumental infrared spectroscopic method. The results are expected to reflect the effect of different extraction conditions on the composition and solubility of these proteins.

4.2.1 COMPARISON BETWEEN KJELDAHL AND MILKOSCAN METHOD

Fig 4.2.1 shows the correlation between Kjeldahl and Milkoscan values for protein estimation by linear regression analysis at three different pHs. The slopes for the protein extracts at pH 2, pH 7 and pH 9 were 0.745, 1.011 and 0.982, respectively. The results obtained by the Milkoscan method agreed with those by the Kjeldahl method, for extracts at pH 7 and pH 9, but the results obtained from the Milkoscan were lower than those from the Kjeldahl for extracts at pH 2.

The influence of pH on infrared readings for milk proteins has been mentioned by Goulden (1964) who found that when the pH of milk dropped from 6.7 to 6.5, the infrared reading increased by 0.03%. In naturally soured milk, carboxylic acids were produced and existed predominantly as carboxylate anions with two most intense absorption bands at 1540 cm^{-1} (6.5 mm) and 1410 cm^{-1} (7.1 mm). With the addition of the first absorption band (6.5 mm), due to the carboxylate anions formed in soured milk, the optical density at 6.46 mm for nitrogen - hydrogen bonding of the peptides increased, resulting in an increase in apparent protein content. In contrast, Robertson *et al.* (1981) found that lowering the pH of a milk

sample from 5.96 to 5.48 caused a slight decrease in the protein reading (0.063%). These studies showed a different effect of pH on the protein reading. However, the protein changes found were small, perhaps due to the small pH changes investigated. The influence of pH on the infrared protein reading under strong alkaline and acidic environments, therefore, warranted further investigation.

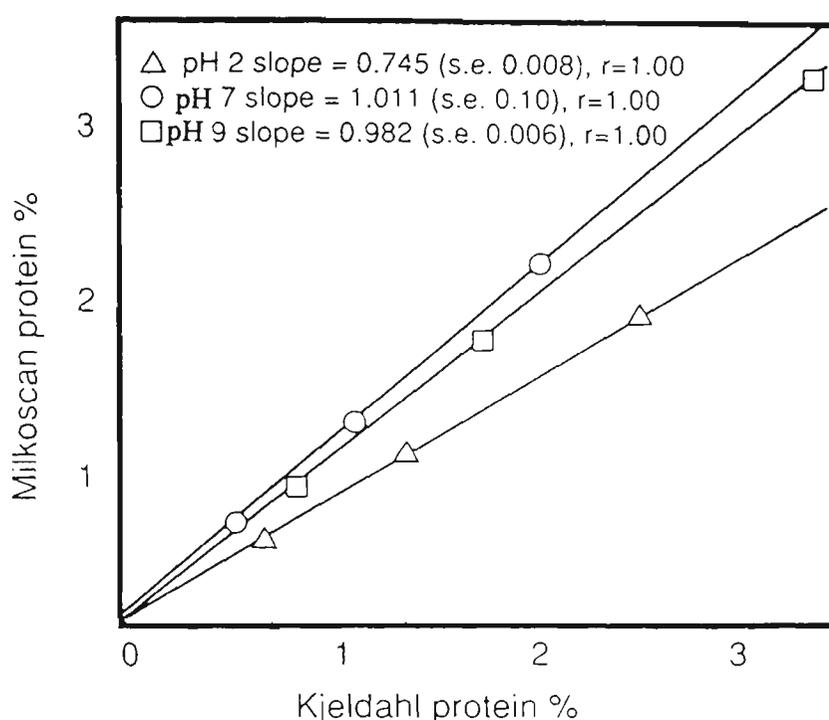


Fig 4.2.1 Protein values obtained by the Milkoscan and by the Kjeldahl procedure on the samples of chickpea protein isolates, extracted at pH 2, pH 7, pH 9.

To investigate the effect of extraction pHs on the accuracy of the Milkoscan protein reading, the solubility of a purified chickpea protein isolate (CPI-3) was measured at a wider range of pH by both methods. The solubility profile obtained by the two methods was different. The Milkoscan gave lower values than the Kjeldahl procedure at very low and very high pHs (pH 2-3 and pH 9-10) but higher values at pHs near the isoelectric point of the proteins (pH 4 - pH 6) (Fig 4.2.2). A similar observation was obtained from the solubility of other chickpea protein isolates extracted at pH 7 and pH 2 (CPI-2 and CPI-1).

The ratio of the Milkoscan to Kjeldahl protein contents was calculated and tabulated for each pH. This procedure was repeated for field pea protein isolate (extracted at pH 9) and a soy protein isolate (Fig 4.2.3).

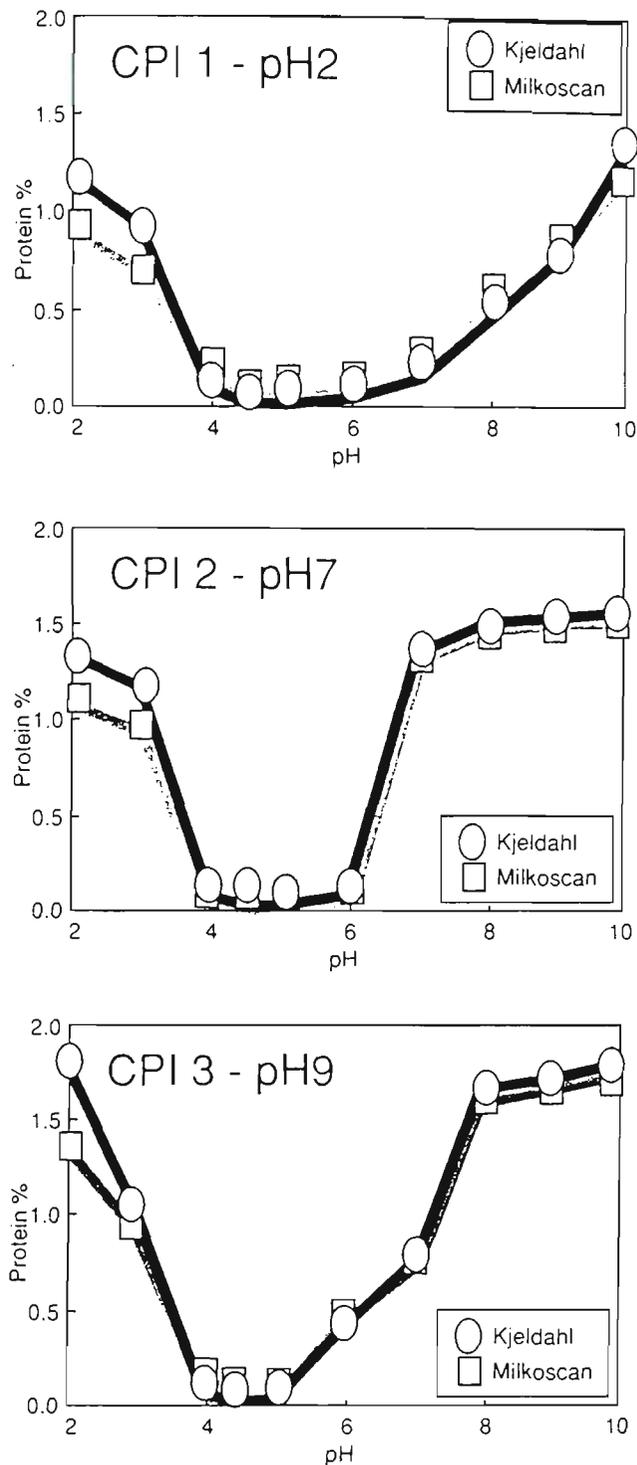


Fig 4.2.2 Solubility of chickpea isolates prepared at pH 2 (CPI-1), pH 7 (CPI-2), pH 9 (CPI-3) measured by the Milkoscan and the Kjeldahl procedure.

4.2.2 MILKOSCAN AND ACETAMIDE SOLUTIONS

The result in Table 4.2.1 indicated that at the pHs at which the proteins are likely to have net negative or positive charge (ie very low, very high pH), the Milkoscan readings were lower than those from the Kjeldahl (ratio less than 1). In a study on aqueous ammonium ion by transmission infrared spectroscopy, Van de Voort *et al.* (1986) found the influence of pH over the range of 3.5-8 had little effect on the infrared reading, but a significant effect was

observed at pH 8.5. The change was consistent with the shift in the equilibrium from NH_4^+ to NH_3 (aqueous). Since the protein measurement by the Milkoscan was based on the infrared energy absorbed by the nitrogen hydrogen bond within the peptide skeleton of the protein molecules, any factor affecting this bond is likely to cause a change in the Milkoscan reading. To examine this, solutions of two acetamide derivatives - N-methyl and N-ethyl acetamide - were analysed on the Milkoscan at a constant concentration but at different pHs. The Milkoscan readings obtained from solutions of each acetamide derivative, over the pH range 2-10, are given in Table 4.2.2. In each case, the Milkoscan reading varied with pH and in each case the highest reading was obtained at the unadjusted pH. The different response to pH change of the two acetamide derivatives appears to be a consequence of the altered strength of the N-H bond caused by the different electron releasing ability of the two alkyl substituents. The behaviour of a protein in aqueous solution is obviously more complicated than either of these simple amide derivatives. However, it seems clear from this investigation of the behaviour of the amides that the lower Milkoscan readings with protein solutions under both strongly alkaline and acidic conditions, are due to the influence of pH on the nature of the peptide bonds.

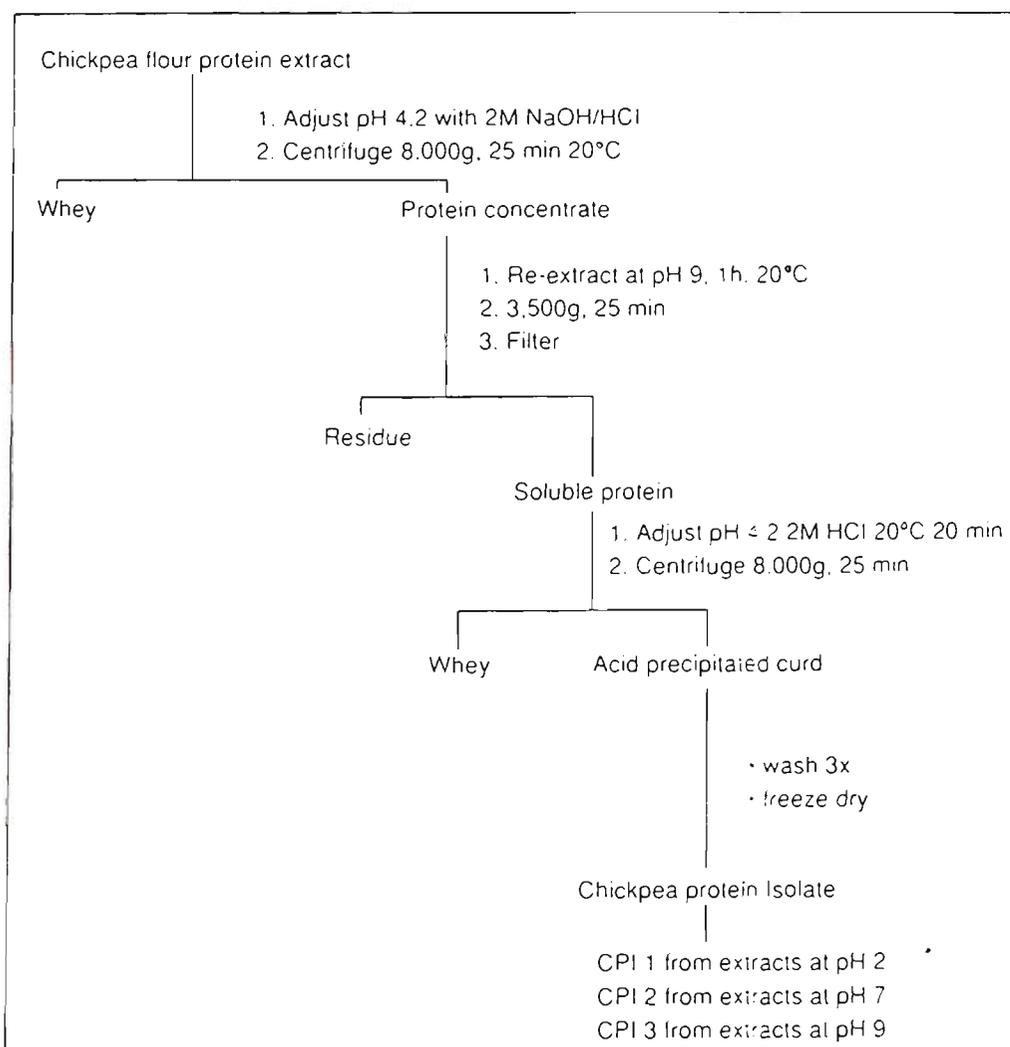


Fig 4.2.3 Process for chickpea / field pea protein isolate preparation.

Table 4.2.1 Ratio of Milkoscan/Kjeldahl protein contents of protein isolates measured over a range of pH values

Extract pH	Chickpea CPI-1	Chickpea CPI-2	Chickpea CPI-3	Field pea FPI	Soybean SPI
2	0.77	0.77	0.77	0.82	0.79
3	0.81	0.82	0.81	0.90	0.85
4	0.82	1.06	1.32	2.27	2.4
4.5	1.71	1.75	5.92	36	3.9
5	1.60	3.2	6.37	-	3.5
6	1.26	1.33	1.06	3.6	1.01
7	1.22	0.94	0.98	1.02	0.95
8	1.00	0.95	0.95	0.98	0.96
9	0.98	0.95	0.95	0.98	0.96
10	0.95	0.95	0.94	0.97	0.95

Table 4.2.2 Milkoscan reading* of N-methyl acetamide and N-ethyl acetamide aqueous solution with constant concentration over pH 2 - pH 10

Measure pH	N-methyl acetamide ^a	N-ethyl acetamide ^b
2	0.300	0.302
3	0.306	0.313
4	0.312**	0.332
5	0.308	0.357
6	0.305	0.372
7	0.306	0.370
8	0.300	0.378**
9	0.300	0.375
10	0.292	0.367

* Means of 6 readings

a. SD= 0.0037 - 0.0081

** Unadjusted pH

b. SD= 0.0040 - 0.0106

Table 4.2.3 Milkoscan and Kjeldahl protein contents (%) of skim milk

Milkoscan	Measured Kjeldahl	Expected Kjeldahl protein
0.39	0.37	0.370
0.19	0.18	0.185
0.11	0.08	0.092
0.06	0.01	0.046

At pHs close to the pI the protein readings by the Milkoscan were higher than by Kjeldahl. In this study the protein content in the extracts at these pHs (pH 4-6) was low, sometimes lower than 0.02%. At such a low concentration the instrumental measurement was likely to be more sensitive than the Kjeldahl. To test the sensitivity of the two methods when measuring low levels of protein, dilute skim milk was assayed by both methods at several low protein levels. The results (Table 4.2.3) indicated that the Milkoscan results were marginally higher across the range with larger differences at very low levels. At the expected protein level of 0.046, the Milkoscan reading (0.06) was much closer than the Kjeldahl value (0.01). At pH 4 - pH 4.5 in this study, the Milkoscan gave a value 6 times higher than that from Kjeldahl (Table 4.2.1) but the calculated mean protein contents obtained from the Milkoscan and the Kjeldahl were only 0.067% and 0.011%, respectively. It suggested that at such a very low concentration the difference found between the two methods should be treated as experimental error. A reasonable conclusion from this work is that the Milkoscan can reliably estimate protein in these solutions down to 0.1%.

The Milkoscan gave a low reading at pH 10 and a lower reading at pH 2 for chickpea, field pea and soy bean protein isolates. Among chickpea isolates, there were similar differences in protein values obtained by the two methods at pH 2-3 and pH 9-10. This suggests that these protein isolates, although prepared under different conditions, retained a similar amino acid profile and/or structural arrangement and consequently were subject to similar effects under acidic and alkaline environments. Indeed, amino acid profiles of the three chickpea protein isolates prepared at three different pHs was very similar (Table 3.3.1). The gel electrophoretic patterns of protein isolates extracted at pH 7 and pH 9 were similar but both were different from the pH 2 extraction (Fig 4.2.4). This observation may indicate that the amino acid composition of the proteins had more influence on the IR reading while the protein structural arrangement had more influence on the solubility. The degree of difference in protein values obtained by the two methods was not the same between chickpea and field

pea or soybean. This suggests, as expected, a different amino acid profile and structural arrangement among these three grain legumes (Table 3.3.1 and Fig 4.2.4).

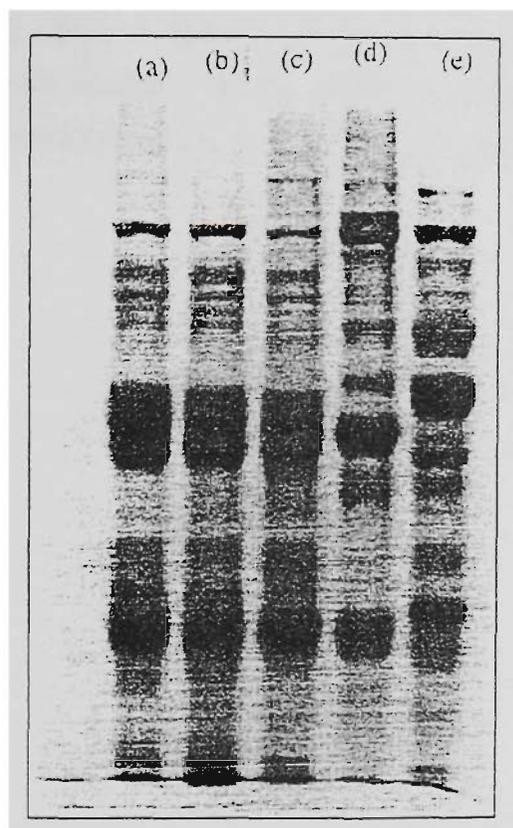


Fig 4.2.4 SDS-PAGE patterns of protein isolates: (a) CPI-3, (b) CPI-2, (c) CPI-1, (d) SPI, (e) FPI.

In conclusion, when protein contents in an aqueous solution are measured there is a significant difference between the values obtained by the Milkoscan and the Kjeldahl procedure at certain pHs. The Milkoscan, however, could still provide a rapid approach to obtain the solubility of different grain legume proteins over pH 7 - pH 9, a most practical range in food applications. A correction factor derived from each grain family needs to be introduced to compensate for the Milkoscan readings at low and high pHs (pH<3 and pH>9). The different response of the three chickpea protein isolates toward infrared measurement reflected their different amino acid composition and conformation, due to the effects of different extraction conditions.

4.3 CONCLUSION

Chickpea proteins extracted at different pHs showed different functional properties. Those extracted at pH 2 and pH 9 showed a high solubility, water absorption and oil absorption and

could be compared with those of a commercial soy protein isolate. The EC of all chickpea isolates increased with increasing protein concentration, suggesting a correlation between their EC and solubility. Also their pH / EC curves correlated with their pH/solubility curves. In addition, the different effect of thermal treatments and salt addition on the EC and FE depended on each protein isolate. These different functional properties reflected their different composition and structures due to different processing conditions.

CHAPTER 5

MODIFICATION OF CHICKPEA PROTEIN ISOLATES BY ACETYLATION AND SUCCINYLATION

MODIFICATION OF CHICKPEA PROTEIN ISOLATES BY ACETYLATION AND SUCCINYLATION

Chickpea proteins have been used as protein ingredients in meat (Verma *et al.*, 1984), beverage (Luz Fermander and Berry, 1987), infant formula (Livingston *et al.*, 1993) and bakery products (Hung *et al.*, 1993) on a laboratory scale. Currently, other novel applications are also being sought. However, like other food proteins, the effective application of chickpea proteins in the food industry depends partly on their nutritional values but mainly on their functional properties. So far only limited information on functional properties of chickpea proteins is available. The effects of extraction conditions on some functional properties such as the solubility, water and oil absorption and surfactant related properties of chickpea proteins were investigated and presented in Chapter 4. The results confirmed that these properties are affected by intrinsic and environmental factors such as protein composition, conformation, salt and pH and at the same time indicated that there was also a need to improve these properties.

There is no single protein which can satisfy all the functional properties required by different food systems. However, the functional properties of a food protein can be altered or modified to improve their behaviour in various food systems (Kinsella, 1976; Olsen and Adler Nissen, 1979; Rhee, 1989). Also proteins are modified for structure-functional relation studies (Damodaran, 1994). There are several chemical and biological means to modify the functional properties of a protein (Vojdani and Whitaker, 1994).

Of several chemical modification approaches, acylation with acetic and succinic anhydride was widely used to improve functional properties of various food proteins (Schwenke and Rauschal, 1980). By introducing acetyl groups into the protein molecules, acetylation improved emulsifying capacity, emulsion stability, foam capacity and stability and water absorption of pea (Johnson and Brekke, 1983; Mendoza-Martinez *et al.*, 1988), soy (Kim and Rhee, 1989), winged bean (Narayana and Narasinga Rao, 1984) and rapeseed proteins (Gwiazda *et al.*, 1989). Similarly, the addition of succinic groups with succinylation improved some functional properties of peanut (Beuchat, 1977; Kim and Kinsella, 1987), egg (Sato and Nakamura, 1977), fish (Chen *et al.*, 1975), casein (Strange *et al.*, 1993), sunflower (Schwenke *et al.*, 1986), field pea (Schwenke *et al.*, 1993) and canola proteins (Paulson and Tung, 1987, 1988; Nitecka *et al.*, 1986).

The modification of chickpea proteins warrants a detail investigation since this can serve as a

means*to improve some of their functional properties and also to enhance its utilisation in processed foods. It also assists in the understanding of the effect of protein's structure on functional performance. Consequently, this chapter reports on the modification of chickpea proteins by acetylation and succinylation, the characterisation and functional properties of the acylated proteins.

The chickpea protein concentrates (CPC) and isolates (CPI) were prepared on a pilot scale plant (40 kg flour) at the food processing centre of the Australian Food Industry Science Centre, using the flour of *C. arietinum* cv Kaniva. Two concentrates (CPC2, CPC3) were extracted at pH 7 and pH 9 and two corresponding isolates (CPI2, CPI3) having 79%, 81% 90% and 92% protein content were acylated with acetic and succinic anhydride at several levels.

Eight acylated proteins (8) from one isolate (CPI3 or NCP) were selected for further investigation. Four succinylated proteins (SCP16, SCP72, SCP73 and SCP79) with 16, 72, 73 and 79% succinylated levels and four acetylated proteins (ACP6, ACP26, ACP45 and ACP49) with 6, 26, 45, 49% acetylated level were subjected to a detailed study.

5.1 CHARACTERISATION OF MODIFIED CHICKPEA PROTEINS

5.1.1 EXTENT OF ACYLATION

The extent of acylation of chickpea protein concentrates and isolates by acetic and succinic anhydride is given in Fig 5.1.1 (a-b). For all investigated protein concentrates and isolates, the level of acylation increased with increasing ratio of acylating reagents to proteins.

However the acylation was almost unchanged above certain acylating reagent / protein ratios. High levels of acetylation for protein isolates extracted at pH 7 (71%) and at pH 9 (72%) were obtained with 20 and 40% acetic anhydride. High levels of succinylation were achieved at 10 and 20% succinic anhydride for proteins isolated at pH 7 (84%) and pH 9 (85%). Protein concentrates also reached high levels of acylation at certain acylating reagent / protein ratios. Canella *et al.* (1979) found that increasing the succinic anhydride from 50% to 100% only increased the level of succinylation of sunflower protein from 86% to 88%. The results indicated that protein isolates were more acylated than concentrates.

The extent of acylation was also affected by different acylating reagents. Eisele and Brekke (1981) found that acetic anhydride was the most effective acylation reagent, compared with

succinic, benzenetricarboxylic anhydride and tetrahydrofuran-tetracarboxylic dianhydride. Ma (1984) also reported that acetic anhydride was more reactive than succinic anhydride. At similar levels of anhydride, oat proteins was acylated to a greater extent (25%) with acetic than with succinic anhydride. Similar results have been reported with sunflower protein isolates (Kabirulla and Wills, 1982). In theory, all nucleophilic groups of the amino acid residues such as sulfhydryl, phenol, amidazole, hydroxyl and epsilon amino acid could be acylated. However, the last group was found to be most reactive because of its low pK and lower steric hindrance (Gounaris and Perlmann, 1967). The high reactivity of acetic anhydride found in some investigations was perhaps due to its high solubility and also to its reduced steric hindrance.

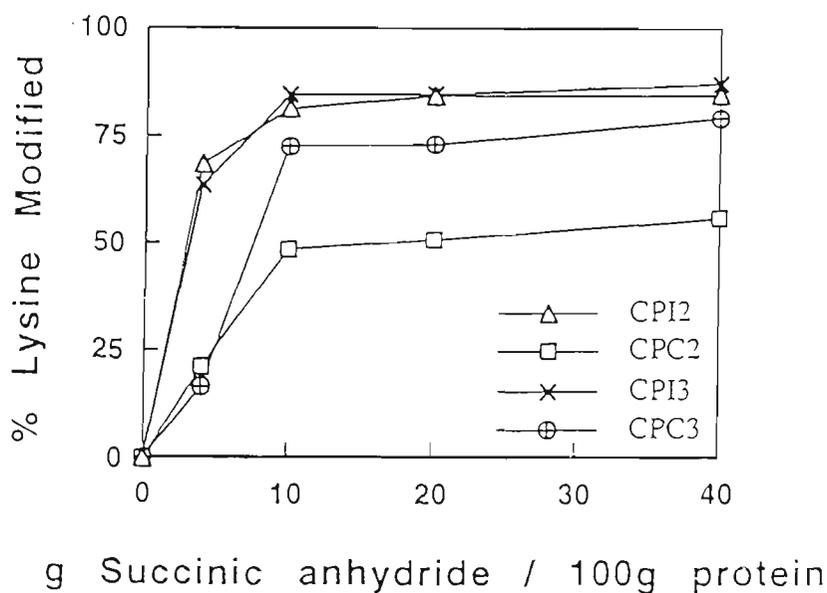
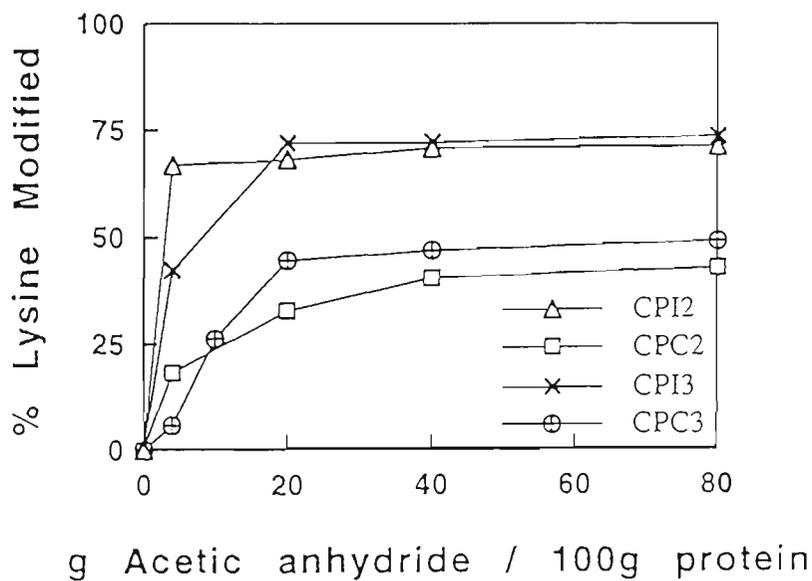


Fig 5.1.1 The extent of acylation of chickpea protein concentrates and isolates by acetic (a) and succinic anhydride (b).

Some investigations indicated that there was no significant difference in the effect of acetic and succinic anhydride on the acylation of winged bean flour (Narayana and Rao, 1984), peanut (Shyma Sunda and Rao, 1980) and cotton seed flour (Rhama and Narasinga, 1983).

In this study, succinic anhydride was found to be more reactive than acetic anhydride. At a 4% of succinic anhydride / protein ratio, more than 63% of epsilon amino groups in lysine were succinylated and the level of succinylation increased to 85% at a 10% succinic anhydride/ protein ratio. The acetylation level increased to 74% and 78%, but it required 40% and 80% acetic anhydride/ protein ratio, respectively. Acetic anhydride was found less reactive than succinic anhydride in the acetylation of sunflower proteins (Canella *et al.*, 1979). The high reactivity of succinic anhydride was demonstrated by a rapid succinylation of rapeseed albumin by a small amount of succinic anhydride (Nitecka *et al.*, 1986). Fungal proteins were succinylated to 20% with only a 2.5% ratio of succinic anhydride / protein and increased greatly to 85% at a ratio of 20% succinic anhydride/ protein. Tobacco leaf (Sheen, 1991), canola (Paulson and Tung, 1988), casein (Kim and Kinsella, 1985), bovine serum albumin (Alford *et al.*, 1984) and soy proteins (Franzen and Kinsella, 1976) were all succinylated to over 90%. The reactivity of succinic anhydride was also affected by the type of protein investigated. Under identical treatment, casein, field bean protein, sunflower globulin was succinylated to levels of 98, 93 and 83%, respectively.

Consequently, the acylation of chickpea proteins indicated that the effect of acylation reagents (acetic and succinic anhydride) on the level of modification varied not only with the type of reagent and its concentration but also with the different types of proteins involved in the system.

5.1.2 AMINO ACID COMPOSITION

The amino acid profile of the native, acetylated and succinylated chickpea protein isolates at several levels of acylation is given in Table 5.1.1.

The essential amino acids of the native protein accounted for 46% of total amino acids. This is higher than those reported for chickpea protein isolated by isoelectric precipitation (39.6%) and micellization (38.2%) (Paredes Lopez *et al.*, 1991). Sulfur containing amino acids and aromatic amino acids accounted for 1.8 and 9.7% while lysine (7.4%) and leucine (9.7%) were the highest essential amino acids. For non essential amino acids, glutamic, aspartic acid and arginine accounted for 36% of the total amino acids.

Succinylation up to 79% and acetylation up to 49% did not alter the essential amino acid

content. The aromatic acids of both succinylated and acetylated proteins remained unchanged but the sulphur containing amino acids increased slightly from 1.8 to 2.5%. Acylation reduced the lysine perhaps due to degradation of lysine residues by long exposure to alkaline treatment and also some loss during dialysis (Franzen and Kinsella, 1976a; Kabirullah and Wills, 1982). As expected, the level of lysine gradually reduced ($P < 0.05$) from 7.4 to 5.81% with increasing level of succinylation. Acetylation also reduced the amount of lysine but to a smaller extent (6.49%). The difference in the loss of lysine reflected the difference in the degree of chemical modification.

Table 5.1.1 Amino acid profile of native (NCP), acetylated (ACP) and succinylated chickpea proteins (SCP) (g/100g protein).

Protein	NCP	ACP6	ACP26	ACP45	ACP49	SCP16	SCP73	SCP75	SCP79	FAO/WHO/UNU		
										Reference protein		
Amino Acid										Infant	child	adult
Essential amino acid												
Histidine	2.62	2.50	2.48	2.36	2.26	2.54	2.58	2.55	2.57	2.6	1.9	1.6
Isoleucine	5.21	4.93	5.22	5.34	5.11	5.06	5.21	5.20	5.16	4.6	2.8	1.3
Leucine	8.48	8.35	8.29	8.52	8.74	8.25	8.43	8.42	8.31	9.3	6.6	1.9
Lysine	7.16	6.70	6.49	6.41	6.59	6.97	6.36	6.39	5.82	6.6	5.8	1.6
Methionine	1.01	1.53	1.65	1.70	1.37	0.98	1.29	1.51	1.39	4.2	2.5	1.7
Cystine and Cysteine	0.88	1.20	1.43	1.32	1.17	0.86	1.16	1.35	1.14			
Phenylalanine	6.50	6.41	6.52	6.61	6.81	6.32	6.51	6.54	6.42	7.2	6.3	1.9
Tyrosine	3.24	3.32	3.38	3.39	3.48	3.15	3.29	3.28	3.29			
Threonine	3.98	3.82	4.00	3.99	3.83	3.87	3.96	3.95	3.99	4.3	3.4	0.9
Tryptophan	0.88	1.01	1.18	1.10	0.78	0.86	0.89	1.10	0.97			
Valine	5.44	5.36	5.53	5.62	5.63	5.29	5.73	5.71	5.70	5.5	3.5	1.3
Nonessential amino acid												
Aspartic acid	11.5	11.9	11.1	9.78	11.8	11.2	11.5	11.4	11.5			
Serine	6.05	5.82	5.86	6.49	5.91	5.88	5.94	5.92	5.95			
Glutamic acid	15.6	16.3	15.4	14.9	14.9	15.2	15.4	15.5	16.0			
Proline	4.41	4.33	5.00	5.03	4.47	4.29	4.50	4.25	4.80			
Glycine	3.94	3.89	3.91	3.99	3.98	3.83	3.94	3.92	3.97			
Alanine	4.52	4.47	4.54	4.62	4.66	4.39	4.56	4.49	4.61			
Arginine	8.68	8.56	8.45	8.38	8.04	8.44	8.61	8.59	8.62			
EAA / TAA (%)	45.4	45.0	46.0	46.5	46.0	45.0	45.5	45.9	44.8			
Protein Recovery (%)	93.3	96.2	86.1	88.4	90.6	95.4	97.4	92.2	96.7			

The data indicated that chickpea protein contained all essential acids required for pre school children and adults as recommended by FAO/WHO (WHO, 1985). Only lysine and threonine were slightly lower than those recommended for infant. Although acylation reduced some lysine, essential amino acids including lysine in acylated chickpea proteins still met the required level for pre school children and adults.

5.1.3 GEL FILTRATION

Native, acetylated (6, 26, 45 and 49%) and succinylated (16, 73, 74 and 79%) protein isolates of chickpea were subjected to Sepharose B6 gel filtration. All these isolates showed very similar resolving patterns by Sepharose with three major fractions. Molecular weight of these three fractions calculated from a standard curve of proteins of known molecular weight were approximate 912, 52.5 and 20.9 kD for the native protein; 923, 58.9 and 22.9 kD for 6% and 45% acetylated proteins. The proportion of these three main fractions from the unmodified protein was approximately 30, 42 and 28%, respectively. Although the chromatogram of these proteins was very similar, the quantity of protein in each individual peak was somewhat different. As the level of acetylation increased, the proportion between the peaks changed. The peak eluted at void volume decreased to 19% while the second peak increased to 44.7% when the protein was acetylated to 49%. The results indicated that some protein rearrangement occurred with the dissociation of high molecular weight proteins and the agglomeration of proteins with low MW.

5.1.4 GEL ELECTROPHORESIS

The SDS-PAGE patterns of the unmodified and acetylated, succinylated chickpea proteins at several levels is given in Fig 5.1.2.

The electrophoretic profile of the native protein showed seven bands with three major bands found in the range of 0.21, 0.33 and 0.83 Rf, respectively. This profile contained less band than those reported for protein isolates prepared by micellization and isoelectric precipitation (Paredes Lopez *et al.*, 1991).

There were some similarities between the electrophoretic patterns of the native and succinylated proteins as illustrated by several major bands. However, the electrophoretic profile of succinylated proteins showed a new distinct band at 0.32 Rf. As the level of succinylation increased several minor bands in the range of 0.34 to 0.83 Rf gradually decreased in their intensity and completely disappeared at 79% succinylation. These

electrophoretic changes indicated that succinylation lead to the formation of larger subunits, perhaps due to the protein agglomeration.

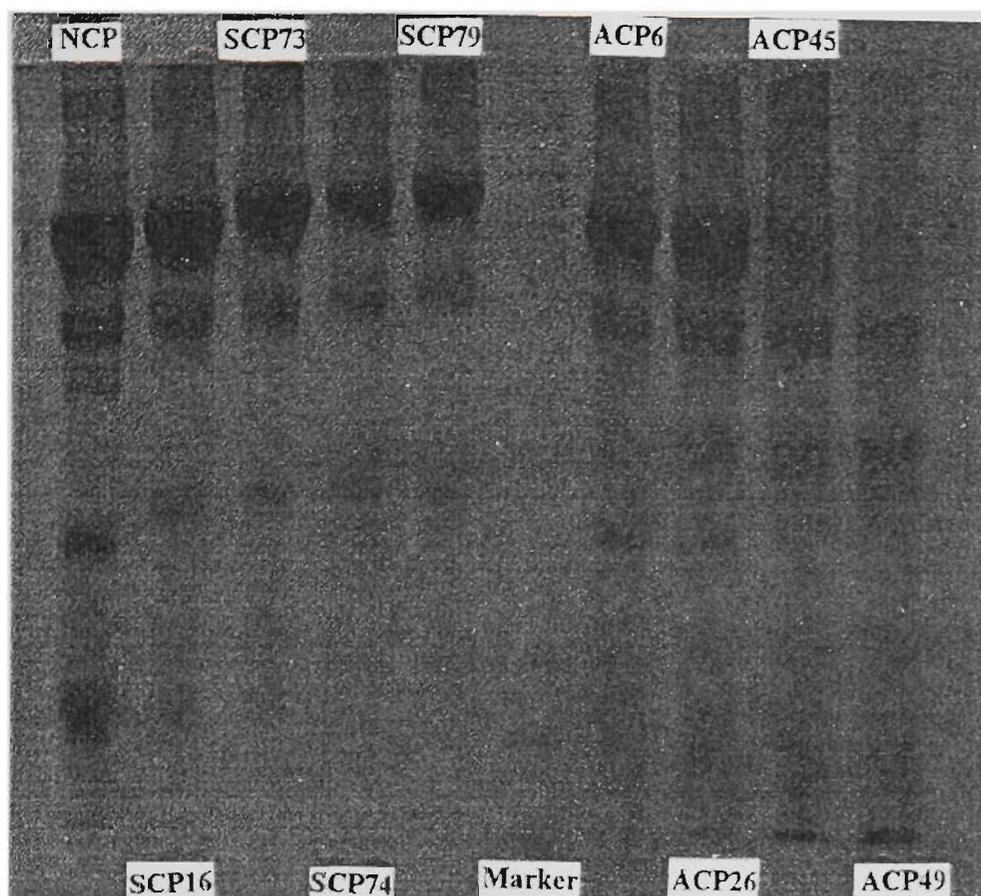


Fig 5.1.2 The SDS-PAGE patterns of the unmodified and acetylated, succinylated chickpea proteins at several levels.

Increasing the level of succinylation would introduce more negatively charged succinic residues to the protein molecules. With increasing net negative charge, the electrophoretic mobility of the succinylated proteins is expected to be faster. Indeed, succinylation of sunflower (Kabirulla and Wills, 1982) and cotton seed flour protein (Choi *et al.*, 1983) did increase their electrophoretic mobility in Polyacrylamide disc gel electrophoresis. The highly succinylated cottonseed flour (treated with 60-100% succinic anhydride / protein) migrated faster than the non treated samples (Choi *et al.*, 1983). However, the electrophoretic mobility of proteins does not always increase by succinylation. For example, the electrophoretic mobility of peanut proteins in PAGE without SDS was reduced with those succinylated at the highest levels (100-130%) (Beuchat, 1977). Succinylation also retarded electrophoretic mobility of corn germ protein isolate (Messinger *et al.*, 1987). When the proteins are subjected to electrophoresis in the presence of SDS, they are separated purely on the basis of differences in the sizes and then by the molecular weight (Andrews, 1988). Consequently, the change in electrophoretic mobility of succinylated proteins in this

investigation could not be affected by an increase in net negative charge but more likely by their unfolding and molecular expansion.

The electrophoretic pattern of the native and acetylated chickpea proteins was outstandingly similar in terms of band number and band intensity. As the level of acetylation increased, a major band at 0.32 Rf and also at 0.83 Rf gradually disappeared and several bands in the region below 0.91 Rf were formed. The formation of new subunits with low molecular weight, together with disappearance of some subunits with high molecular weight, showed that acetylation lead to the dissociation of some protein fractions with larger molecular weight.

5.1.5 DENSITOMETRIC SCANNING PROFILES

The effect of acylation on the structural changes of proteins could be demonstrated clearly by using densitometric scanning of SDS-PAGE of the proteins investigated. Densitometric scanning profiles of the native, acetylated and succinylated proteins at several levels are given in Fig 5.1.3.

The densitometric profile of the unmodified chickpea protein consisted of 7 bands. The molecular weight of the subunits calculated from a standard curve of proteins of known molecular weight were 15.8, 21.9, 24.5, 36.3, 41.7, 44.7 and 52.5 kD, respectively. Of these, those of 15.8, 44.7 and 52.5 kD were the major subunits.

Succinylation at a level of 16% resulted in two major changes. Firstly, the major subunit of 15.8 kD and few other subunits in the molecular weight range of 24.5 and 15.8 kD disappeared. Secondly, the subunit with highest molucular weight (52.5 kD) was found at the range of 53.7 kD. As the level of succinylation increased, this subunit moved upward gradually to 63.1 kD while subunits with low molecular weight either disappeared or formed new larger subunits. The densitometric pattern of the protein succinylated at 79% showed only three subunits having molecular weight of 63.1, 49 and 30.9 kD, respectively. These changes reflected some rearrangement of proteins caused by succinylation. Hurrell *et al.* (1963) found that cross linking between peptide chains of proteins could occur through epsilon-amino groups of lysine.

Compared with the native protein, the electrophoretic pattern of 6% acetylated protein showed a clearly retarded mobility for all six subunits. As the level of acetylation increased, an increase in retarded mobility of acetylated proteins was observed. The subunit having molecular weight of about 53 kD at 6% acetylation increased to 61.7 kD at 49% acetylation.

Acetylation resulted in the formation of a new subunit having the highest molecular weight in faba bean protein (Schwenke *et al.*, 1991). Acetylation also increased remarkably the molecular mass of faba bean legumin and protein isolates and subsequently retarded their SDS-PAGE mobility.

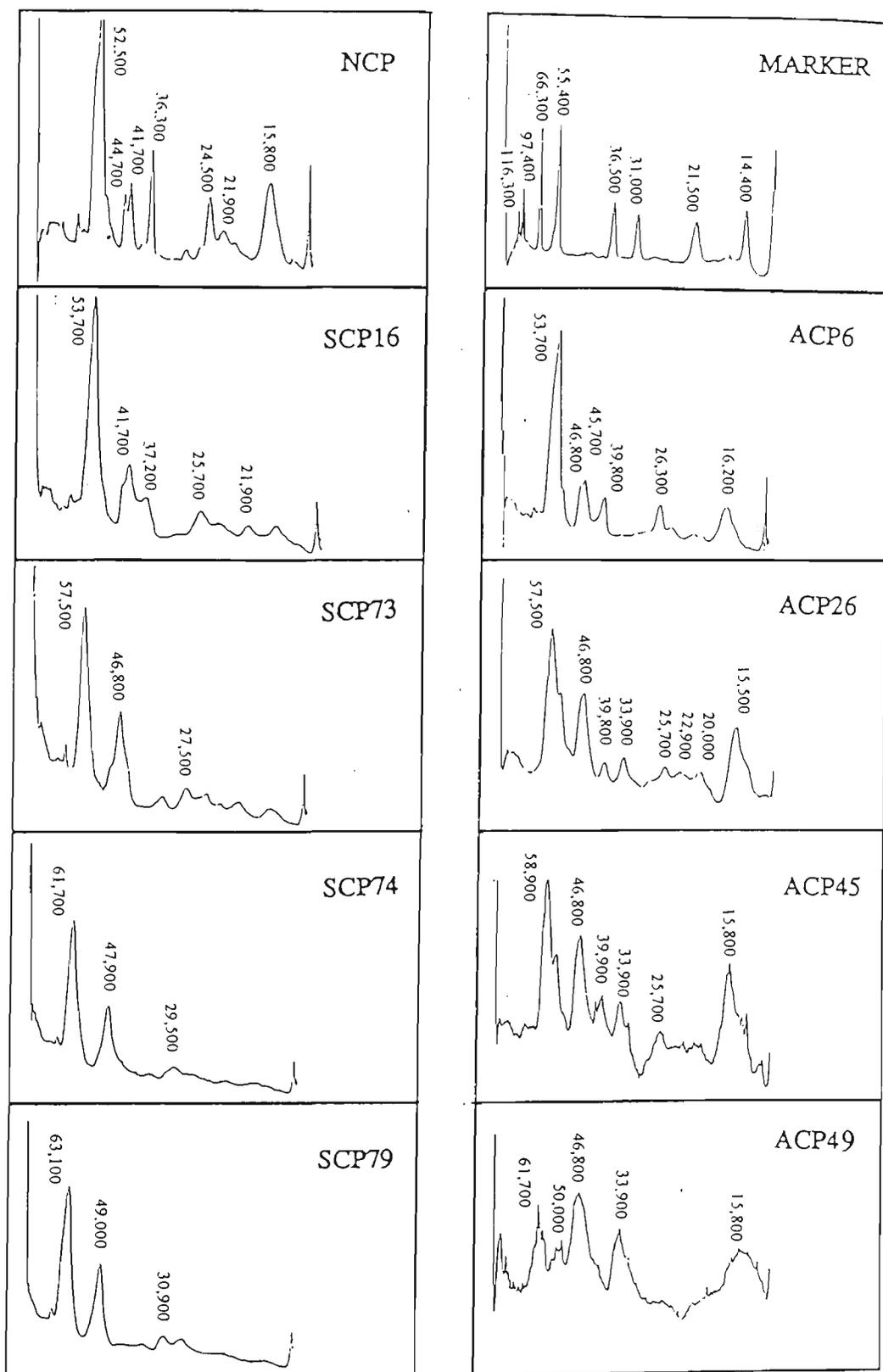


Fig 5.1.3 Densitometric scanning profiles of the native, acetylated and succinylated proteins at several levels.

The electrophoretic profiles revealed some structural differences between acetylated and succinylated proteins. Some subunits with low molecular weight (<20 kD) of succinylated proteins gradually disappeared while those of acetylated proteins associated as the level of acylation increased. At high level of acetylation (45-49%) these subunits agglomerated to form a new subunit having molecular weight of 16.2 kD while subunits with high molecular weight (45 kD) partly dissociated. These structural change may lead to some change in functional properties of ACP45 and ACP49 (Liu and Hung, 1996).

5.1.6 *IN VITRO* DIGESTIBILITY

The *in vitro* digestibility of the native, acetylated and succinylated chickpea proteins by trypsin, chymotrypsin, pepsin and papain, presented as relative activity (%RA) is given in Fig 5.1.4 a-d and Fig 5.1.5 a-d.

5.1.6.1 Acetylated proteins

Papain

The papain degradation of native and acetylated proteins is given in Fig 5.1.4a. The effect of acetylation on the digestibility of papain was only shown after a long digestion time (24 hours). After 6 hours of digestion there was no difference in the protein hydrolysis of all isolates, except ACP6. The relative activity of papain to all protein isolates increased about 4 to 8 fold after 24 hours of digestion. Acetylated proteins were hydrolysed much more than the native protein but the increase in RA of papain did not vary proportionally with the level of acetylation.

Trypsin

Trypsin poorly degraded all chickpea proteins compared with other three proteases. After 24 hours of digestion, its RA increased only to about 2 fold. In addition, the result indicated that acetylation reduced the digestibility of proteins by trypsin (Fig 5.1.4b).

Pepsin

Although there was no significant difference in the protein hydrolysis between the native and 6% acetylated protein, digestibility of highly acetylated proteins (ACP26, ACP45 and ACP49) was improved by pepsin. After 6 and 24 hours of digestion, the digestibility of these three acetylated proteins increased up to 3 to 6 folds (Fig 5.1.4c). The difference in

digestibility of mildly and exhaustively acetylated proteins indicated acetylation unfolded the proteins, made them more susceptible to the reactivity of pepsin, leading to some structural changes of proteins.

Chymotrypsin

This enzyme showed a high protein degradation capacity with all isolates (Fig 5.1.4d). After 6 hours of digestion, its RA increased to 2.5 - 5 folds. Prolonged the digestion time to 24 hours, increased its RA 10 fold. The degree of protein hydrolysis by chymotrypsin increased with increasing levels of acetylation, followed the order of NCP<APC6<ACP26, ACP45, ACP49.

5.1.6.2 Succinylated proteins

Papain

The digestibility of chickpea proteins by papain was depressed by succinylation (Fig 5.1.4a). Native protein was hydrolysed more than succinylated proteins by papain after 6 hours of digestion. Prolonged digestion for 24 hours did not improve the digestibility of succinylated proteins. Highly succinylated proteins (SCP73 and SCP79) were digested less than the slightly succinylated protein (SCP16).

Trypsin

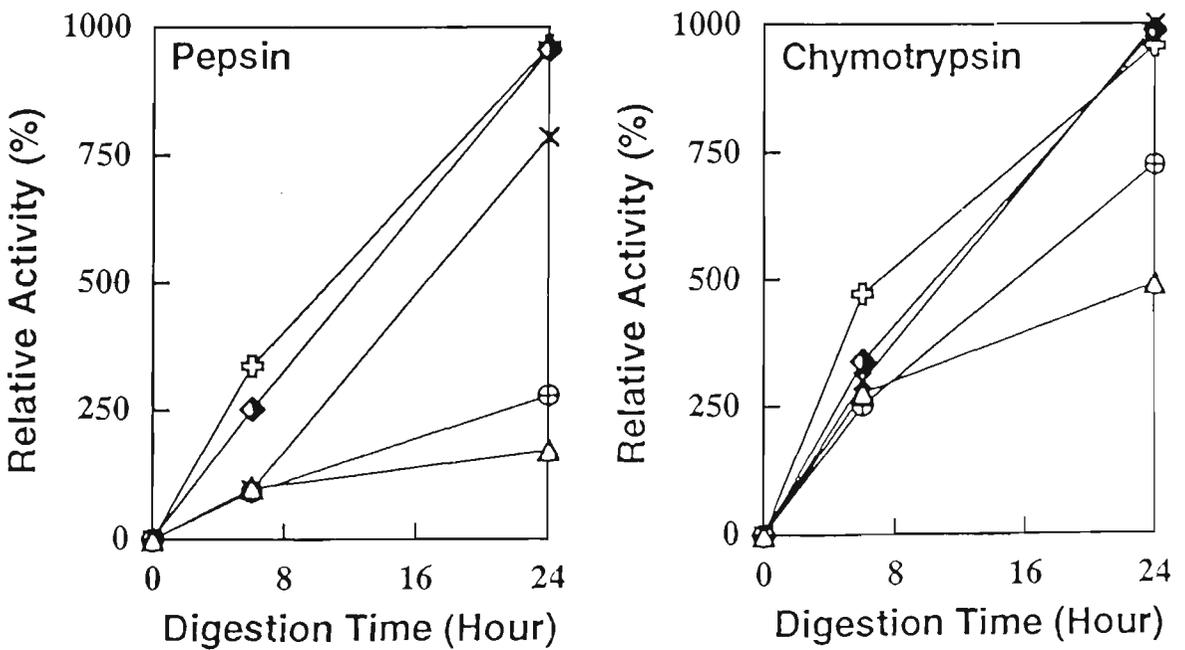
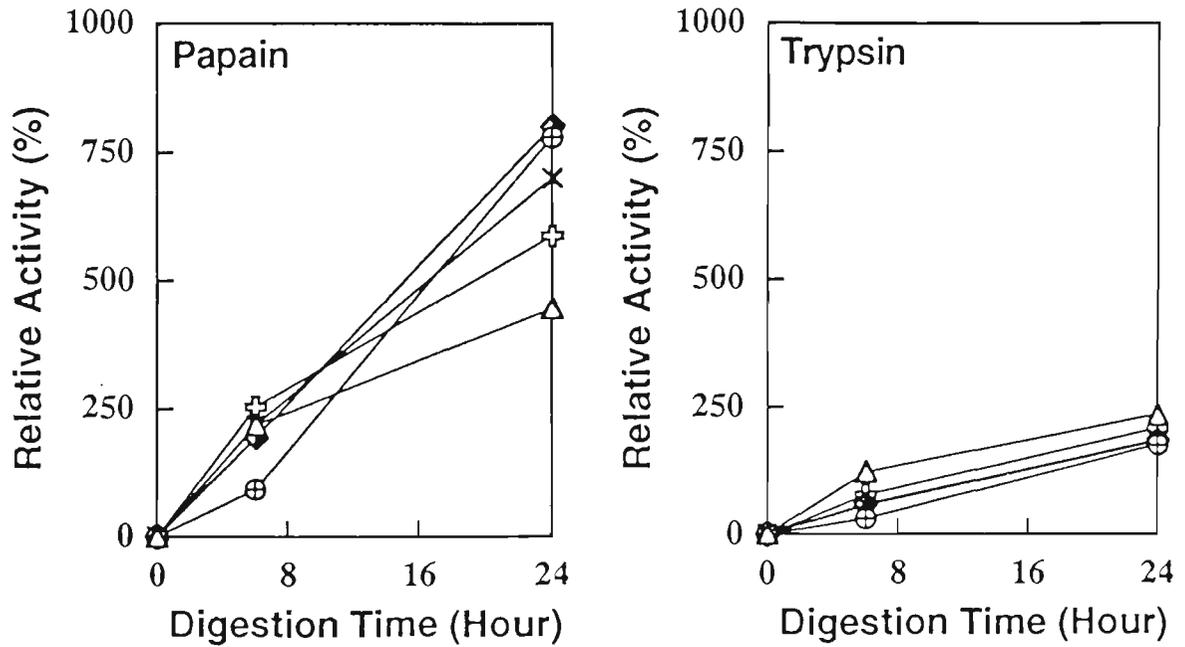
The activity of trypsin on chickpea proteins was depressed by both acetylation and succinylation (Fig 5.1.5b). After a 6 hour digestion, there was no difference in digestibility of the native and succinylated proteins. Prolonging the digestion time for 24 hours did not improve the hydrolysis of succinylated proteins by this proteases.

Pepsin

Pepsin showed a high degradation capacity with succinylated proteins (Fig 5.1.5c). Succinylated proteins were hydrolysed much more than acetylated proteins after only 6 hour of digestion. The highly succinylated proteins were hydrolysed more than the mildly succinylated proteins by pepsin under similar treatment. The RA of pepsin acting on SCP72, 73 and 79 ranged from 6 to 8 fold while with NCP and SCP16 the RA increased only 2 fold.

Chymotrypsin

Like papain, succinylation depressed the digestibility of chickpea proteins by chymotrypsin (Fig 5.1.5d). All succinylated proteins were hydrolysed less than the native protein and the increasing level of succinylation led to a reduction in the level in hydrolysis.



—△— NCP —⊕— ACP6 —×— ACP45 —◆— ACP47 —⊕— ACP49

Fig 5.1.4 (a-d) The *in vitro* digestibility of the native and acetylated chickpea proteins by papain, trypsin, pepsin and chymotrypsin is presented as relative activity (%) of these enzymes.

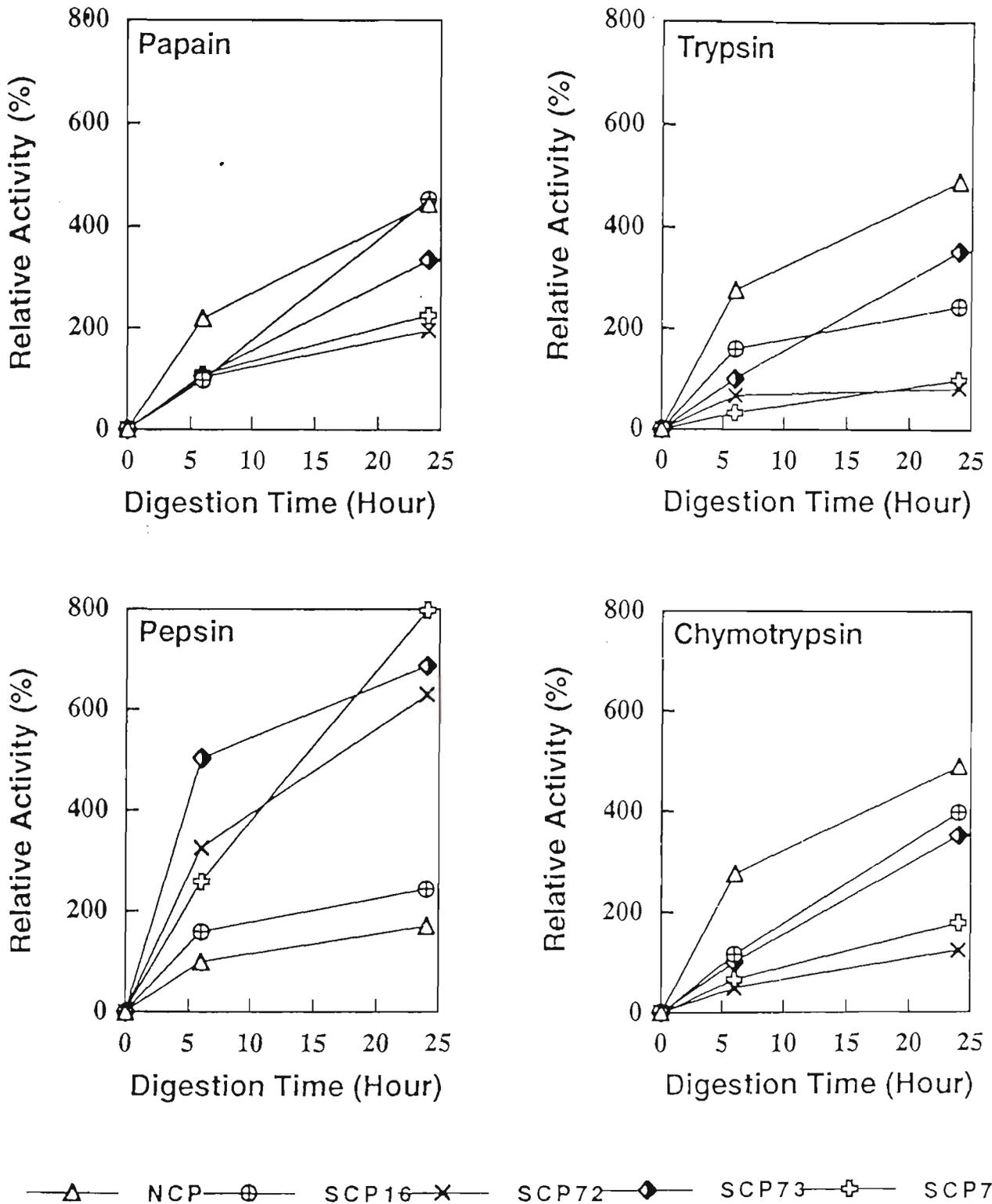


Fig 5.1.5 (a-d) The *in vitro* digestibility of the native and succinylated chickpea proteins by papain, trypsin, pepsin and chymotrypsin is presented as relative activity (%) of these enzymes.

5.1.7 CONCLUSION

Chickpea proteins were acylated by succinic and acetic anhydride to certain levels. The levels of modification varied with the type of reagent, its concentration and also with different types

of proteins involved in the system. The lysines were slightly reduced but the proportion of essential amino acids of acylated proteins was unchanged and still met the required nutrition level for children and adults. Acylation did not significantly alter the gel filtration profile of the modified proteins but the SDS-PAGE patterns showed their electrophoretic mobility was retarded. Densitometric scanning of SDS-PAGE confirmed some structural changes occurred in chickpea proteins due to protein aggregations. Acetylation improved the *in vitro* digestibility of the modified proteins by papain, pepsin and chymotrypsin while succinylation only improved the modified protein hydrolysis of pepsin.

5.2 FUNCTIONAL PROPERTIES OF ACETYLATED CHICKPEA PROTEINS

In previous Section (5.1) the effects of acetylation on the characteristics of chickpea proteins have been described. This chemical modification resulted in several changes in the structure and composition of the modified proteins as reflected in their amino acid composition, *in vitro* digestibility, gel filtration and SDS-PAGE patterns. A structural modification normally lead to some changes in the functional properties of a protein.

Functional properties of proteins such as solubility, emulsifying and rheological properties can be chemically or enzymatically modified (Campbell *et al.*, 1992; Hamada, 1992; Lahl and Braun, 1994). Enzymatic modification could improve functional properties of proteins but bitter peptides could also be formed from extensive protein hydrolysis (Shiraishi *et al.*, 1973; Arai *et al.*, 1975). Of various chemical approaches, acetylation has been the most widely employed to alter functional properties of food proteins by introducing acetyl groups into the protein.

Acetylation improved the solubility of winged bean (Narayana and Narasinga Rao, 1984), glandless cottonseed flour (Childs and Park, 1976), soy (Franzen and Kinsella, 1976a), leaf protein (Franzen and Kinsella, 1976b), arachin (Shyama Sunda and Rajagopal Rao, 1982) rapeseed (Gwiazda *et al.*, 1989) and castor bean pomace protein (Yoon, 1980). Acetylation also improved water absorption and oil adsorption of winged bean, field pea, soy and rapeseed proteins. Emulsifying capacity and stability of glycinin (Kim and Rhee, 1990), faba bean (Muschiolik, 1989) and arachin (Shyama Sundar and Rajagopal Rao, 1982) were improved by acetylation.

These improvements were attributed to the conformational changes in these protein molecules. The conformational changes and subsequent changes in functionality of these

proteins are affected by the extent of acetylation. For example, extensive acetylation increased hydrophobicity of glycinin while moderately acetylated glycinin showed low hydrophobicity (Kim and Rhee, 1989). The viscosity of field bean and fat absorption of winged bean depend on the degree of acetylation (Schmidt and Schmandke, 1987, Narayana and Narasinga Rao, 1984). Barman *et al.*, (1977) showed that the effect of acetylation could be predicted and the functional properties of soy protein could be altered by varying degrees of acetylation. Consequently, acetylation not only provides a means to improving the functionality of chickpea proteins but also allows a further understanding of their behaviour in food systems.

The objective of this section was to examine the functional properties of chickpea proteins acetylated at different levels.

5.2.1 SOLUBILITY IN WATER

The nitrogen solubility curves of native and acetylated chickpea proteins in water are shown in Fig 5.2.1. The typical U shape of solubility curve of grain legume proteins was obtained with all isolates. Compared with native chickpea protein, the acetylated protein isolates were more soluble at high pHs (7.5-10) and less soluble at low pHs (pH 4 - pH 2). The highly acetylated chickpea protein (ACP49) was more soluble (43%) than the moderately acetylated protein (ACP6) at high alkaline pHs but less soluble than ACP6 at low acidic pHs.

Acetylation also shifted the isoelectric point (pI) of acetylated proteins from pH 5 to pH 4. The shift of pI of acetylated chickpea protein to lower pHs can be explained by the change of overall charge characteristic. Acetylation alters a normally protonated amino acid group by linking to a neutral acetyl group. This produces proteins with a greater net negative charge which shifts the pIs of acetylated proteins to lower pHs. $(\text{Protein-NH}_2 + (\text{CH}_3\text{CO})_2\text{O} = \text{Protein-NHCOCH}_3 + \text{CH}_3\text{COO}^- + \text{H}^+)$.

The introduction of hydrophobic groups into the protein by acetylation, particularly at high levels, would be expected to reduce the solubility of acetylated proteins in water. Indeed, acetylation of cottonseed protein has been shown to decrease its N solubility (Rhama and Narasinga-Rao, 1983). However, the solubility of rapeseed protein (Thompson and Cho, 1984; Gwiazda *et al.*, 1989), castor bean protein (Yoon, 1980), oat protein (Ma, 1984; Ma and Wood, 1987) and broad bean protein (Schneider *et al.*, 1985) was improved by acetylation. In this study, the solubility of acetylated chickpea proteins was decreased over the pH range of 2-7, as expected, but at high alkaline pHs, acetylated proteins became more soluble than the native proteins. Similarly, Kabirullah and Wills (1982) found acetylated

sunflower protein was less soluble than native proteins at pH<4 but more soluble at pH>7. The solubility of acetylated peanut protein was also improved in the pH range 6.5-8.5 (Shyama Sundar and Rajagopal Rao, 1978).

The different results observed in these investigations indicated that there were several factors that could affect protein solubility. If the hydrophobic groups introduced by acetylation was a dominant factor, then the solubility would be reduced. However, the effect of hydrophobic groups could be outweighed by other factors such as salt concentration, pH and composition of the proteins. If this was the case, then, a reverse effect on solubility may be observed.

At acidic pHs, upon acetylation, the number of hydrophilic cation groups could not exceed the aggregate force, formed by hydrophobic bonds between the alkyl and aromatic groups of constituent amino acid residues. Consequently, the solubility of acetylated proteins decreases (Carbonaro *et al.*, 1993). In contrast, at high alkaline pHs (pH 10), insoluble protein aggregates bounded by hydrophobic interactions or by hydrogen bonds could be resolved into soluble components (Carbonaro *et al.*, 1993). Prakash and Narasinga Rao (1986) also found that alkaline peptidization at high alkaline pH was predominantly responsible for the increase in protein solubility. As observed in other studies (Cheryan, 1980; Yanagi, 1983), the exposure of native chickpea protein to a large amount of sodium hydroxide at pH 9 during acetylation could affect the stability of the protein's quaternary complex, making it more susceptible to pH changes and affecting its solubility. These factors may contribute to the increase in solubility of acetylated proteins at alkaline pHs.

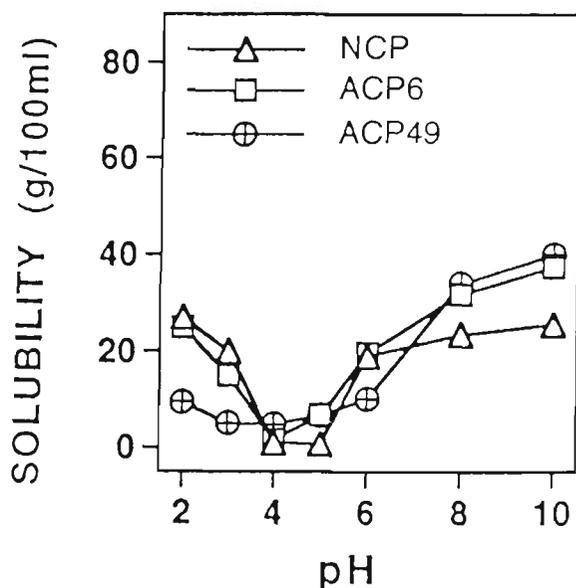


Fig 5.2.1 The nitrogen solubility of native and acetylated chickpea proteins in water.

5.2.2 SOLUBILITY IN SALT SOLUTIONS

The solubility of native and acetylated chickpea proteins at different pHs in NaCl and CaCl₂ solutions is shown in Fig 5.2.2 a-b and Fig 5.2.3 a-b, respectively.

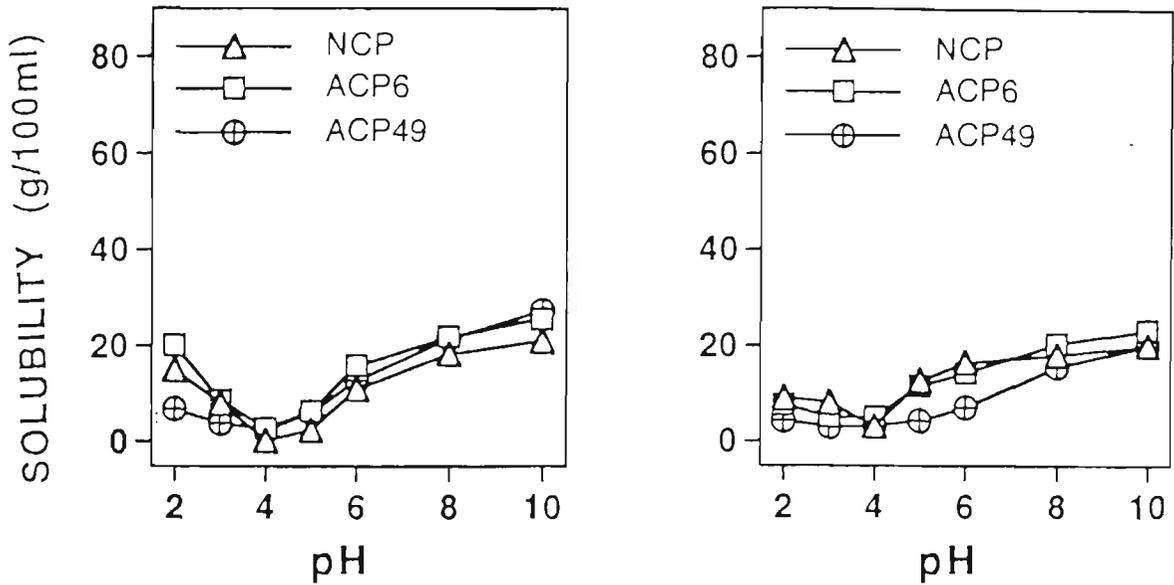


Fig 5.2.2 The solubility of native and acetylated chickpea proteins at different pHs in NaCl solutions (a) 0.25M NaCl, (b) 0.75M NaCl.

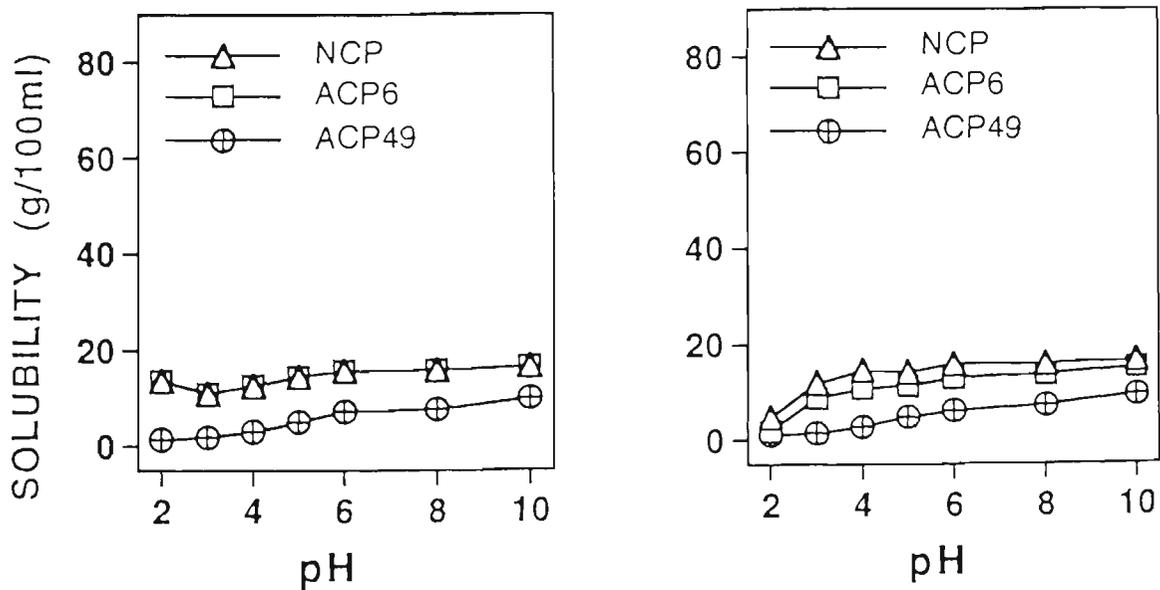


Fig 5.2.3 The solubility of native and acetylated chickpea proteins at different pHs in CaCl₂ solutions (a) 0.25M CaCl₂, (b) 0.75M CaCl₂.

In dilute NaCl (0.25M) solution, the solubility of the native chickpea protein was decreased, perhaps due to the salting out effect and its pI was shifted to more acidic pH. This pI shift could be attributed to the binding of anionic chlorides with the protein. In a protein

dispersion, inorganic anions due to their smaller hydrated radii could attain a closer proximity to the protein molecule and bind to proteins more strongly than the inorganic cations. Consequently, with the addition of NaCl and the binding of the chloride anions, the negative charge of the protein at the original isoelectric point would increase. More acid is therefore required to establish a new pI which is located at a more acidic pH (Paulson and Tung, 1987; Schut, 1976).

Acetylated chickpea proteins were more soluble than the native protein at pHs above the pI but less soluble at pHs below the pI. The decrease in solubility at pHs below the pI could be due to a combined effect of increased net negative charge due to acetylation, resulting in the formation of more rigid protein complexes together with the competition with NaCl for the water in the system. The highly acetylated chickpea protein (ACP49) could have less net negative charge, making it possible for increasing the amount of insoluble protein complexes and leading to lower solubility, compared with the moderately acetylated protein (ACP6). The increase in solubility at alkaline pHs, once again could be a combined effect of several factors. At high alkaline pHs, it is likely that more anionic chlorides are bound to the protein rather than Na⁺ cations, resulting in an increase in the net negative charge and hence solubility (Hamm, 1975, Gillberg and Toernell, 1976).

The effect of increasing salt concentration (0.75M) on protein solubility was more pronounced with acetylated than native proteins. In more concentrated NaCl (0.75M) solutions, the solubility of all proteins decreased. In a study of the effect of salt at several pHs on the solubility of soy protein isolates, Van Megen (1974) found that under certain salt contents, the solubility of the proteins appeared to be independent of salt concentration but in fact at high salt concentrations, salting out might occur. Since there were small fixed, charged sites to which ions could bind selectively and strongly, this type of salt binding was the most extensive at low levels of salt addition. The effect of anionic chlorides at high salt concentrations was not profound. In contrast, Na⁺ cations may also bind with protein, neutralise some negatively charged groups and reduce the net negative charge of the protein, resulting in a decrease of protein solubility.

Regardless of the salt concentration, the addition of CaCl₂ greatly changed and reduced the solubility of both native and acetylated proteins. The typical U shape of the protein solubility curve was replaced by an almost linear line for native chickpea protein in 0.25M CaCl₂ solution. This, was due to an increase in solubility in the pI region and a decrease at alkaline pHs. In the study of solubility of rapeseed protein, Gillberg (1978) found that at high pHs, Ca²⁺ cations had a higher tendency than Na⁺ cations to bind with carboxyl groups in the protein. This reduced the net negative charge and, under some circumstances, created the most favourable conditions for the formation of insoluble complexes. However, at pHs close

to their pI, caseinate (Konstance and Strange, 1991) and soy globulin (Megen, 1974) could be solubilised by CaCl_2 . CaCl_2 is a salting out salt and protein destabiliser. Its behaviour under different pHs may explain the drastic change in solubility of the native protein. When the CaCl_2 concentration increased (0.75M), the solubility of the native chickpea protein was further decreased. Similar observations were reported by Gillberg (1978) who found that at pH 2, solubility of rapeseed protein decreased with increasing CaCl_2 concentration because the binding of chloride anion created favourable conditions for the formation of insoluble protein complexes.

Acetylated chickpea proteins are more soluble at acidic pHs in CaCl_2 solutions than in NaCl solutions. This can be explained as a result of more Ca^{2+} binding to carboxyl groups on the proteins and also due to the higher ionic strength of CaCl_2 , compared with NaCl at the same concentration (Gillberg, 1978).

5.2.3 WATER ABSORPTION CAPACITY

Water absorption of acetylated chickpea proteins was higher than that of the native chickpea protein but lower than that of a commercial soy protein isolate (Fig 5.2.4). Water absorption capacity increased with increasing level of acetylation up to 45% and then remained constant. Water absorption of the following proteins was also improved by acetylation: glandless cottonseed flour (Choi *et al.*, 1981), cotton seed flour (Rahma and Narasinga Rao, 1983), peanut flour (Beuchat, 1977), winged bean (Narayana and Narasinga Rao, 1984), sunflower (Kabirullah and Wills, 1982), pea protein isolates (Johnson and Brekke, 1983) and rapeseed protein (Gwiazda *et al.*, 1989).

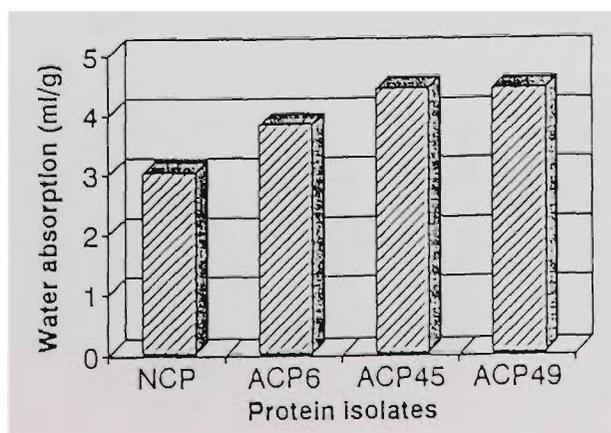


Fig 5.2.4 Water absorption of native and acetylated chickpea proteins.

Acetylation introduced hydrophobic groups into the protein molecules which is expected to reduce the water absorption capacity of acetylated proteins, particularly at high levels of acetylation. Canella *et al.* (1979) found that acetylation reduced the water absorption capacity of sunflower. Similar results were obtained with oat (Ma, 1984) and soy (Barman *et al.*, 1977). Hermansson (1973) reported that highly soluble proteins have poor water absorption. Consequently, the unexpected increase in the water absorption capacity (56%) found in this study and in some other investigations indicated that the protein dissociation and unfolding caused by acetylation might expose more hydrophilic groups than hydrophobic groups, thereby, increasing the number of hydrophilic binding sites. Water absorption capacity could also be affected by the water physically entrapped within the unfolded protein and also by different degrees of protein denaturation (Fiora *et al.*, 1990).

5.2.4 OIL ABSORPTION CAPACITY

Oil absorption capacity of acetylated chickpea protein was higher than the native chickpea protein and soy isolate. The increase in oil absorption capacity increased with increasing level of acetylation (Fig 5.2.5). The published effect of acetylation on oil absorption capacity of food proteins is conflicting. Acetylation improved the oil absorption capacity of glandless cotton seed flour (Childs and Park, 1976; Choi *et al.*, 1981) and winged bean flour protein (Narayana and Narasinga Rao, 1984). However, the oil absorption capacity of acetylated cotton seed flour (Rahma and Narasinga Rao, 1983) and acetylated peanut flour (Beuchat, 1977) was not improved. It is believed that oil absorption capacity of a protein depends on its capacity to entrap the oil (Kinsella, 1976). The relatively high oil absorption capacity of acetylated chickpea proteins may be attributed to the degree of denaturation by chemical modification.

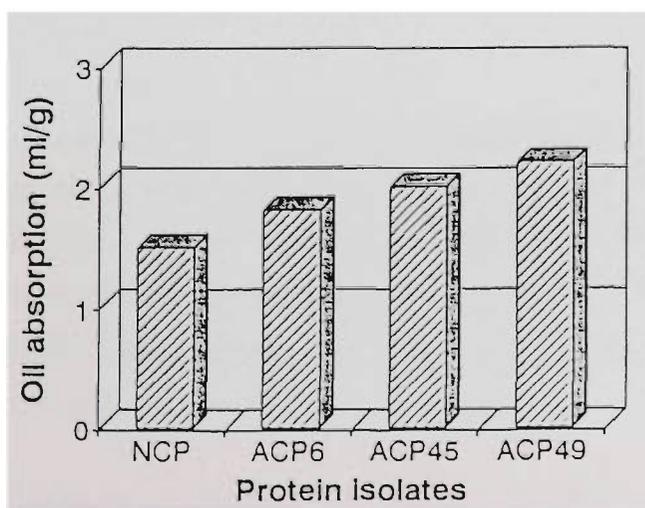


Fig 5.2.5 Oil absorption capacity of native and acetylated chickpea proteins.

5.2.5 EMULSIFYING CAPACITY

The effect of protein concentration on the emulsifying capacity (EC) of native and acetylated chickpea proteins is given in Fig 5.2.6. The protein concentration effect on EC of each protein is different although all proteins showed a high EC at high protein concentrations. The increased EC of food proteins associated with increasing protein concentration has been noted (Liu *et al.*, 1995; Crenwelge *et al.*, 1974; Pearson *et al.*, 1965). There is a close relationship between the EC of a protein and its protein solubility (Kinsella, 1976) as well as hydrophobicity (Voutsinas *et al.*, 1983). At a low concentration (0.025%), EC of acetylated chickpea proteins was lower than that of the native protein but was equal to or greater than the EC of native proteins at higher concentrations.

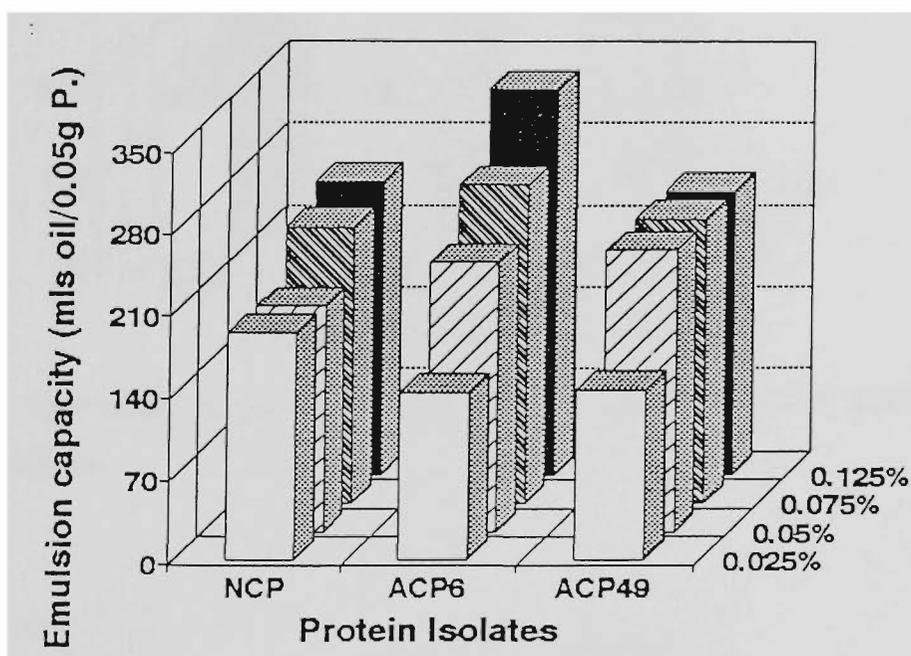


Fig 5.2.6 The effect of protein concentration on the emulsifying capacity (EC) of native and acetylated chickpea proteins.

The effect of pH on the EC of both native and modified chickpea proteins is given in Fig 5.2.7. The EC of all proteins increased with increasing pH and the magnitude difference reflected their solubility. EC of acetylated chickpea proteins was higher than that of the native protein as seen by their corresponding solubility (Fig 5.2.8). Similar observations were obtained with peanut, soy, cotton seed and milk protein (Ramanatham *et al.*, 1978; Crenwelge *et al.*, 1974).

The effect of salt addition on the EC of the isolates is given in Fig 5.2.9. The EC of native and slightly acetylated chickpea protein (ACP6) increased at low salt concentrations (0.125-0.25M) but gradually decreased at higher salt concentration (0.5-1.0M). At low ionic

strength, salt contributes to the decrease of intermolecular electrostatic interactions between oppositely charged groups leading to an increase in solubility and in EC (Dench, 1982; McWatters and Holmes, 1979). At high ionic strengths, salt exerted a salting out effect, decreased the protein solubility and the subsequent EC. The EC of extensively acetylated chickpea (ACP49) was sharply decreased even at low salt concentrations.

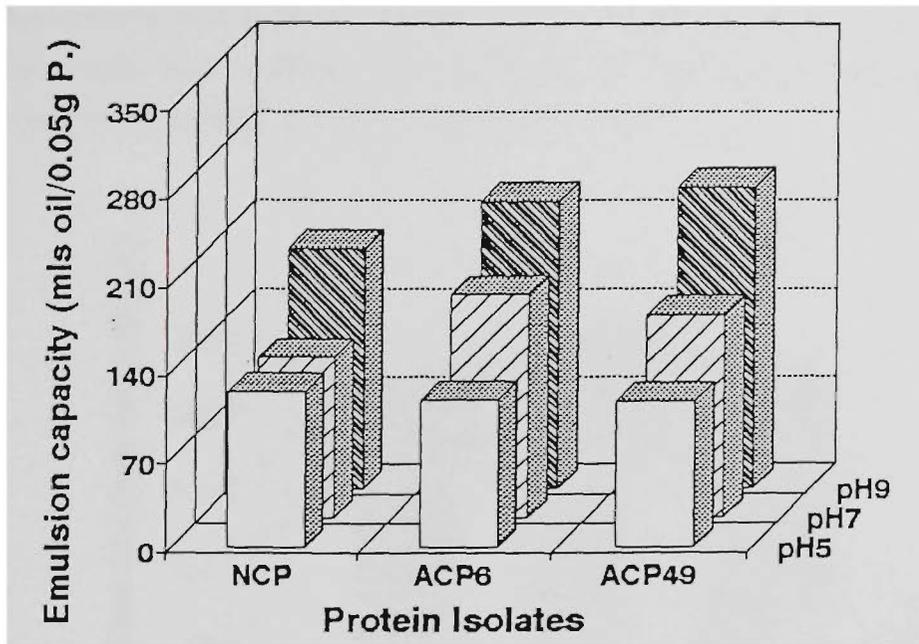


Fig 5.2.7 The effect of pH on the emulsifying capacity (EC) of native and acetylated chickpea proteins.

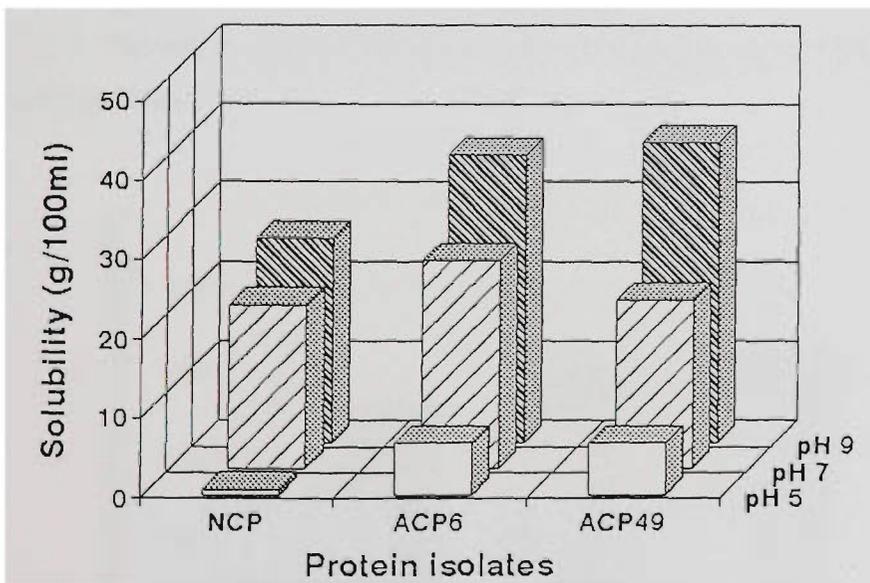


Fig 5.2.8 The solubility of acetylated chickpea protein.

The effect of temperature on the EC of both native and acetylated chickpea proteins is shown in Fig 5.2.10. The EC of all proteins decreased with increasing temperature, particularly above 45°C but there was no significant difference in EC between the native and acetylated

proteins over the same temperature range. Thermal effects on the EC of proteins is not uniform, but depends on protein type and composition. Although solubility had a significant effect, EC of a protein was also affected remarkably by its hydrophobicity (Voutsinas *et al.*, 1983; Kato and Nakai, 1980). Heat treatment caused protein denaturation and gradually exposed hydrophobic amino acid residues which were normally buried in the interior of native protein molecules. The exposing of the hydrophobic groups could increase the hydrophobicity and improve the EC (Morr, 1979). However, these exposed hydrophobic groups could also undergo some hydrophobic interactions, leading to a decrease in the hydrophobicity as well as the EC (Voutsinas *et al.*, 1983).

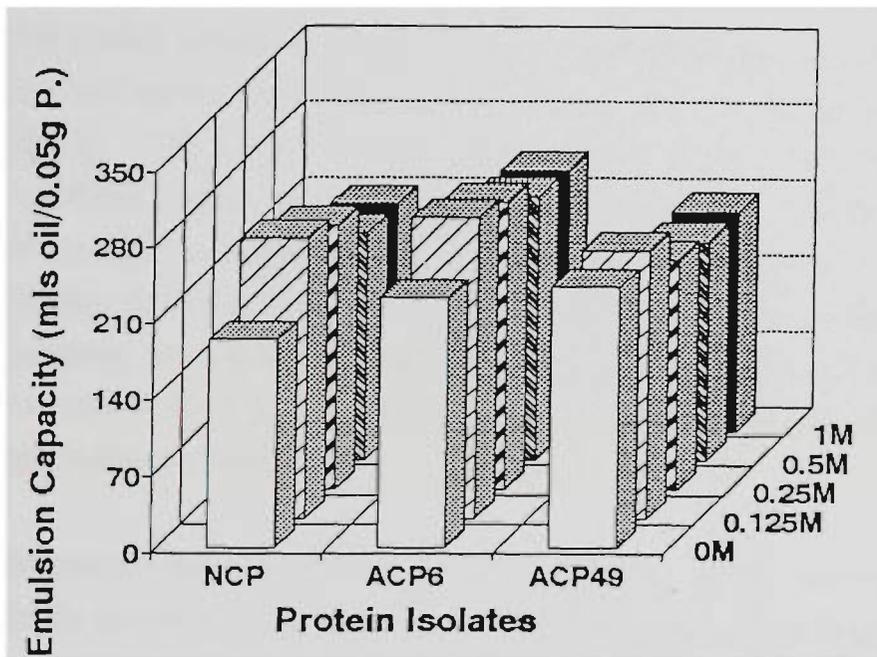


Fig 5.2.9 The effect of salt addition on the emulsifying capacity (EC) of native and acetylated chickpea proteins.

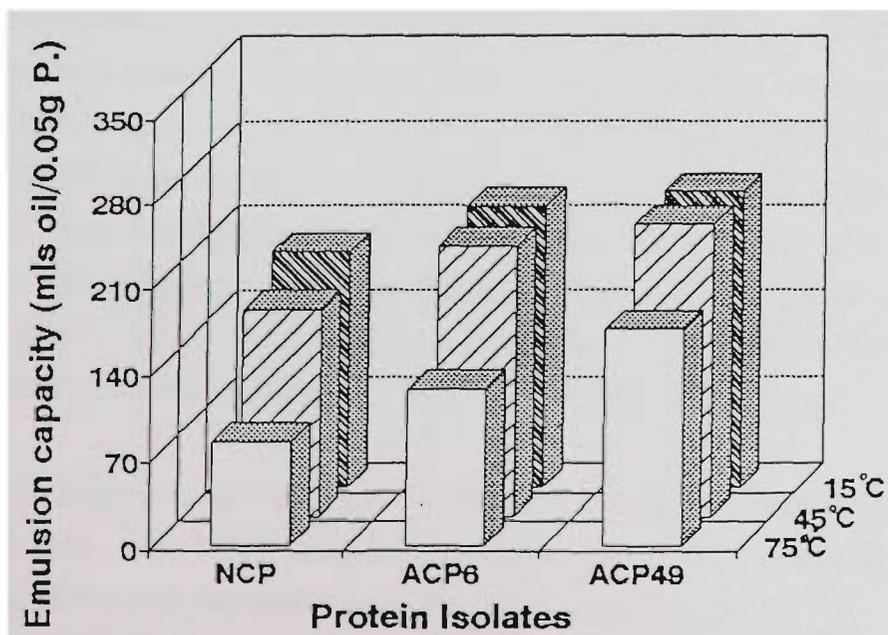


Fig 5.2.10 The effect of temperature on the emulsifying capacity (EC) of native and acetylated chickpea proteins.

5.2.6 EMULSION STABILITY (ES)

The effect of protein concentration on the emulsion stability (ES) of native chickpea protein is greater than that of acetylated chickpea proteins. For short time stability (within 3 hours), there is no significant difference in emulsion stability between native and modified chickpea proteins at all concentrations (Fig 5.2.11a). After 4 days, the native chickpea protein could retain about 30% of its stability at 0.025 % protein concentration and less than 10% at 0.125% protein concentration. The ACP 49 could retain about 40% its stability at both of the above concentrations (Fig 5.2.11b).

The ES of all protein isolates was highest at pH 9 but less at pH 5 and neutral pH (Fig 5.2.12 a-b). Effect of pH on the ES of acetylated chickpea proteins is greater than that of the native chickpea protein. The native chickpea isolate could retain about 60-90% of their initial ES while the modified isolates could retain only 15-60% after 3 hours (Fig 5.2.12b). The emulsion of the highly acetylated isolate ACP49 is even less stable than that of the moderately acetylated isolate ACP6, showing the effects of different degrees of acetylation. At pH 5 - pH 7, the emulsion of ACP49 was almost collapsed within 60 minutes, retaining only 15% of its original emulsion (Fig 5.2.12a). The difference in emulsion stability reflected the behaviour of the protein isolates at different pHs.

The greatest emulsifying capacity of a protein is not necessarily associated with the highest level of soluble protein (Turgeon *et al.*, 1992; McWatters and Holmes, 1979). It is more likely that emulsion stability depends on the suitable balance between the hydrophilic and hydrophobic groups (Aoki *et al.*, 1980). At pH 9 all protein isolates were more soluble and their net negative charges did not create favourable conditions for the aggregation of soluble protein, contributing to the emulsion stability. Consequently, ACP49, ACP6 and native chickpea protein isolate could retain about 50, 45 and 40% respectively of their initial emulsions after 4 days. In contrast, at pH 7 and particularly at pH 5, the net negative charge of the acetylated proteins were decreased and more hydrophobicity groups were exposed. This would have created favourable conditions for hydrophobic interactions and protein aggregation, influencing the emulsion stability. The effect on ES was stronger with highly acetylated ACP49 because of the lipophilization effect due to acetylation (Aoki *et al.*, 1980). Their emulsion had almost collapsed at pH 5 - pH 7 after only one day.

Effect of salt concentration on the ES of native and modified chickpea proteins was uniform (Fig 5.2.13 a-b). ES of all isolates decreased with increasing salt concentrations. The destabilising effect also depends on the level of acetylation with the ES of ACP49 being less than that of ACP6. Salting out effects combined with the possible Na⁺ cations binding to the protein could explain the overall instability of emulsions studied in this work.

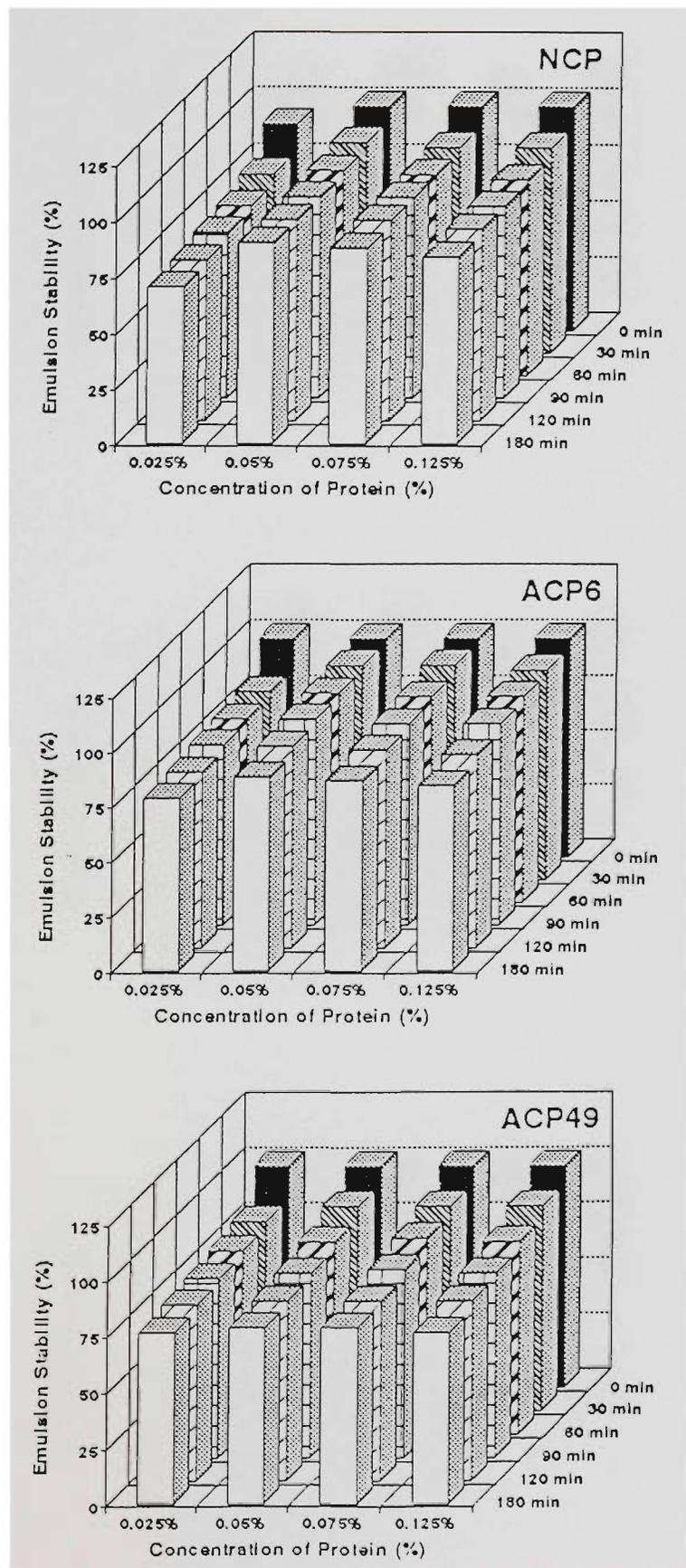


Fig 5.2.11a The effect of protein concentration on the emulsion stability (ES) of native and acetylated chickpea proteins over 3 hours.

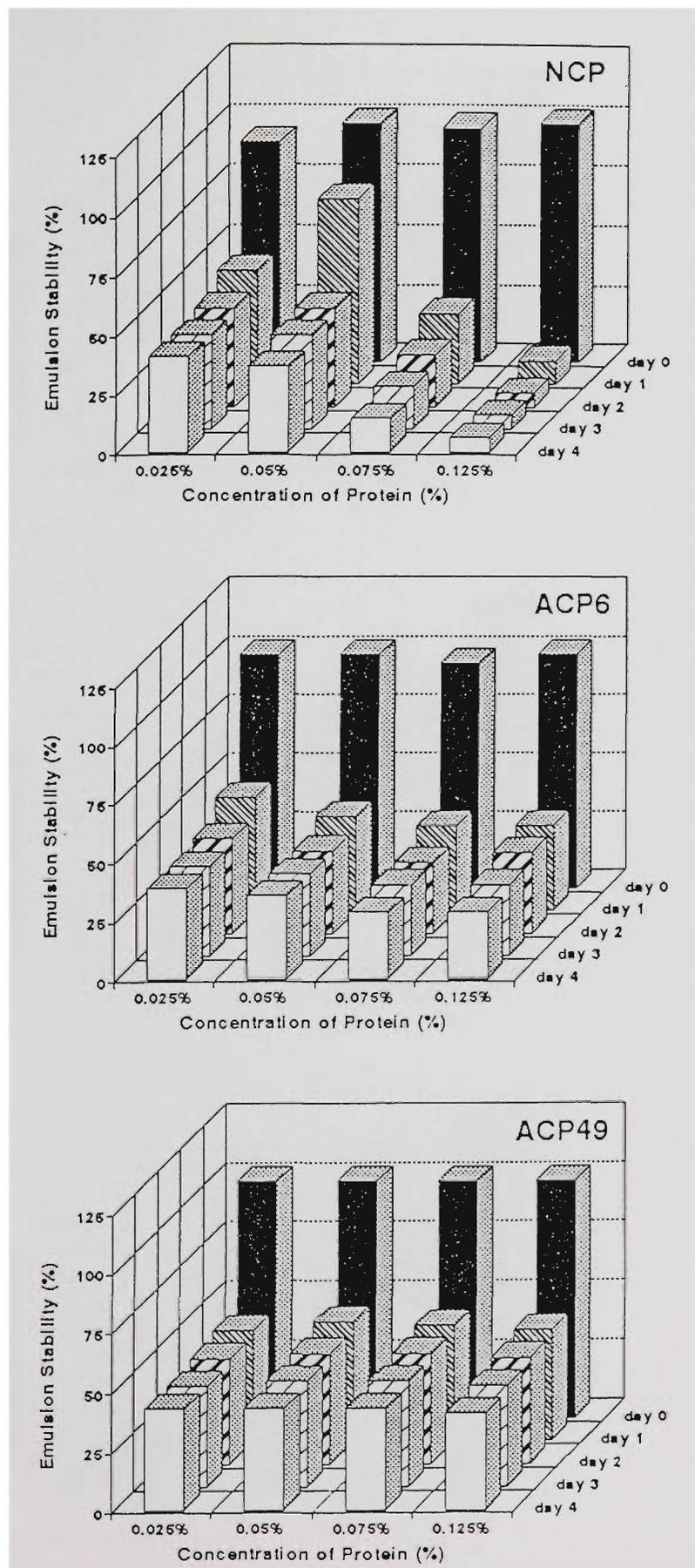


Fig 5.2.11b The effect of protein concentration on the emulsion stability (ES) of native and acetylated chickpea proteins over 4 days.

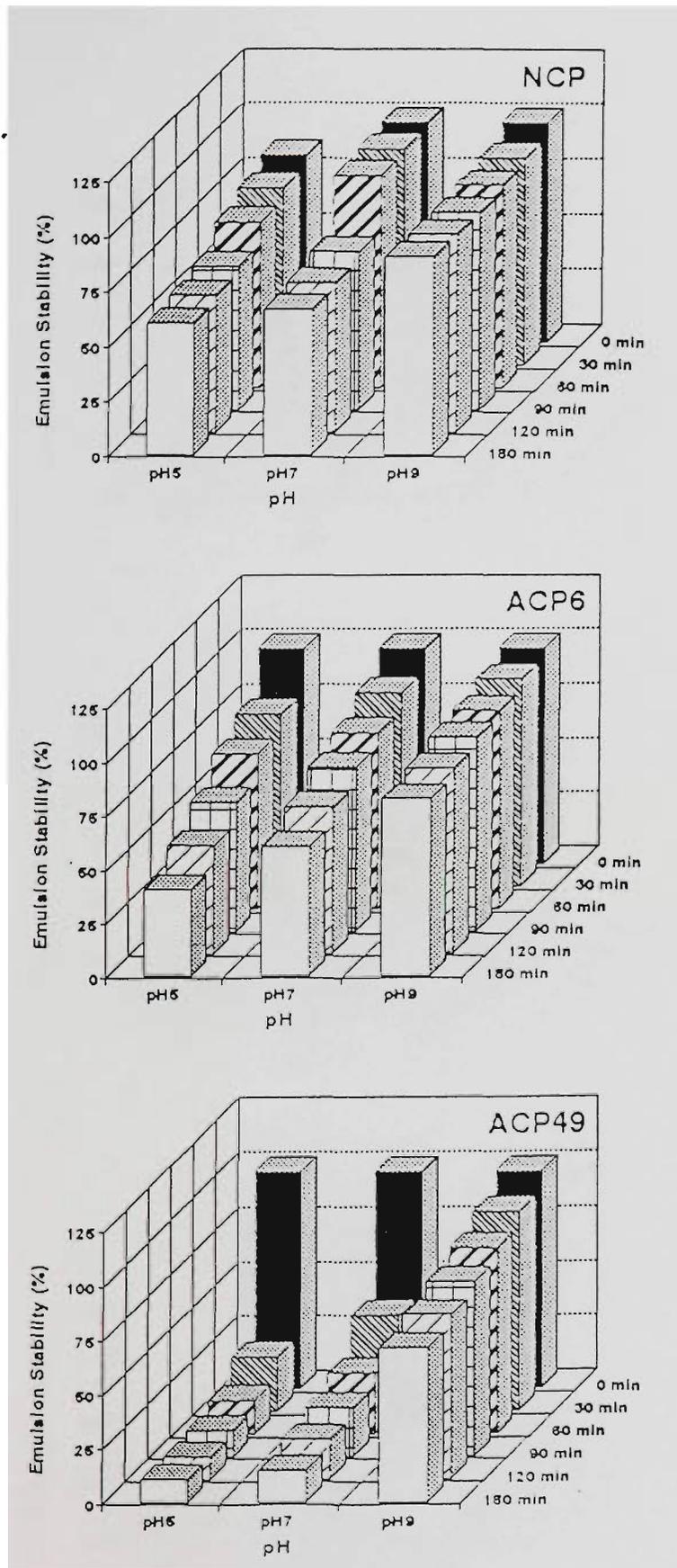


Fig 5.2.12a The effect of pH on the emulsion stability (ES) of native and acetylated chickpea proteins over 3 hours.

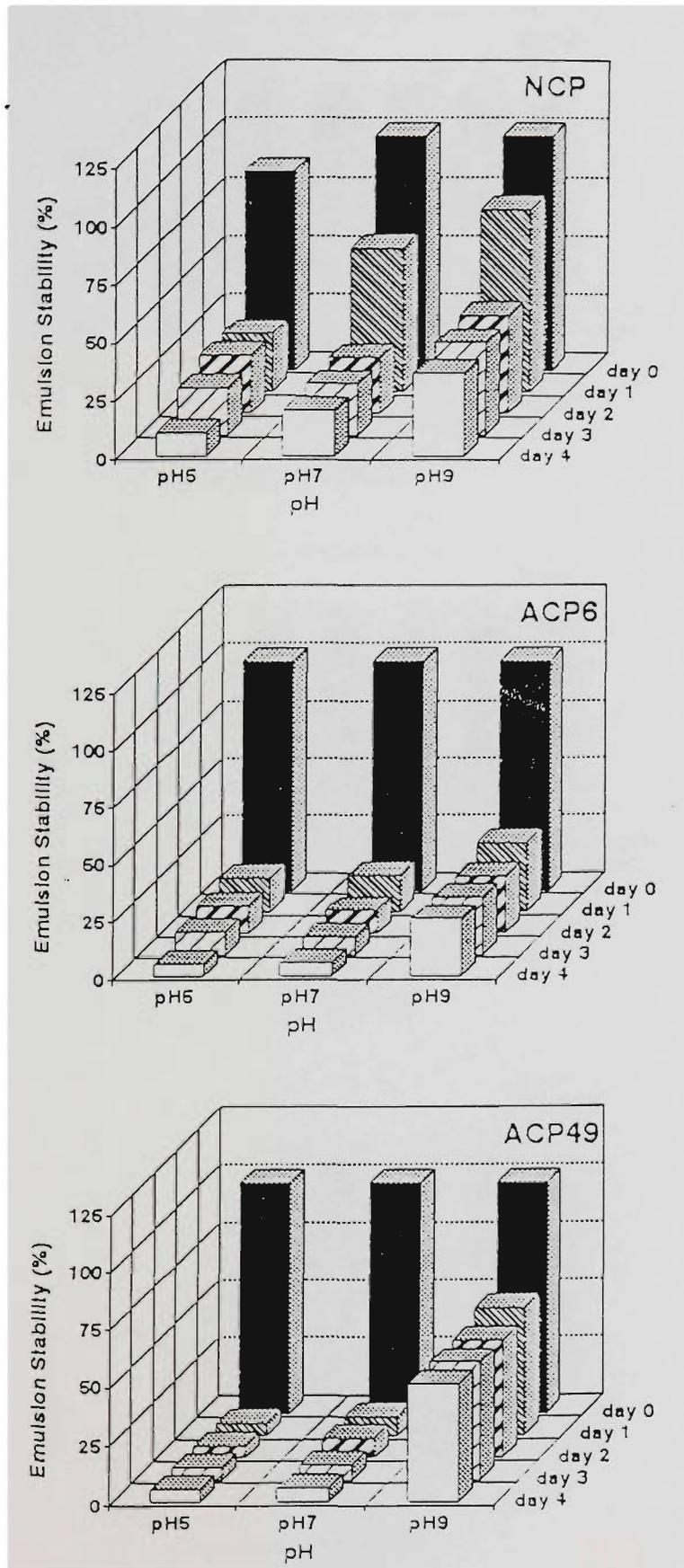


Fig 5.2.12b The effect of pH on the emulsion stability (ES) of native and acetylated chickpea proteins over 4 days.

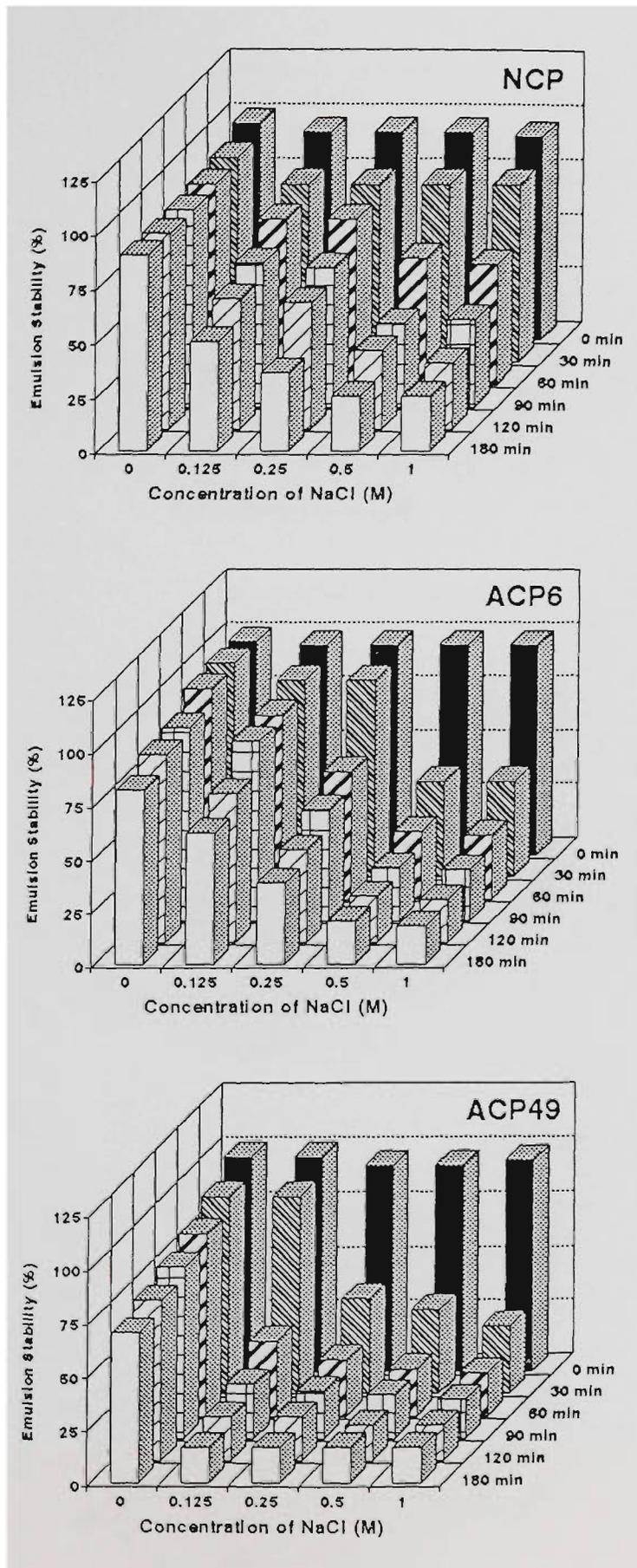


Fig 5.2.13a The effect of salt concentration on the emulsion stability (ES) of native and acetylated chickpea proteins over 3 hours.

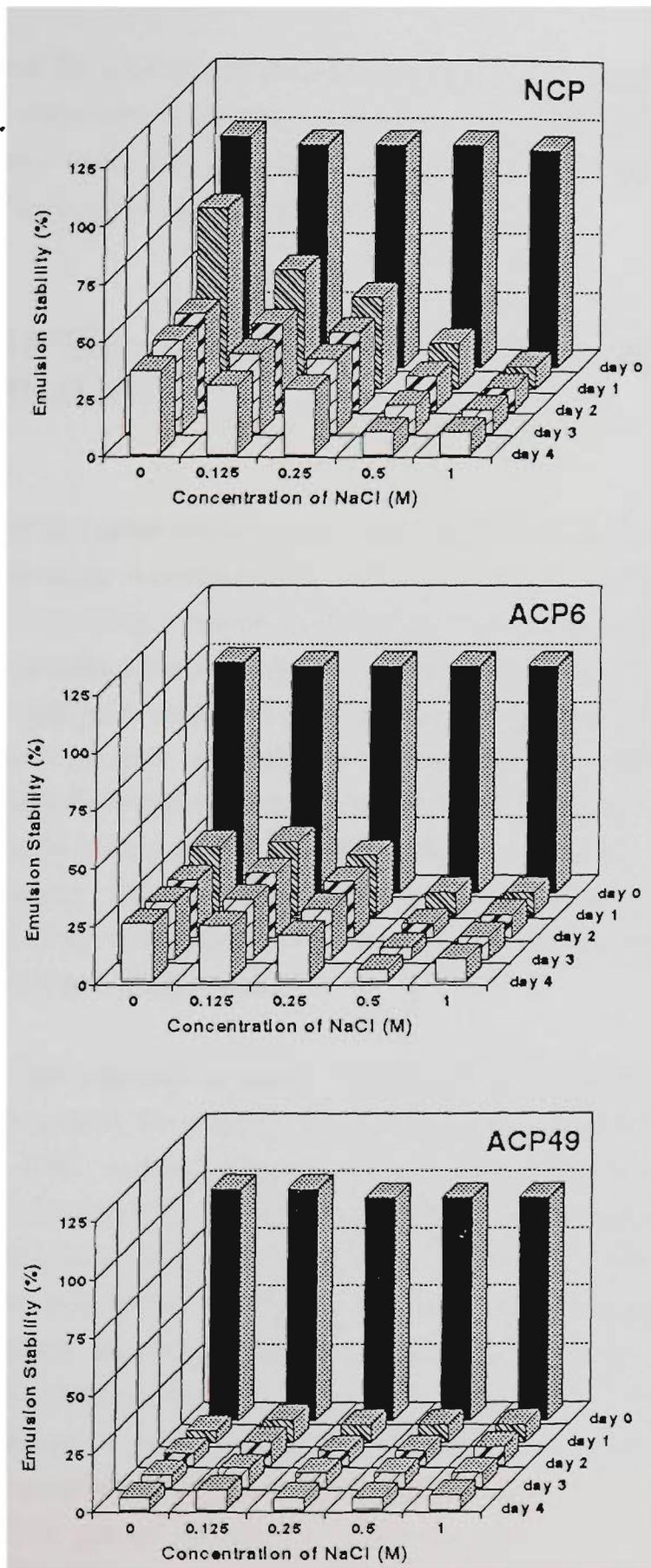


Fig 5.2.13b The effect of salt concentration on the emulsion stability (ES) of native and acetylated chickpea proteins over 4 days.

5.2.7 CONCLUSION

Acetylation improved the solubility of chickpea proteins at high alkaline pHs. Acetylation also improved their water and oil absorption capacities. The emulsion capacity of acetylated chickpea proteins was higher than that of the native proteins but these emulsions were less stable than those of unmodified chickpea proteins.

5.3 FUNCTIONAL PROPERTIES OF SUCCINYLATED CHICKPEA PROTEINS

Acetylation demonstrated large effects on the functional properties of many food proteins including chickpea proteins. Acetylation only converts a normally protonated amino group to a neutral group by introducing a neutral acetyl group. This results in proteins having more negative net charge and the subsequent changes in their functional properties. Succinylation is another chemical approach which has been widely used to alter proteins for food uses. From a chemical point of view, succinylation should exert more effect on the functional properties of the modified proteins than acetylation since this procedure can not only increase the net negative charge of the protein but also convert a positively charged group to a negatively charged group. This dramatic change could lead to more drastic changes in the functional properties of the modified proteins. Thus this technique could be used to prepare proteins with selected functional properties.

In fact, succinylation has improved solubility of many grain legume and food proteins over a wide range of pHs (rapeseed, Schwenke *et al.*, 1991; cottonseed, Choi *et al.*, 1982; canola, Paulson and Tung, 1987; sesame, Hasegawa *et al.*, 1981; peanut, Shyama Sundar and Rajagopal Rao, 1978; leaf, Franzen and Kinsella, 1976). Water absorption of wheat (Barber and Warthesen, 1982), cottonseed flour (Choi *et al.*, 1983), yeast (Giec *et al.*, 1989) and sunflower (Kabirullah and Wills, 1982) were improved by succinylation. Oil absorption capacity of succinylated sunflower (Canella *et al.*, 1979), whey protein concentrate (Thompson and Reyes, 1980), leaf protein (Sheen, 1991) and casein (Schwenke *et al.*, 1981) were increased. Rheological properties of food proteins such as viscosity, gel forming capacity were also changed by succinylation. Viscosity of cottonseed protein, whey, faba bean (Schwenke *et al.*, 1990) and gel of soy, canola (Paulson and Tung, 1988) and cottonseed (Choi *et al.*, 1983) were influenced by succinylation. Interfacial properties of proteins such as emulsion and hydrophobicity were also affected by succinylation. Foam and emulsion capacity and stability of canola (Paulson and Tung, 1988), cottonseed flour (Choi *et al.*, 1983), wheat (Barber and Warthesen, 1982) and soy (Franzen and Kinsella, 1976) could be

positively altered and hydrophobicity of canola (Paulson and Tung, 1987, 1988) was also changed by succinylation.

Although several desirable modification of food proteins were achieved with succinylation, this procedure may also result in some negative effects. For example, succinylation could improve the solubility of many food proteins but it can also give a reverse effect on the solubility of others (Shyama Sundar, 1978). The change in emulsion capacity and foam forming capacity of napin (Schwenke *et al.*, 1991), rapeseed (Gueguen *et al.*, 1991) and cotton seed flour (Choi *et al.*, 1983) varied with the level of succinylation. The degree of sunflower protein, field pea (Schwenke *et al.*, 1990) dissociation also depended on the level of modification. Like acetylation, succinylation not only alters functional properties of food proteins but also provides a means to understand their conformational changes and their structure-function relationship. This section reports on the functional properties of chickpea proteins succinylated at different levels.

5.3.1 SOLUBILITY IN WATER

The nitrogen solubility curves of native and succinylated chickpea proteins (SCP16, SCP79) in water are shown in Fig 5.3.1.

Compared with native chickpea protein, the succinylated protein isolates were more soluble at high pHs (pH 4 - pH 10) and less soluble at low pHs (pH 2 - pH 4). This change has also been obtained with acetylated chickpea proteins.

At pHs above the pI, the addition of anionic succinate residues, covalently bonded to the ε-amino groups of the lysine increased the net negative charge of the protein and enhanced the electrostatic repulsive forces (Habeeb *et al.*, 1958). These changes promoted an excessive unfolding of the polypeptide chain, created favourably conditions for the water molecules to penetrate into the protein molecular structure, resulted in an increase in solubility (Franzen and Kinsella, 1976; Cheftel, 1977). In contrast, at pHs lower than the pI, succinylated chickpea proteins were less soluble than the native protein. This low solubility can be explained by several factors. Since succinylation blocked free amino groups, the charged groups were reduced. The pK of the succinate radical is about 4.16, thus, carboxyl groups of succinylated protein were neutral at this or lower pHs. This reduction in total charge made the repulsive forces between oppositely charged molecules insignificant, creating favourable conditions for the proteins to be aggregated, thus, reducing its solubility. At a strong acidic pH (pH 2), there was little difference in solubility of all three chickpea proteins. Perhaps under strong acidic conditions, the non-succinylated free amino groups were protonated,

making the net charge of the protein more positive and the repulsive forces between protein molecules stronger, therefore, hindering the formation of protein aggregates. The difference in solubility between the native and each succinylated chickpea protein at pHs above and below the pI reflected the degree of succinylation. The highly succinylated chickpea SCP79 was more soluble than the less succinylated SCP16 at pH 6-10. In contrast, the SCP16 was more soluble at pH 4-2 than the SCP79 at acidic pHs (pH 2 - pH 4).

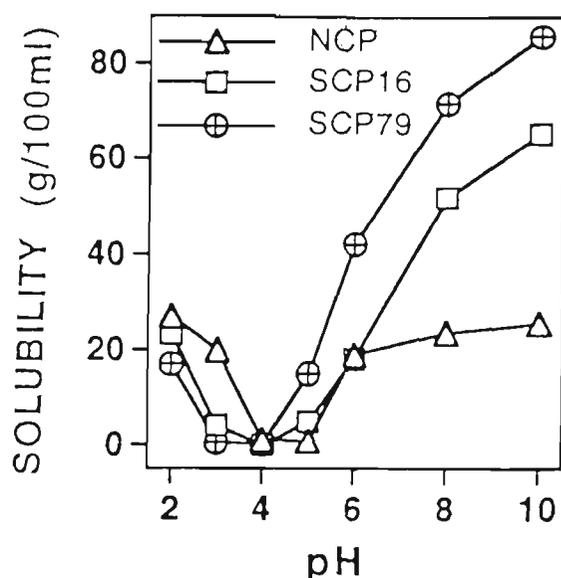


Fig 5.3.1 The nitrogen solubility of native and succinylated chickpea proteins in water.

5.3.2 SOLUBILITY IN SALT SOLUTIONS

The solubility of native and succinylated chickpea proteins at different pHs in NaCl and CaCl₂ is shown in Fig 5.3.2 a-b and Fig 5.3.3 a-b, respectively.

The pI of the native chickpea protein was shifted from pH 4.8 to pH 4 in 0.25M NaCl and its solubility at pI region was slightly increased in 0.75M NaCl solution. These changes could be attributed to the selective binding of the anionic chlorides to the protein molecules which increased its net negative charge and shifted its pI to a more acidic region. Perhaps the binding of chlorides increased with increasing salt concentrations, resulting in an increase in the solubility of protein at pH 4 in 0.75M NaCl solution.

Solubility of all native and succinylated chickpea proteins was decreased in dilute NaCl solutions. At pHs below the pI, solubility of succinylated chickpea proteins were greatly decreased with increasing levels of modification. The highly succinylated chickpea protein SCP79 was almost insoluble. This observation can be explained by several factors. The pK of succinate residues is 4.16, the net charge of the succinylated protein became almost neutral

at pHs below the pI. Thus, the highly succinylated protein could more greatly reduce the repulsive forces between the oppositely charge molecules, creating more favourable conditions for protein aggregation. The succinylated proteins also had to compete with NaCl for the water in the system. The competition for water between NaCl and the proteins became more vigorous in concentrated salt solution (0.75M NaCl). Consequently, even the moderately succinylated SCP16 became more insoluble at pHs below the pI in 0.75M NaCl solution.

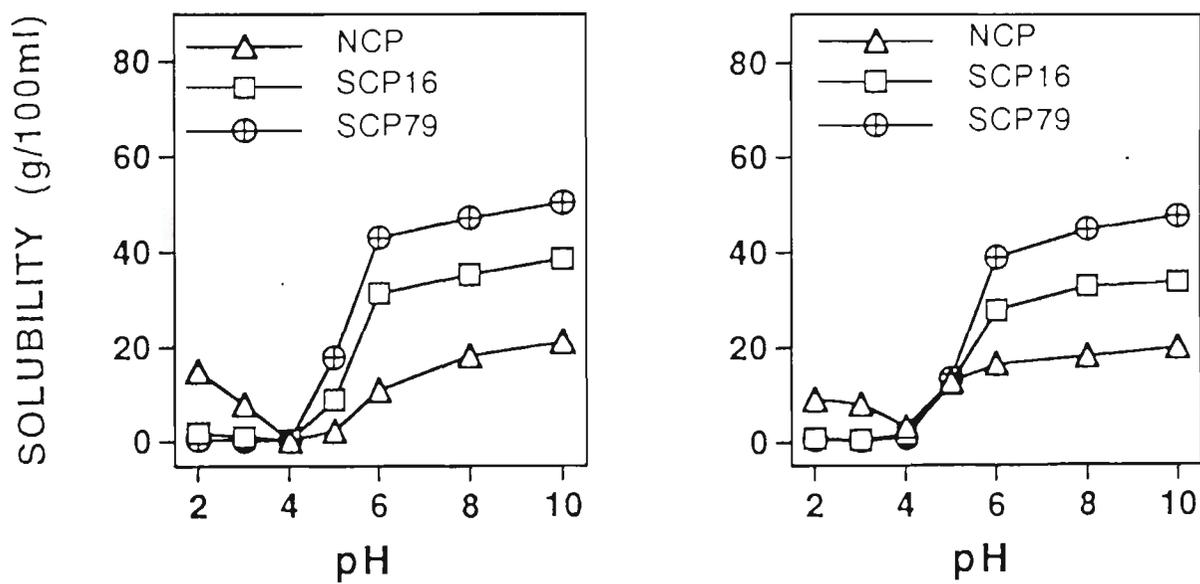


Fig 5.3.2 The solubility of native and succinylated chickpea proteins at different pHs in NaCl solutions (a) 0.25M NaCl and (b) 0.75M NaCl.

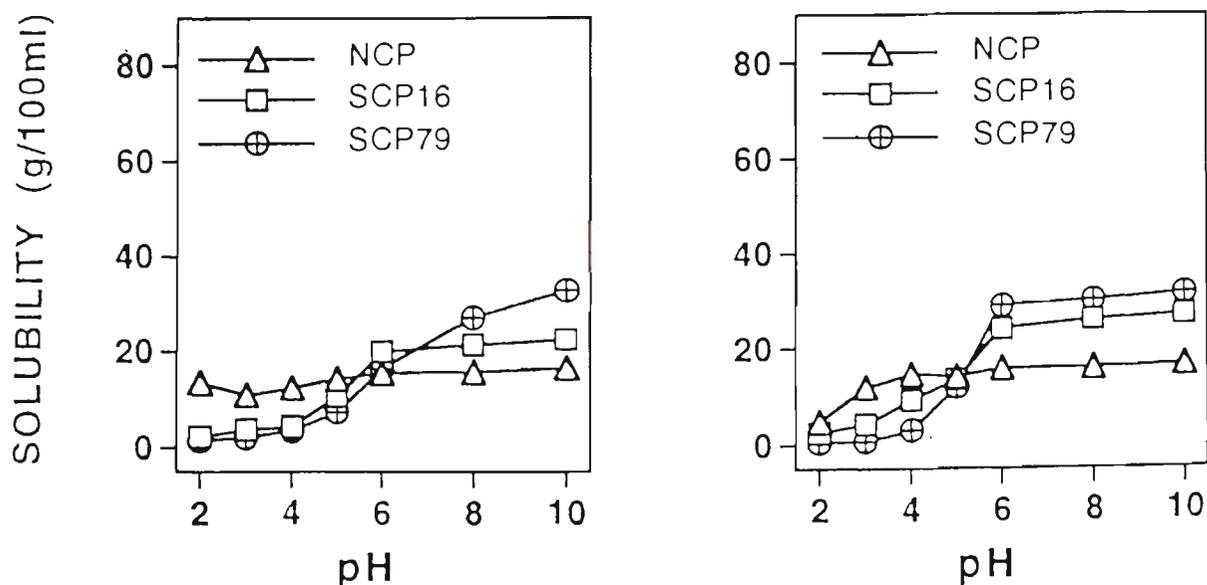


Fig 5.3.3 The solubility of native and succinylated chickpea proteins at different pHs in CaCl₂ solutions (a) 0.25M CaCl₂ and (b) 0.75M CaCl₂.

The addition of CaCl_2 greatly reduced the solubility of all these proteins and their solubility profiles were changed dramatically regardless of the concentration. The typical U shape of solubility for the native chickpea protein was replaced by an almost straight line in the 0.25M CaCl_2 solution. In the more concentrate solution (0.75M CaCl_2), the native protein became more insoluble at pH 2. CaCl_2 is a protein destabiliser and a salting-in salt which behaves very differently under different pHs (Ven Hippel and Schleich, 1969). At high pHs, Ca^{2+} cations had a higher tendency than Na to bind with carboxyl groups in the protein which reduced the net negative charge and created more favourable conditions for the aggregation of proteins. At pHs close to their pI, CaCl_2 could completely solubilise caseinate and soy globulin (Konstance and Strange, 1991; Megen Van, 1974). At pH 2, the solubility of rapeseed protein was decreased with increasing CaCl_2 concentration due to the binding of anionic chlorides to the proteins, rendering favourable conditions for the formation of insoluble protein complexes (Gillberg, 1978)

5.3.3 WATER ABSORPTION CAPACITY

Compared with the native chickpea protein, succinylated proteins had higher water absorption capacities (Fig 5.3.4). Like acetylated chickpea proteins, the water absorption capacity of succinylated proteins increased with increasing level of succinylation up to 72% and then remained constant. Even the mildly succinylated chickpea protein (SCP16) had a higher water absorption capacity than the extensively acetylated chickpea protein (ACP49).

An increase in water absorption capacity of succinylated chickpea proteins is expected since succinylation introduces hydrophilic succinate groups into the protein molecules, thus, increasing its hydrophibicity. Similar results have been obtained with sunflower, cottonseed and other food proteins. The bulky residues may also contribute to the physical entrapment of the water.

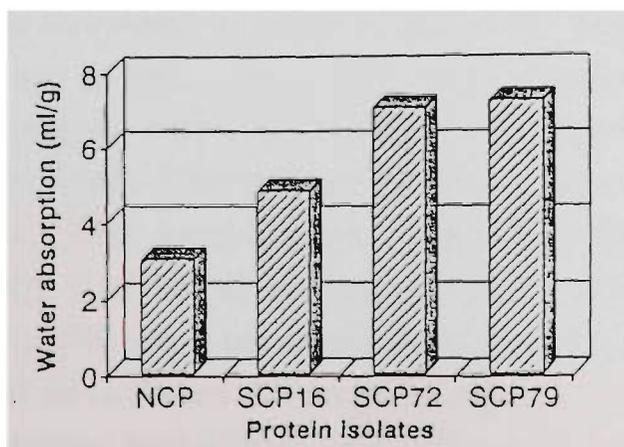


Fig 5.3.4 Water absorption capacity of succinylated chickpea proteins.

5.3.4 OIL ABSORPTION CAPACITY

The oil absorption capacity of succinylated chickpea proteins is given in Fig 5.3.5. Like acetylation, succinylation improved the oil absorption capacity of chickpea proteins. The oil absorption capacity of succinylated proteins is higher than that of both soy and unmodified chickpea protein and increased with increasing level of succinylation. This increase, after succinylation could be due to the exposure of hydrophobic residues which are normally buried inside the protein molecule. Oil absorption capacity of sunflower (Canella *et al.*, 1979), whey protein concentrate (Thompson and Reyes, 1980) leaf protein (Sheen, 1991) and casein (Schwenke *et al.*, 1981) were increased by succinylation.

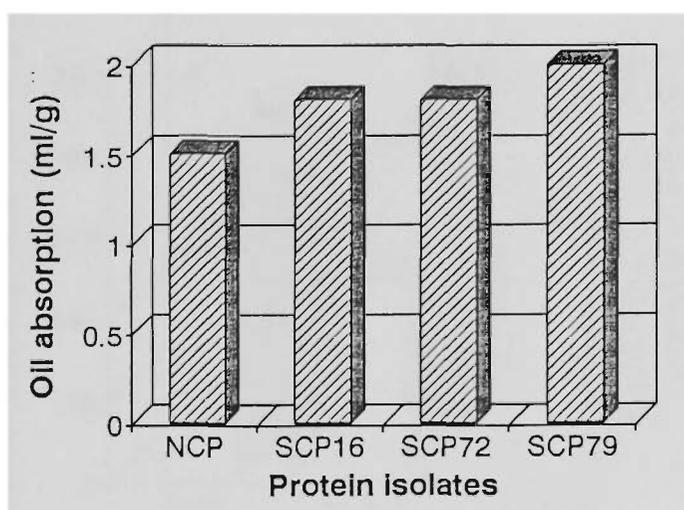


Fig 5.3.5 Oil absorption capacity of succinylated chickpea proteins.

5.3.5 EMULSIFYING CAPACITY

The effect of protein concentration on the emulsifying capacity of succinylated chickpea proteins is given in Fig 5.3.6. The EC of the investigated proteins increased with the increasing protein concentration. Similar observations have been reported with other food proteins such as cottonseed flour (Choi *et al.*, 1983), wheat (Barber and Warthesen, 1982), soy (Franzen and Kinsella, 1976) and canola (Paulson and Tung, 1987, 1988). At concentrations ranging from 0.05 to 0.125%, all succinylated proteins had slightly higher emulsifying capacities (3-17%) than that of the unmodified protein at the same concentration levels. Kinsella (1976) found a positive relationship between the EC and solubility of a protein. A high solubility of succinylated protein could contribute to its higher EC. However, this contribution is not always proportionally related to the solubility. Moreover, different level of succinylations did not greatly influence the EC of succinylated proteins. At 0.125% concentration, both SCP16 and SCP79 had the same EC (295 and 293 ml oil/0.05 g protein).

The effect of solubilising pH on the EC of both unmodified and succinylated chickpea proteins is given in Fig 5.3.7. The EC of all proteins increased with increasing pH. The effect of levels of succinylation on the EC of a modified protein varied with different pHs. The EC of SCP79 is almost double of that of SCP16 and the unmodified protein at pH 7 but both succinylated proteins had much higher EC than that of the unmodified protein at pH 9. This observation confirmed a positive relationship between EC and solubility of a protein as suggested by Kinsella (1976). A similar tendency has been reported with peanut, soy, cotton seed and milk protein (Ramanathan *et al.*, 1978; Crenwelge *et al.*, 1974).

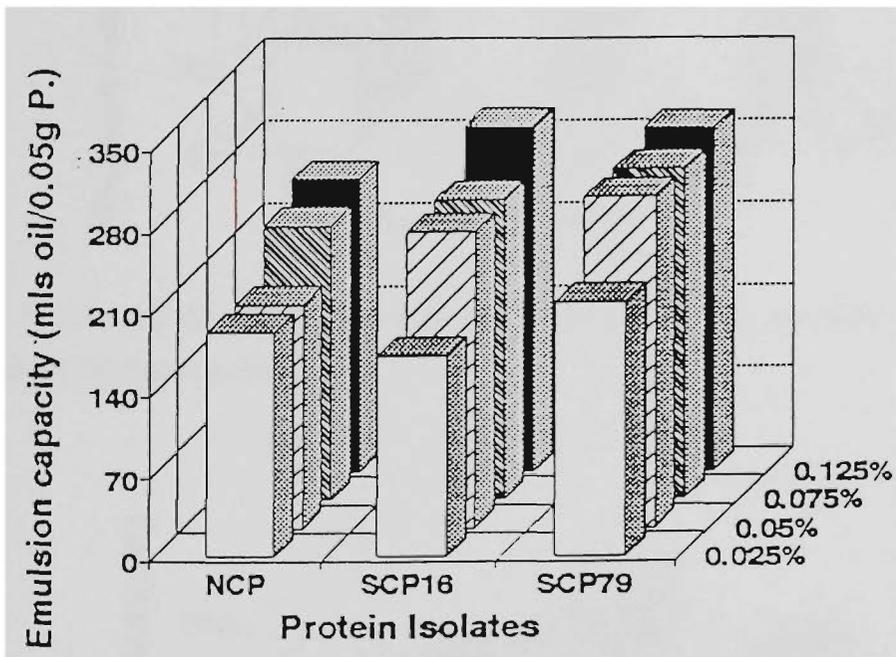


Fig 5.3.6 The effect of protein concentration on the emulsifying capacity (EC) of native and succinylated chickpea proteins.

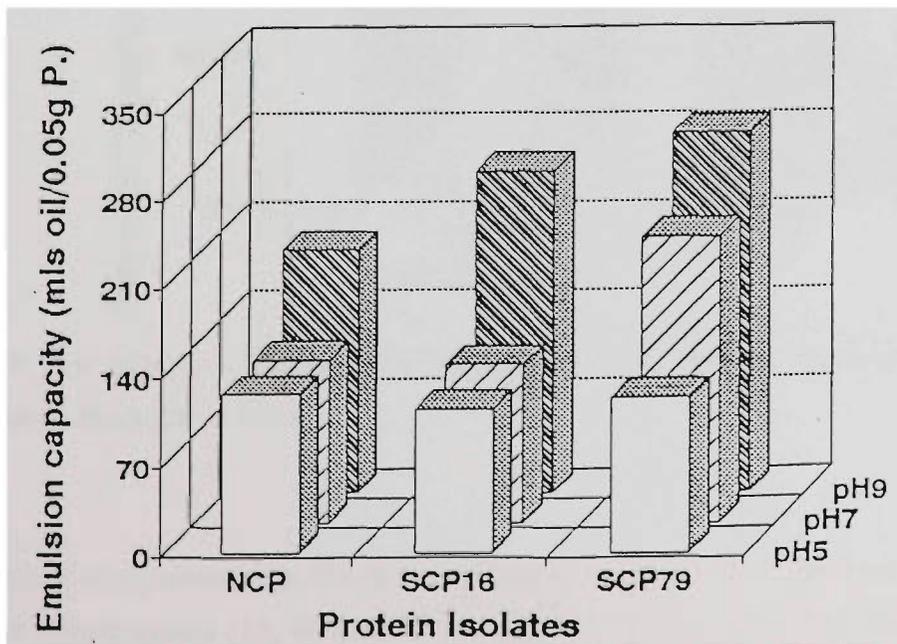


Fig 5.3.7 The effect of pH on the emulsifying capacity (EC) of native and succinylated chickpea proteins.

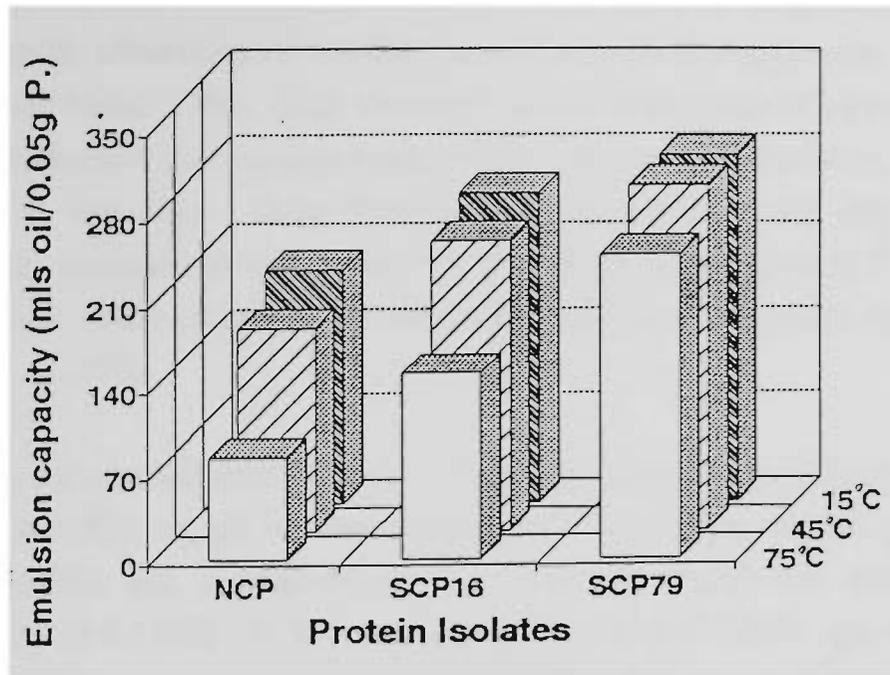


Fig 5.3.8 The effect of temperature on the emulsifying capacity (EC) of native and succinylated chickpea proteins.

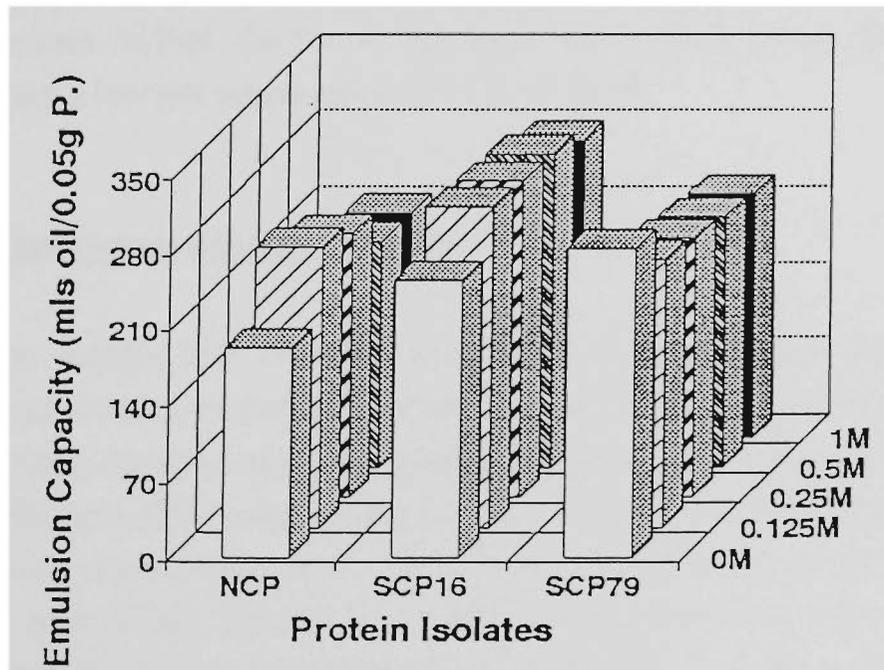


Fig 5.3.9 The effect of salt concentration on the emulsifying capacity (EC) of native and succinylated chickpea proteins.

The effect of temperature on the EC of succinylated proteins is given in Fig 5.3.8. At three measured temperatures (15, 45 and 75°C), the EC of all modified proteins is higher than that of the unmodified protein. The great effect of different levels of succinylation on the EC of a protein was clearly reflected at different temperatures. At 75°C, the unmodified and slightly

succinylated SCP16 could only retain about 30-45% of its EC at 15 or 45°C while the EC of the highly succinylated SCP79 could retain its EC at all three temperatures. The EC of a protein is greatly affected by its solubility and also by its hydrophobicity (Voutsinas *et al.*, 1983; Kato and Nakai, 1980). Heat treatment causes denaturation of a protein and gradually exposes its hydrophobic amino acid residues which are normally buried in the interior of the native protein molecules. Once these hydrophobic groups were exposed, the protein hydrophobicity increased as well as its EC. The highly succinylated SCP79 could be more sensitive to heat treatment as more hydrophobic residues were exposed, thus, contributing to its higher EC at 75°C.

The effect of salt concentration on the EC of unmodified and succinylated proteins is given in Fig 5.3.9. The effect of salt is rather complicated. For the unmodified protein, the EC is increased at low salt concentrations (0.125-0.250M NaCl) but decreased at higher concentrations (0.5-1.0M). At low concentrations (0.125-0.250M), salt contributed to the decrease of intermolecular electrostatic interactions between oppositely charged groups resulting in an increased solubility. In contrast at a high salt concentration (1M), a salting out effect is observed and consequently, the protein solubility and its EC were decreased. A similar tendency is observed with the moderately succinylated SCP16. Like the highly acetylated protein ACP49, the EC of the highly succinylated protein SCP79 was sharply decreased even at low salt concentrations (0.125-0.250M).

5.3.6 EMULSION STABILITY (ES)

The emulsion stability (ES) of succinylated chickpea proteins was influenced by several factors such as protein concentration, solubilised pH and salt concentration. Like acetylated chickpea proteins, the effect of protein concentration on ES of succinylated proteins was less than that of the unmodified proteins (Fig 5.3.10a). For a short time stability (180 minutes) the effect of protein concentration on the ES of both unmodified and succinylated proteins was very similar. After 4 days, the SCP16 and SCP79 could retain over 50% of their stability at high concentrations (0.05-0.125%) while the native chickpea protein could only retain about 10% of its stability at 0.125% concentration (Fig 5.3.10b).

The emulsions of all isolates were more stable at pH 9 than pH 7 and pH 5. For the short time stability, the ES of native proteins was higher than that of succinylated proteins at all three pHs (Fig 5.3.11a). Among the succinylated proteins, the ES of SCP79 was higher than that of SCP16. In contrast, for long time stability, both succinylated chickpea proteins were more stable than the native protein at pH 9 but similar or less stable at other pHs (Fig 5.3.11b).

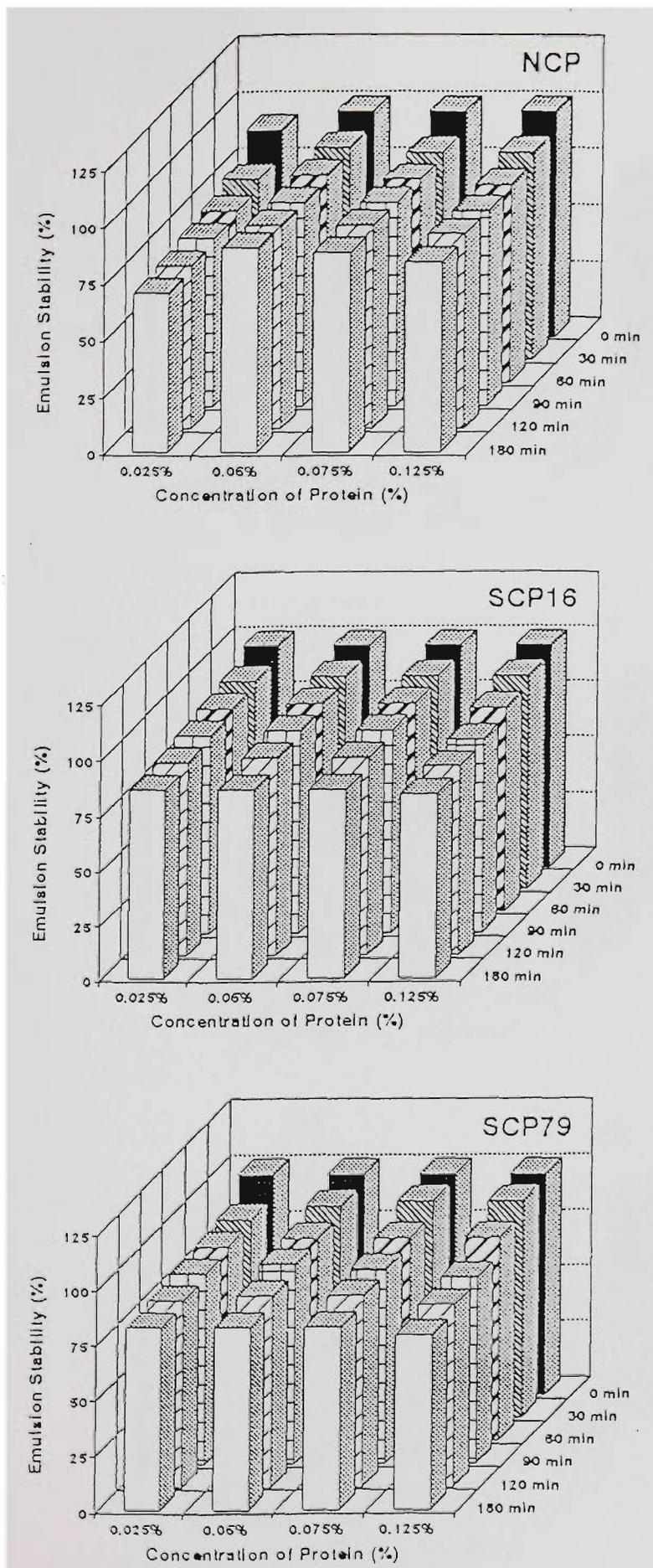


Fig 5.3.10a The effect of protein concentration on the emulsion stability (ES) of native and succinylated chickpea proteins over 3 hours.

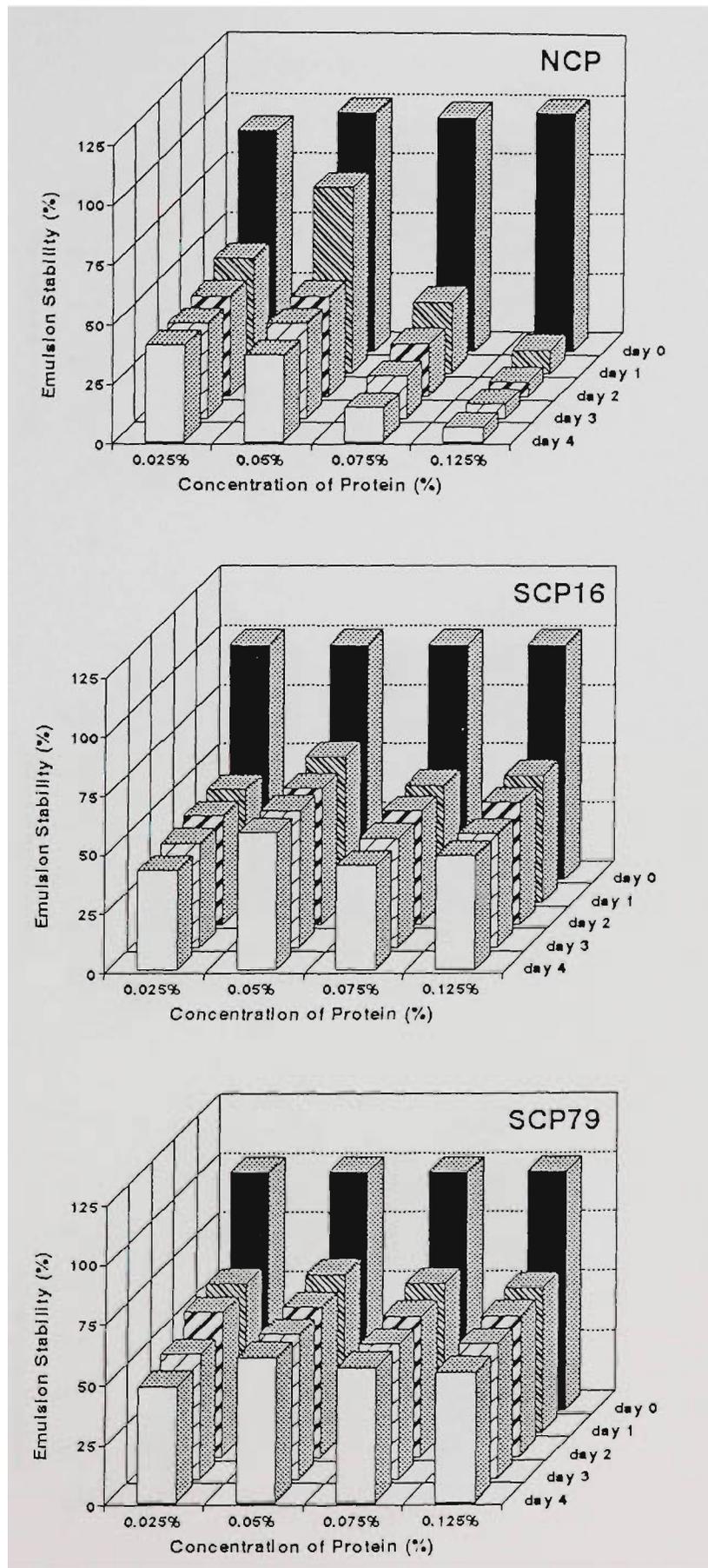


Fig 5.3.10b The effect of protein concentration on the emulsion stability (ES) of native and succinylated chickpea proteins over 4 days.

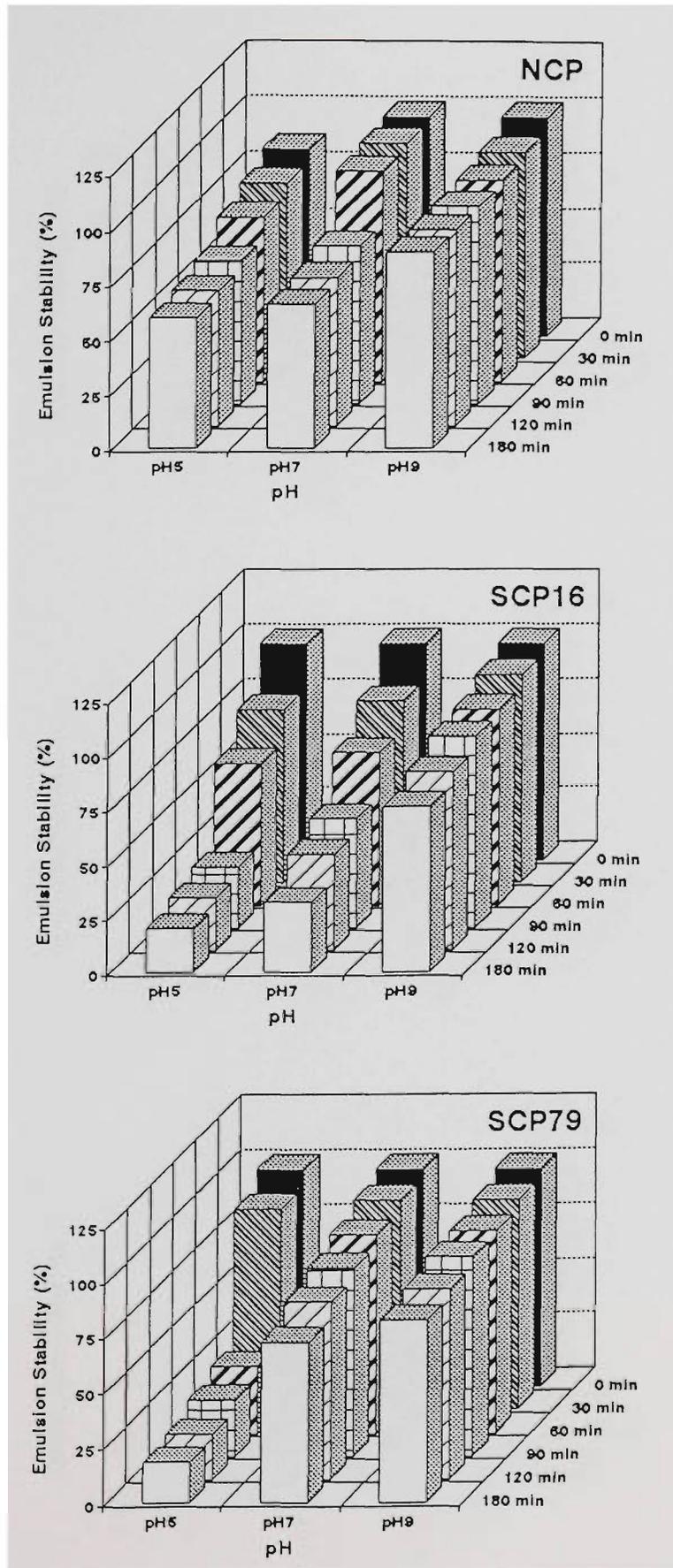


Fig 5.3.11a The effect of pH on the emulsion stability (ES) of native and succinylated chickpea proteins over 3 hours.

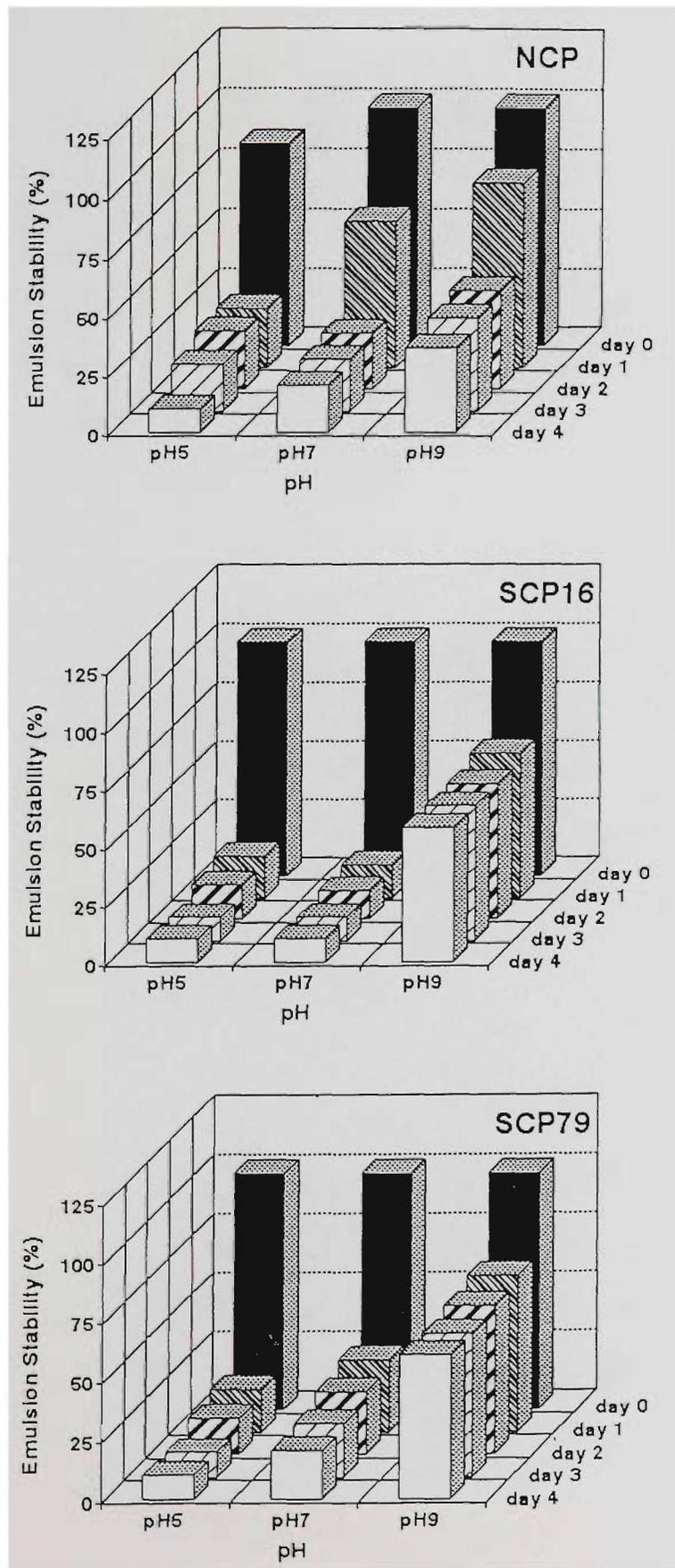


Fig 5.3.11b The effect of pH on the emulsion stability (ES) of native and succinylated chickpea proteins over 4 days.

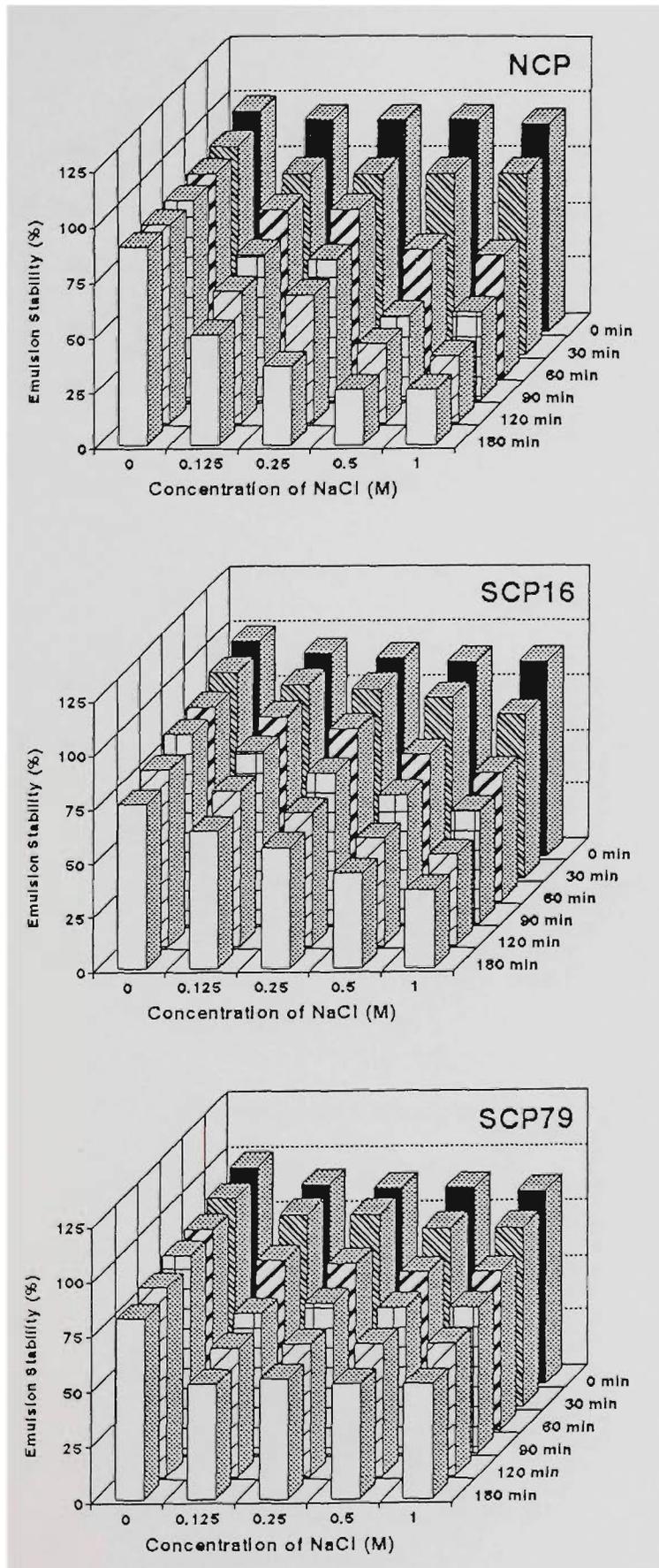


Fig 5.3.12a The effect of salt concentration on the emulsion stability (ES) of native and succinylated chickpea proteins over 3 hours.

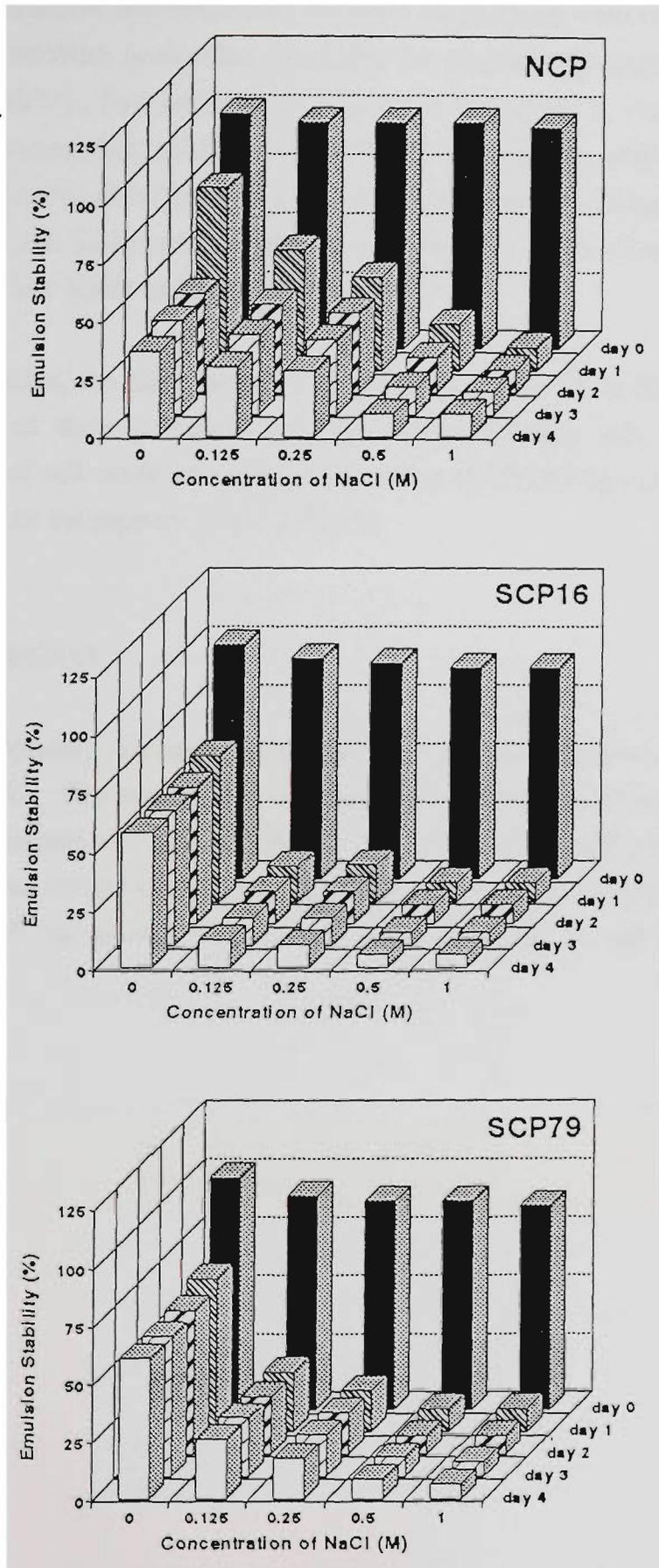


Fig 5.3.12b The effect of salt concentration on the emulsion stability (ES) of native and succinylated chickpea proteins over 4 days.

The difference in the emulsion stability of these proteins at different pHs reflected the difference in their structure and behaviour. At pH 9, all proteins were more soluble and their net charge did not establish favourable conditions for protein aggregation. This contributed to the emulsion stability. Succinylation increased the net negative charge of the modified proteins, thus, enhancing their solubility at pH 9. This might also contribute to their higher ES. In contrast at lower pHs (pH 7 and pH 5) the net negative charge of the succinylated proteins decreased and favourable conditions were formed for protein aggregation, which contributed to their less stable emulsions at low pHs.

Like acetylated proteins, the effect of salt concentrations on the ES of all protein isolates was similar. The ES of these proteins decreased with increasing salt concentrations. The destabilising effect of salt could be explained in terms of the salting out effect and possible Na^+ cations binding to the proteins (Fig 5.3.12a-b).

5.3.7 CONCLUSION

Compared with acetylation, succinylation greatly improved the solubility of chickpea proteins at high alkaline pHs. The water and oil absorption capacities of succinylated chickpea proteins were also improved. The emulsion capacity of succinylated chickpea proteins was higher than that of native and acetylated chickpea proteins. These emulsions were also more stable than those of native and acetylated chickpea proteins at high concentrations and particularly at pH 9.

CHAPTER 6

FLOW PROPERTIES OF CHICKPEA PROTEINS

· FLOW PROPERTIES OF CHICKPEA PROTEINS

Fluid foods make up the major portion of human's food intake. As infants, they can rely entirely on fluid foods to grow and develop. At a later stage, humans can rely, once again, on fluid foods when sick or old. Fluid foods are still important parts of our current food system. Chickpea proteins have been used as ingredients in only a few fluid foods such as imitation milk or beverages but fluid foods can be used as a means to introduce these proteins in wider applications in processed foods

Flow properties are an important factor in deciding how a protein dispersion can be utilised in fluid foods. There are numerous investigations on the flow properties of liquid or fluid foods (Rao, 1977). These studies highlighted the diverse and anomalous flow behaviour of food materials. The investigation of the rheological properties of a protein solution is normally based on the measurement of the viscosity. This, in turn, relates to shear stress and shear rate. Flow behaviour of the protein dispersion is investigated by measuring its viscosity over a range of shear rates. A flow curve is constructed by plotting the measured shear stress against the corresponding shear rate. Although the flow properties of a food material, including its protein dispersion, are rather diverse and anomalous, they can normally be described by the so called power law equation.

$$\sigma = m \gamma^n \quad [6.1.1]$$

$$\text{or } \log \sigma = n \log \gamma + \log m \quad [6.1.2]$$

σ is the shear stress (mPa), γ is the shear rate (sec^{-1}), n is the flow index, m is the consistency coefficient. Depending on the values of these constants, the flow characteristic of a fluid can be characterised as Newtonian or non-Newtonian behaviour.

The flow characteristics of a fluid can also be described by the yield value ($\sigma=k_0^2$) of the protein dispersions which is related to the network structure of the material. This can be calculated by the Casson equation which is normally used to describe the yield stress of chocolates.

$$\sigma^{1/2} = k_0 + k_1 \gamma^{1/2} \quad [6.1.3]$$

For a number of dispersions, shear stress appears to be constant at low shear rates. This tendency indicates the existence of a yield stress. This is the minimum amount of stress

required to initiate a flow. The yield stress value ($\sigma_y = k_0^2$) can be obtained by extrapolating the rheogram of $\sigma^{1/2}$ versus $\dot{\gamma}^{1/2}$ to zero shear rate. Finally, the apparent viscosity of a dispersion (η) can be calculated by the following equation

$$\eta = m \dot{\gamma}^{n-1} \quad [6.1.4]$$

A fluid with no yield stress (σ_y) and a flow behaviour index of unity ($n=1$) is called a Newtonian fluid. True solutions, low molecular weight solvents, dilute macromolecular dispersions in low molecular weight solvents, non interacting polymer solutions and thin solid pastes are examples of Newtonian fluids. However, most fluid foods are non Newtonian. These non Newtonian fluids can be divided into two groups: time independent and time dependent. The time independent group includes Bingham plastic, pseudoplastic and dilatant materials. The time dependent group includes the thixotropic and rheopectic fluids. The flow properties of various liquid and fluid foods are summarised in Table 6.1 by Rha (1978) and the typical rheological patterns of liquid foods, as described by Glicksman (1969), are illustrated in Fig 6.1.

Table 6.1 The flow properties of various liquid and fluid foods.

Flow characteristics of fluids	Typical fluids having these flow characteristics	Consistency index, m	Flow behaviour index, n	Yield stress, σ_y	Examples of food products
Newtonian	True solutions, colloidal dispersions, thin emulsions, dilute macromolecular solutions,	Viscosity $m>0$	$n = 1$	$\sigma_y = 0$	Clarified juices, clear soup, oils, milk, confectionery syrup,
Shear-thinning (pseudoplastic)	Polymer solutions, pastes, emulsions, suspensions	Apparent viscosity $m>0$	$0 < n < 1$	$\sigma_y = 0$	Concentrated fruit or vegetable juice, purees, and paste, starch and protein paste
Bingham plastic	Suspensions, colloids	Plasticity constant $m>0$	$n = 1$	$\sigma_y > 0$	Salad dressing such as French dressing, tomato catsup, fudge sauce
Mixed-type	Suspended particles of irregular shape in thick medium	Consistency index $m>0$	$0 < n < 1$	$\sigma_y > 0$	Sandwich spread, jelly, marmalade
Shear-thickening	Nearly saturated or concentrated suspensions, emulsions	Consistency index $m>0$	$1 < n < \infty$	$\sigma_y = 0$	Homogenised peanut butter, Sausage slurry

Fig 6.1a shows a typical Newtonian fluid in which viscosity is independent of shear rate. The typical behaviour of a Bingham plastic flow is illustrated in Fig 6.1b in which viscosity decreases with increasing shear rate, after the yield stress has been reached. The flow behaviour is similar to a Newtonian fluid when the threshold shear stress is reached. When

the viscosity decreases with increasing shear rates, the flow becomes pseudo plastic or shear thinning (Fig 6.1c). When the viscosity increases with increasing shear rate, the fluid is dilatant or shear-thickening (Fig 6.1d). The characteristic of a thixotropic fluid is reflected in its reversible gel-sol-gel capacity. A gel is formed as a result of a definite structure within the material but it becomes a sol by shaking or stirring. A sol formed can be reversed to a gel if it is allowed to be undisturbed. If the shear stress is plotted against the shear rate, at increasing and decreasing rates, a hysteresis loop is obtained. This indicates the stress required to break the gel structure and reverse the solution back to its normal apparent viscosity (Fig 6.1e). A rheopectic fluid is a reverse thixotropic fluid in which the viscosity increases with a constant increasing force (Fig 6.1f).

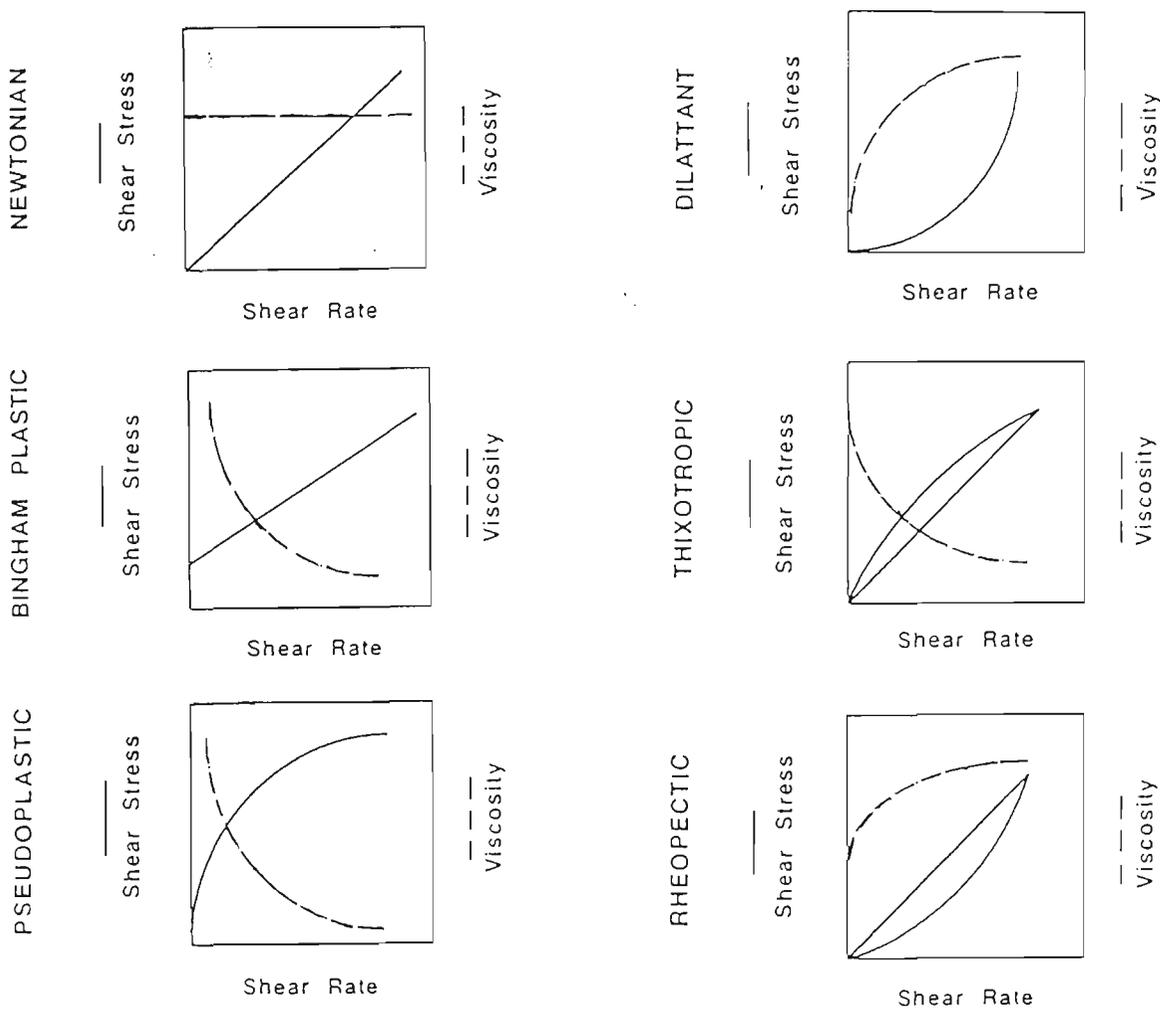


Fig 6.1 Typical rheological patterns of liquid or fluid foods as described by Glisksman (1969).

Information on flow properties of protein dispersions are important for both food processors and academic researchers. This information is useful for manufacturing processes and for the optimal design of unit processes such as pumping, piping and spray drying (Tung, 1978).

They can also be used to predict or select suitable fields of application for new protein based products (Hermansson, 1975). For example, the texture quality of the finished products based on proteins is related to flow properties. On the other hand, flow properties of protein dispersions are affected by their shape, size, charge, nature of the protein and also the processing conditions used to produce the protein. Studies on the flow properties of a protein will provide a means of understanding the protein structure and also its protein-protein interactions (Hermansson and Akesson, 1975a and b).

Only limited information on the flow properties of chickpea protein is available in the literature. For food applications, there is a need to investigate the flow properties of chickpea protein dispersions and to define factors affecting its behaviour as a fluid food. The effect of processing conditions on the characteristics and also the functional properties of these proteins have already been discussed in chapter 3 and 4. In this study, isolated chickpea proteins were extensively modified with different chemical reagents and enzymes. Certainly the effects of these modifications will be reflected in the flow properties of these protein dispersions. Consequently, the objectives of this chapter were to investigate the flow properties of chickpea proteins and to examine the effects due to the protein acetylation and succinylation.

6.1 FLOW PROPERTIES OF CHICKPEA PROTEIN DISPERSIONS

Firstly, aqueous dispersions of unmodified chickpea proteins with different levels of protein concentrations were prepared and their flow properties under different conditions were investigated. The experimental data was collected by using the Rheometer-30 and analysed by using the power law equation [6.1.2] and other equations [6.1.3 and 6.1.4] described previously. The flow characteristics of these protein dispersions were defined either by their flow curves or associated constants.

6.1.1 EFFECT OF CONCENTRATION

The shear stress-shear rate flow curves of chickpea protein dispersions at different concentrations are shown in Fig 6.1.1. The slope of these flow curves increases with increasing concentration. Up to a concentration of 8%, the flow curves were linear, indicating Newtonian or near Newtonian behaviour. Shear stress (σ) as a function of shear rate, on a logarithmic scale, of these chickpea protein dispersions are presented in Fig 6.1.2. Flow properties of many food protein dispersions were reported to be characterised by the power

equation, at several concentrations. Hermansson (1975) found that the flow properties of soy protein isolate, caseinate and whey protein concentrate conformed with the power equation at several concentration levels. Similar observations were obtained with aqueous sunflower protein dispersions (Lefebvre and Sherman, 1977). Mita and Matsumoto (1980) found an excellent linearity of shear stress - shear rate plots at a shear rate change of 100-1000 sec^{-1} for gluten dispersions at different concentrations. Thus, these protein dispersions also conform with the power law equation. A similar result was observed in this study, with a good linearity of shear stress - shear rate plots at shear rates ranging from 100 to 1750 sec^{-1} , obtained for chickpea protein dispersions at different concentrations.

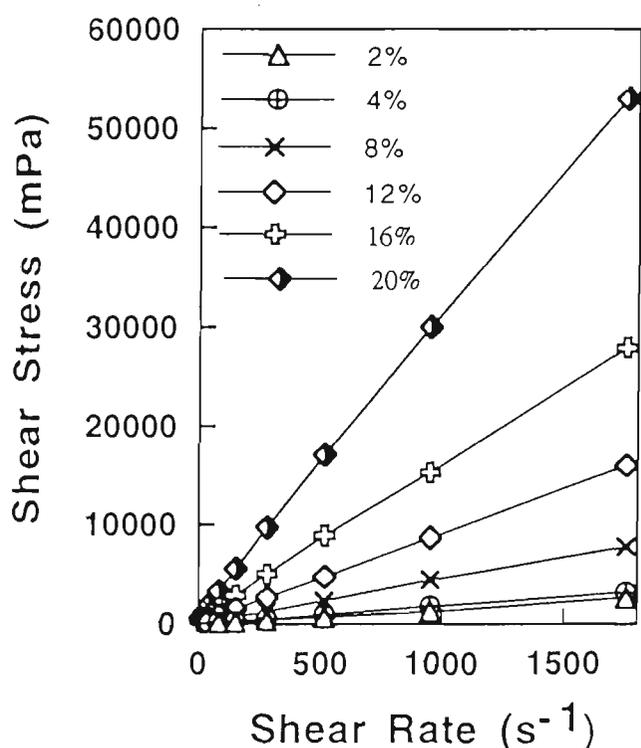


Fig. 6.1.1 Flow curves of unmodified chickpea protein (NCP) dispersions at different concentrations (% based on dry weight).

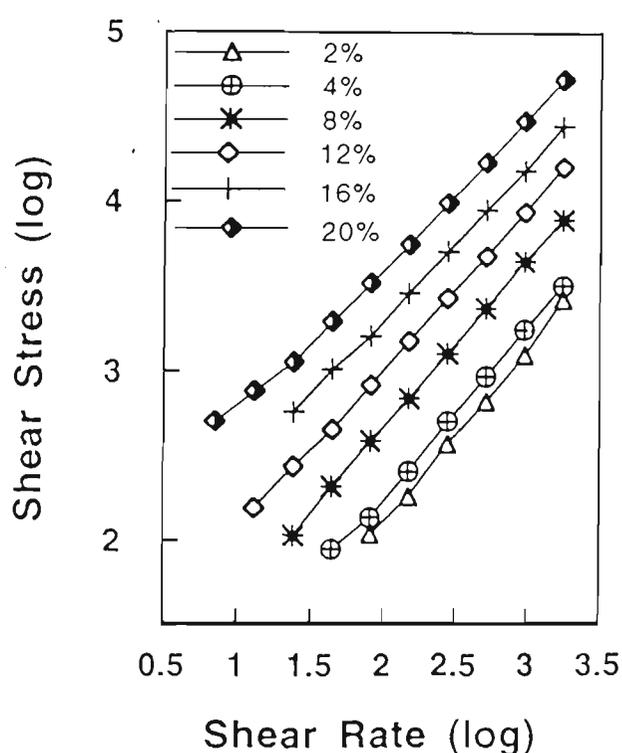


Fig. 6.1.2 Shear stress (σ) as a function of shear rate on a logarithmic scale for chickpea protein dispersions at different concentrations.

The effect of protein concentrations on the power law constants and apparent viscosity of chickpea protein dispersions is shown in Table 6.1.1. Up to 8% concentration, the dispersions are Newtonian or near Newtonian ($n=1$) because the apparent viscosities are unchanged with different shear rates (81.4 and 950 sec^{-1}). At higher concentrations, non-Newtonian behaviour becomes more pronounced ($n=0.94$ to 0.85). The apparent viscosities were affected by the change in shear rates (Fig 6.1.3). The consistency coefficient (m) increased

markedly with increasing concentrations. In fact m , an index of viscosity, is a function of concentration on a semilogarithmic scale (Fig 6.1.4).

At a concentration of 16% a yield stress was apparent and both yield stress and apparent viscosities were markedly increased with increasing concentrations (Table 6.1.1). The capacity of the Casson equation to describe the yield stress of chickpea protein dispersions is shown in Fig 6.1.5. The consistency coefficient (m) increased but the flow behaviour index (n) decreased progressively with increasing concentrations. Consequently, the chickpea protein dispersions exhibited a clear tendency towards pseudoplastic behaviour with increasing concentrations.

The concentration dependence of viscosity of chickpea protein in this study and of soy protein (Hermansson, 1975), faba bean protein (Schmidt *et al.*, 1986), oat protein (Ma, 1993), beta-lactoglobulin (Pradipasena and Rha, 1977), single cell protein concentrate (Huang and Rha, 1971) in other investigations suggests a protein network was formed at high concentrations, due to protein-protein interactions (Hermansson, 1975).

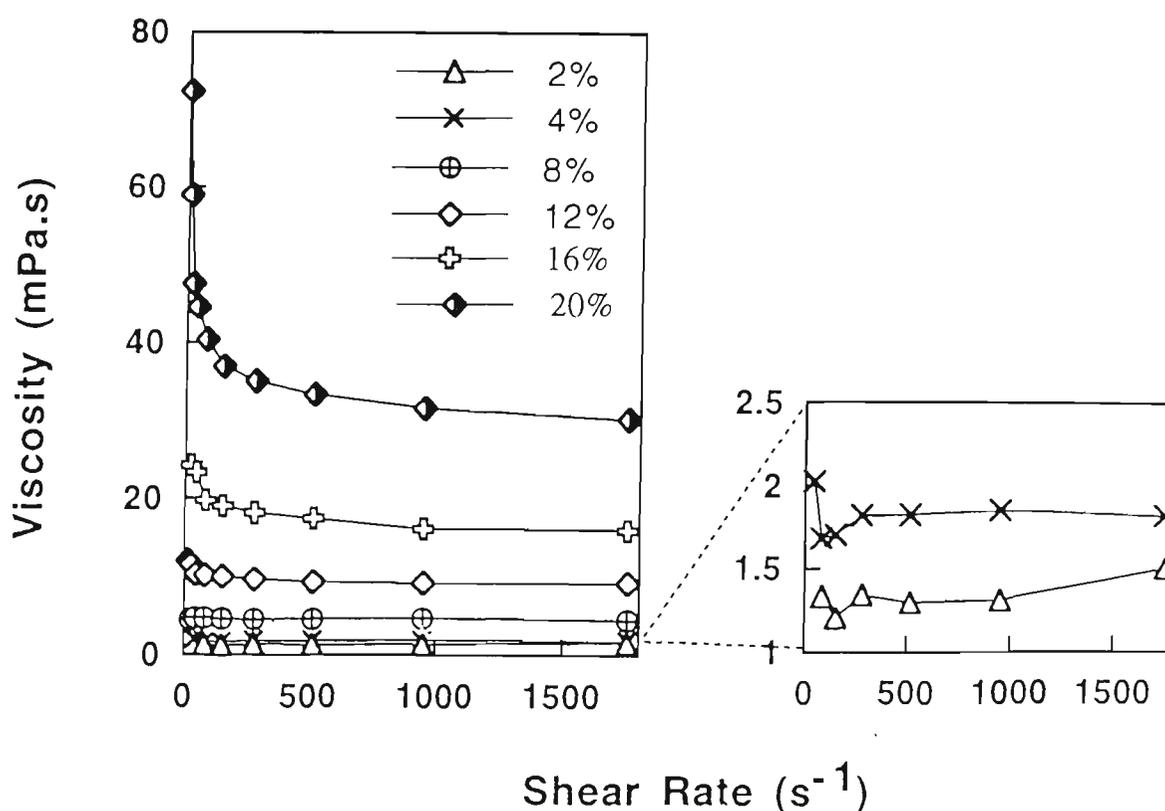


Fig 6.1.3 Effect of shear rate on the apparent viscosities of chickpea protein dispersions at different concentrations. At low concentrations (<4%) shear rate had almost no effect on the apparent viscosity of dilute protein dispersions, indicating Newtonian behaviour.

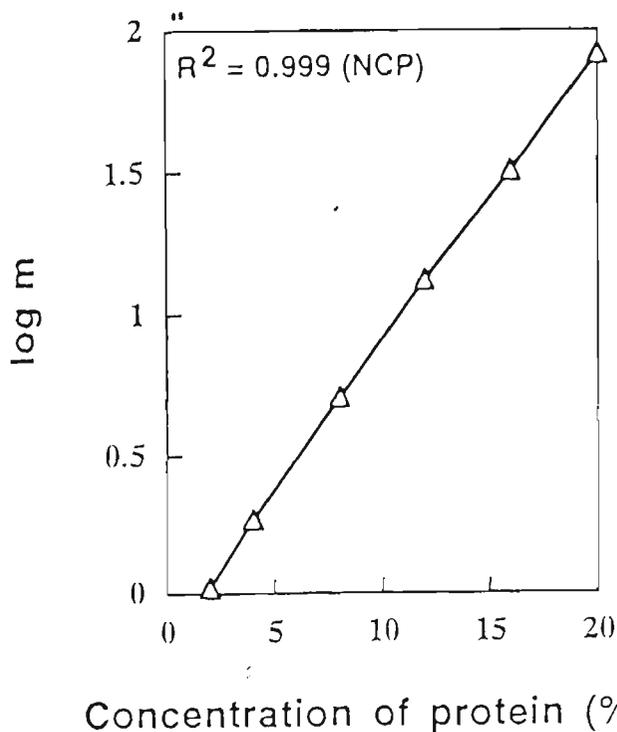


Fig 6.1.4 An index of viscosities, m , is a function of protein concentration on a semilogarithmic scale.

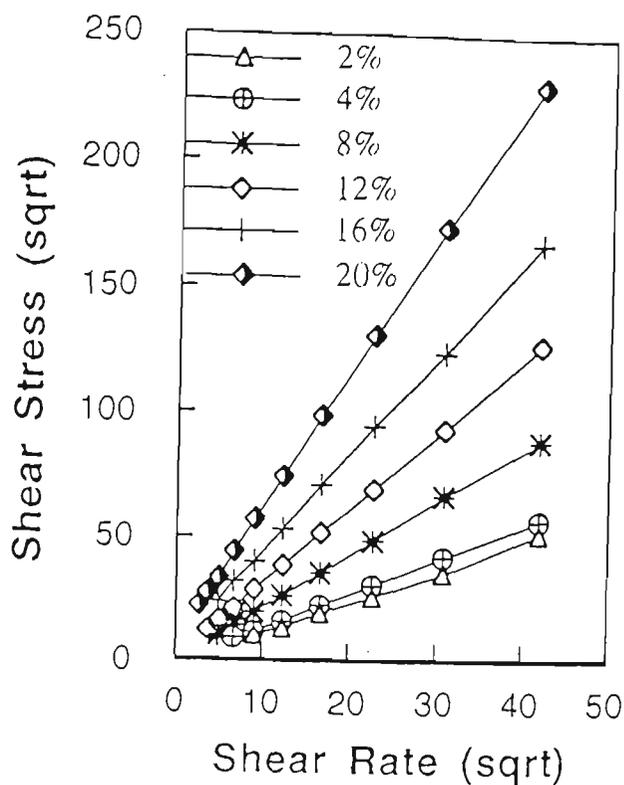


Fig 6.1.5 Shear stress (σ) as a function of shear rate on a square root scale for chickpea protein dispersions at different concentrations.

Table 6.1.1 Effect of concentration (% by weight) on the power law constants and apparent viscosities of chickpea protein dispersions.⁽¹⁾

Concentration (%)	$n^{(2)}$	$m^{(3)}$ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
				81.4 sec ⁻¹	950 sec ⁻¹
2	1.00 ($r^2 = 0.998$)	1.06	0.00	1.33	1.31
4	0.99 ($r^2 = 0.998$)	1.85	0.02	1.68	1.85
8	0.99 ($r^2 = 1.000$)	4.68	0.17	4.75	4.65
12	0.94 ($r^2 = 1.000$)	13.3	3.40	10.6	9.13
16	0.89 ($r^2 = 0.999$)	32.4	36.3	19.7	16.1
20	0.85 ($r^2 = 0.997$)	82.4	80.2	40.4	31.6

- (1) Averages of four determinations at pH7 and 25°C
- (2) n = flow behaviour index
- (3) m = consistency coefficient

6.1.2 EFFECT OF TEMPERATURE

The effect of temperature on the power law constants and apparent viscosity of 8% protein dispersions is given in Table 6.1.2. Up to 35°C there was no change in any power law

constant and apparent viscosity. At 55°C, the flow behaviour index exponentially decreased, the consistency coefficient increased greatly and a yield stress became apparent. The flow behaviour became more pseudoplastic.

Apparent viscosities of chickpea protein dispersions slightly increased when the temperature increased from 35 to 55°C (Fig 6.1.6). The apparent viscosity at 55°C decreased at a high shear rate (950 sec⁻¹), indicating that the flow deviated from Newtonian behaviour. Increase in the viscosity of protein dispersions associated with increasing temperatures have been reported with blood plasma protein (Howell and Lawrie, 1987), and also in whey protein (Voutsinas *et al.*, 1983), faba bean (Schwenke *et al.*, 1990) and oat protein (Ma, 1993). The sensitivity of protein dispersions to thermal increase depends on the type of protein. Blood plasma protein is more thermally sensitive than beta lactoglobulin (Plock and Kessler, 1992; Howell and Lawrie, 1987). The viscosity of blood plasma protein increased greatly at relatively low temperature (76°C) while whey protein was only affected at higher temperatures (>80°C). The increase in viscosity of these protein dispersions indicated a change in the shape of proteins as a result of protein unfolding which is due to the thermal treatment provided (Lee and Rha, 1979; Catsimpoolas and Myer, 1970; Ma, 1993).

In contrast to these observations, Mita and Matsumoto (1980) found that the apparent viscosities of 12% gluten and gluten methyl ester dispersions decreased over the range of temperatures from 20° to 50°C. When the thermal effects outweighed the intermolecular interactions, contributed by hydrogen bonding, the apparent viscosity decreased. Consequently, these investigations indicated that the thermal effects on the flow properties of the proteins are not uniform, as seen with their emulsifying properties (Voutsinas *et al.*, 1983).

Table 6.1.2 Effect of temperature (°C) on the power law constants and apparent viscosities of 8% chickpea protein dispersions.⁽¹⁾

Temperature (°C)	n ⁽²⁾	m ⁽³⁾ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
				81.4 sec ⁻¹	950 sec ⁻¹
15	0.98	5.13	0.22	4.82	4.70
25	0.99	4.68	0.17	4.75	4.65
35	0.97	5.50	1.09	4.90	4.43
55	0.68	44.7	25.4	11.2	5.02

(1) Averages of four determinations of 8% protein dispersions at pH7

(2) n = flow behaviour index

(3) m = consistency coefficient

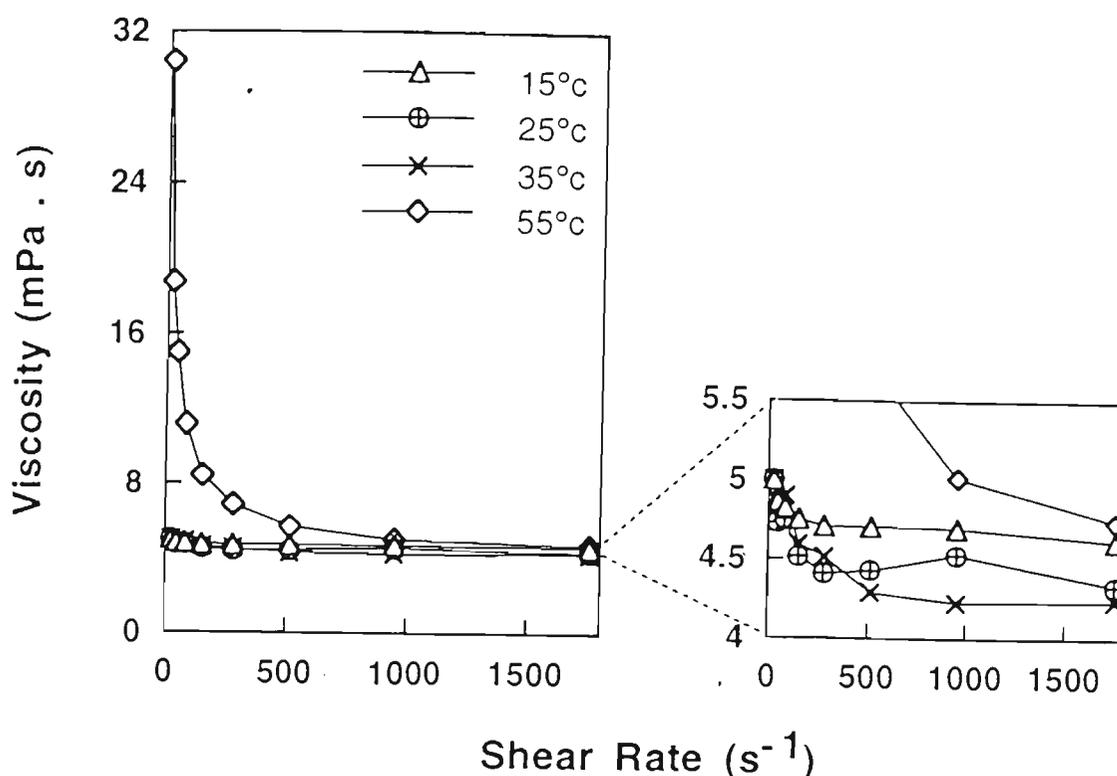


Fig 6.1.6 Thermal effects on the apparent viscosities of 8 % chickpea protein dispersions. At 55°C heat treatment increased the apparent viscosities of chickpea protein dispersions.

6.1.3 EFFECT OF SALTS

The effect of salt concentration (M) on the power law constants and apparent viscosity of 8% protein dispersions are given in Table 6.1.3.

The flow curve was linear up to 0.2M NaCl, indicating a Newtonian flow (Fig 6.1.7). Between 0.2M and 1M NaCl, the flow curves progressively deviated from Newtonian behaviour but a reversal was observed at 2M NaCl.

The flow behaviour index progressively decreased with increasing salt concentrations up to 1M ($n=0.99-0.73$), indicating a shear thinning effect. In contrast no consistent trend was noted for the consistency coefficient which initially dropped at 0.2M, but increased up to 1M.

The apparent viscosities reduced with salt addition and varied with a change of shear rate (81.4-950 sec⁻¹). This indicated a progressive deviation of flow curves from Newtonian

behaviour. Reverses in the apparent viscosity and other power law constants (n , m) occurred between 1 and 2M NaCl. Ma (1993) observed a similar tendency with oat protein dispersions at 1M salt concentration. Hermansson (1975) also noted a similar reversal in apparent viscosity of a soy protein isolate between 0.5 and 1M NaCl. This reversal was attributed to a critical salt concentration for the protein solubilization (Van Megen, 1974).

Table 6.1.3 Effect of salt concentration (NaCl/M) on the power law constants and apparent viscosities of 8% chickpea protein dispersions.⁽¹⁾

NaCl conc (M)	$n^{(2)}$	$m^{(3)}$ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
				81.4 sec ⁻¹	950 sec ⁻¹
0	0.99	4.68	0.17	4.75	4.65
0.2	0.98	3.02	0.05	1.71	0.21
0.5	0.84	9.33	0.25	1.87	0.22
1.0	0.73	22.1	55.6	1.98	0.22
2.0	0.78	16.2	36.3	1.95	0.22

- (1) Averages of four determinations of 8% protein dispersions at pH7 and 25°C.
 (2) n = flow behaviour index
 (3) m = consistency coefficient

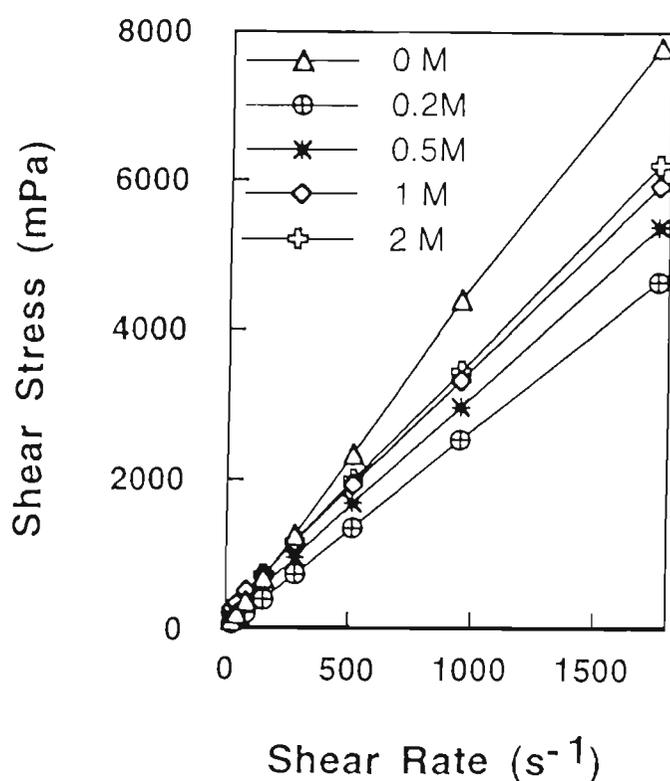


Fig 6.1.7 Flow curves of chickpea protein dispersions in salt solutions at different concentrations (0.2-2M NaCl).

Flow properties of protein dispersions are markedly influenced by ionic strength. Salt concentrations (NaCl) could either increase or decrease the apparent viscosities of protein isolate dispersions. At a low shear rate (10 sec^{-1}), the apparent viscosities of 11.4% canola protein dispersions increased with salt concentrations but at a high shear rate ($1,000 \text{ sec}^{-1}$) the apparent viscosities decreased (Paulson and Tung, 1988). Hermansson (1975) found that the apparent viscosity of soy protein dispersions decreased with increasing salt concentrations but a reversal was observed with caseinate. The different response of soy protein and caseinate to an ionic environment was influenced by a structural difference between the two proteins. Similarly, the flow behaviour index of oat globulin dispersions (15%) was decreased progressively with increasing salt concentrations, showing an increasing pseudoplastic tendency. The addition of salt produced an insignificant effect on the apparent viscosity of chickpea protein dispersions. The results suggest that chickpea protein had a rather rigid structure which barely altered in a salt medium.

6.1.4 EFFECT OF pH

Table 6.1.4 Effect of pH on the power law constants and apparent viscosities of 8% chickpea protein dispersions.⁽¹⁾

pH	n ⁽²⁾	m ⁽³⁾ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
				81.4 sec ⁻¹	950 sec ⁻¹
2	0.91 ($r^2 = 1.000$)	12.9	10.9	8.70	7.14
5	0.84 ($r^2 = 0.995$)	6.01	11.8	2.85	1.96
7	0.99 ($r^2 = 0.998$)	4.68	0.17	4.75	4.65
9	0.85 ($r^2 = 0.999$)	20.4	28.7	10.3	7.62

(1) Averages of four determinations of 8% protein dispersions at 25°C.

(2) n = flow behaviour index

(3) m = consistency coefficient

The effect of pH on the power law constants and apparent viscosities of 8% protein dispersions are given in Table 6.1.4 and Fig 6.1.8. At pH 7, the flow was Newtonian with $n=0.98$, no yield stress and apparent viscosities were unchanged at 81.4 and 950 sec^{-1} . The flow progressively deviated from Newtonian behaviour at both alkaline and acidic pHs with a decrease in the flow behaviour index and lower viscosities at high shear rates.

The viscosity-pH curve resembled the typical solubility curve of chickpea protein. Minimum solubility occurred at the isoelectric pH (pH 4-5) region with much higher solubilities at acidic or alkaline pH (pH 2 or pH 9). Since the apparent viscosities were concentration dependent, the viscosity was high at the protein's most soluble pHs (Fig 6.1.9). Like soy and canola protein (Ishino and Okamoto, 1975; Lee and Rha, 1979; Paulson and Tung, 1988) the high viscosity of chickpea protein at pH 9 was a combined effect of alkaline induced protein unfolding, dissociation into subunits and an increased protein solubility.

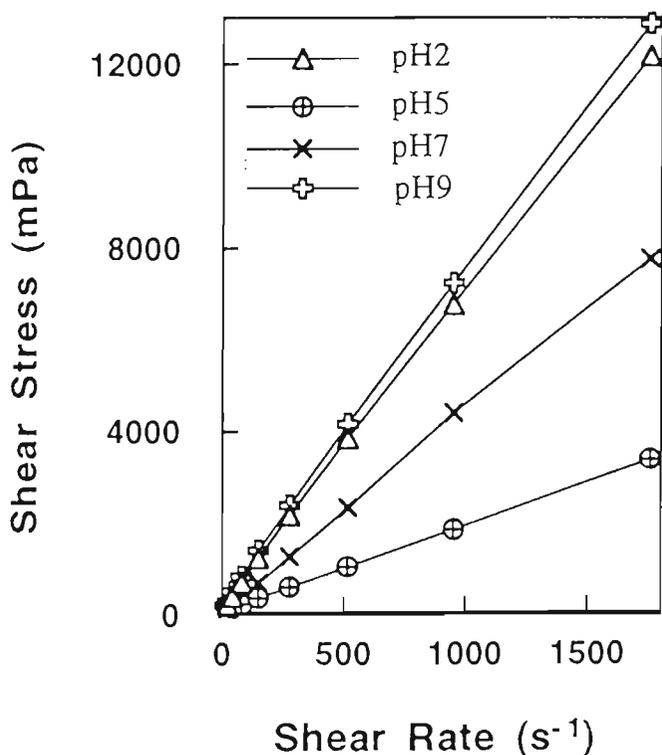


Fig 6.1.8 Flow curves of chickpea protein dispersions at pH 2, pH 5, pH 7 and pH 9.

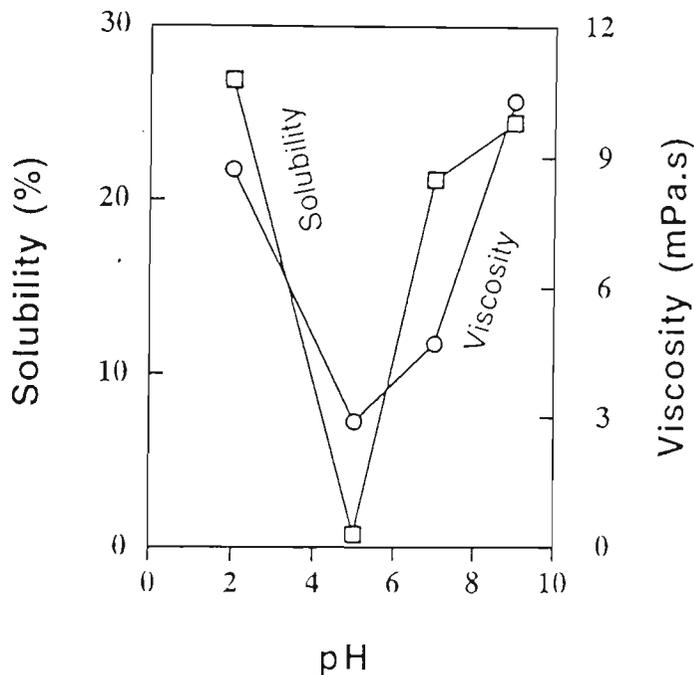


Fig 6.1.9 Solubility and apparent viscosity of chickpea protein dispersions measured at four different pHs.

6.1.5 EFFECT OF PROTEIN DENATURING AGENTS

The effect of sodium dodecyl sulphate (SDS) and urea on the power law constants and apparent viscosity of chickpea protein dispersions are given in Table 6.1.5.

SDS has been used to improve the solubility of rapeseed, sunflower and soy protein (Nakai *et al.*, 1980; Arce *et al.*, 1991). The pronounced effect of 6-8M urea on the unfolding of soy protein was noted (Shibasaki *et al.*, 1969). In this study, urea treatment caused a decrease in the "n" value, increase in the "m", yield stress and apparent viscosity. The effect of 1% SDS

treatment on the flow characteristic of chickpea protein was much more pronounced. A dramatic decrease in n value, increase in m , yield stress and viscosity were observed. The flow behaviour was much more pseudoplastic. A similar result was obtained with oat globulin when treated with SDS and urea (Paulson and Tung, 1993). These investigators suggested that the marked increase in apparent viscosities of oat proteins was attributed to the unfolding effect of the protein denaturing agents, SDS and urea. Arce *et al.* (1991) found that the dispersability of soy protein concentrate could be improved by up to 65% with SDS addition. Consequently, the improved solubility of chickpea protein caused by SDS and urea did contribute to the change of flow behaviour through changes in the conformation by unfolding of the proteins.

Table 6.1.5 Effect of chemical modification (Urea/SDS) on the power law constants and apparent viscosities of 8% chickpea protein dispersions.⁽¹⁾

Sample	n ⁽²⁾	m ⁽³⁾ (mPa)	Casson yield stress (mPa)	Apparent viscosity (mPa.s)	
				81.4 sec ⁻¹	950 sec ⁻¹
Chickpea protein	0.99	4.70	0.17	4.70	4.60
6M urea treated*	0.84	39.5	24.5	17.3	13.9
1% SDS**	0.54	327	525	36.5	14.1

(1) Averages of four determinations of 8% protein dispersions at pH7 and 25°C

(2) n = flow behaviour index

(3) m = consistency coefficient

* 8% protein dispersion in 6M urea solution

** 8% protein dispersion in 1% SDS

6.1.6 CONCLUSION

Flow properties of aqueous chickpea protein dispersions are considerably influenced by a number of factors. Chickpea protein dispersions possessed Newtonian flow behaviour at concentration levels up to 4% by weight. At higher concentrations (>8%), non-Newtonian flow behaviour became more progressive with increasing concentration.

Its Newtonian flow behaviour remained intact in dilute salt solutions of only 0.2M NaCl. The strength in apparent viscosities of protein dispersions was affected by pH, following the order of pH 9, pH 2, pH 7 and pH 5, indicating the influence of the protein solubility at these pHs.

Within the investigated temperatures (15, 25, 35 and 55°C), all the power law constants were unchanged up to 35°C but the flow behaviour became non-Newtonian at 55°C. This suggested a formation of a protein network induced by thermal treatment.

Denaturation by urea and sodium dodecyl sulphate (SDS) increased the consistency index (m), Casson yield stress and apparent viscosities, and the flow became more pseudoplastic with a decrease in the flow index (n). These changes were attributed to the unfolding effect of the protein denaturing agents.

In conclusion, changes in flow behaviour of these protein dispersions resulted from the formation of a protein network, dissociation, ionic and hydrophobic interactions and particularly the solubility of chickpea proteins under different environmental conditions.

6.2 FLOW PROPERTIES OF ACETYLATED CHICKPEA PROTEINS

As briefly discussed in Chapter 5, functional properties of proteins can be chemically or enzymatically modified (Matheis and Whitaker, 1984; Campbell *et al.*, 1992). Acetylation has been used to improve several functional properties of some native proteins with low functionalities. By introducing acetyl groups to the protein molecules, acetylation improved the emulsifying capacity, emulsion stability, foam capacity and stability and water absorption in pea (Johnson and Brekke, 1983), soy (Kim and Rhee, 1989), winged bean (Narayana and Narasinga Rao, 1984) and rapeseed proteins (Gwiazda *et al.*, 1989).

The effect of acetylation on these functional properties varied with the degree of modification and also with different types of proteins. For example, acetylation could increase or decrease the interfacial tension at the oil/water interface of rapeseed protein dispersions, depending on the degree of modification (Schwenke *et al.*, 1991). Hydrophobicity of glycinin could increase at high degrees of acetylation but could also decrease at low degrees of acetylation (Kim and Rhee, 1989). These observations suggested composition and also conformation changes in the protein molecules at different levels due to modifications, leading to different changes in several functional properties of the acetylated proteins.

Acetylation, therefore, does not only improve functionality, tastes and appearances of some proteins for wider usage in the food industries (Rhama and Rao, 1983; Franzen and Kinsella, 1976a,b) but it also provides a means to more clearly understand their functional properties (Rhee, 1989). The effect of acetylation on the flow and other rheological properties of some

food proteins has been investigated (Ma, 1993). Acetylation caused an increase in the specific viscosity of glycinin since its globular conformation was expanded and denatured due to its chemical modification (Kim and Rhee, 1989). Yamauchi and co workers (1979) showed that highly acetylated glycinin underwent drastic conformational changes in which most of the modified proteins were polymerised or dissociated to smaller protein molecules. Their conformational changes affected their viscosities. Acetylation increased the viscosity of both low and high density lipoproteins of egg yolk (Tsutsui *et al.*, 1980). Viscosity behaviour of faba bean and field peas protein dispersions were also affected by the degree of acetylation (Schmidt *et al.*, 1986; Schmidt and Schmandke, 1987).

The rheological behaviour of native chickpea protein dispersions was investigated and presented in the previous section. This section reports on the flow properties of acetylated chickpea protein dispersions in an attempt to elucidate the effect of acetylation at different levels on the flow properties of protein dispersions.

6.2.1 EFFECT OF PROTEIN CONCENTRATION AND EXTENT OF ACETYLATION

The flow curves of 6% acetylated chickpea protein (ACP6) dispersions at different concentrations are given in Fig 6.2.1. The slope of these flow curves increases remarkably with increased concentrations. At 1% concentration, the flow curve was linear, indicating Newtonian behaviour. At higher concentrations (74%), the flow curves became non linear, showing a deviation from the Newtonian characteristics. The effect of protein concentration on the power law constants and apparent viscosities of the acetylated chickpea dispersions is illustrated in Table 6.2.1. For comparison, the previously published data on native chickpea protein (NCP) is also given.

The flow exhibited both shear thinning and thickening behaviour, depending on the protein concentration. At a very dilute concentration (1%), the flow index increased ($n=1.10$) while at higher concentrations (4-20%) the flow index decreased ($n=0.77-0.38$), indicating a great deviation from Newtonian behaviour. The pronounced increasing pseudo plasticity associated with increasing protein concentration of acetylated chickpea proteins was reflected by a drastic increase in the consistency coefficient (m), yield stress and apparent viscosities. Fig 6.2.2 shows the consistency coefficient on a semi logarithmic scale for acetylated chickpea proteins which was much higher than that of the native one at the same protein concentration. Apparent viscosities of acetylated chickpea proteins increased exponentially from 6.4 mPa.s to 1,298 mPa.s while those of native chickpea proteins only increased from 1.68 to 40.4 mPa.s when protein concentrations increased from 4 to 20%.

Schmidt and Schmandke (1987) reported a pronounced effect of acetylation on the apparent viscosities of field bean protein isolate. At a shear rate of 145.8 sec, the apparent viscosity of unmodified and 80% acetylated field bean protein isolate dispersion (16% concentration) was 50 mPa.s and 700 mPa.s, respectively. Similar results were also obtained with unmodified and acetylated *Vicia faba* bean protein isolates (Schmidt *et al.*, 1986).

Acetylation increased the solubility of rapeseed (Cho and Thompson, 1984), soy (Sung *et al.*, 1983; Franzen and Kinsella, 1976) beef protein (Eisele and Brekke, 1981) and also increased the apparent viscosity of beef heart myofibrillar (Eisele and Brekke, 1981), faba bean and field bean protein isolates (Schmidt and Schmandke, 1987; Schmidt *et al.*, 1986). Like other food proteins, the increase in apparent viscosity of the acetylated chickpea proteins was partly due to the increase in their solubility. The formation of smaller protein aggregates and the addition of acetyl groups, bounded to protein molecules by acetylation, could also promote protein-protein interactions, competing with the predominant protein solvent interactions. This resulted in an increase in apparent viscosity.

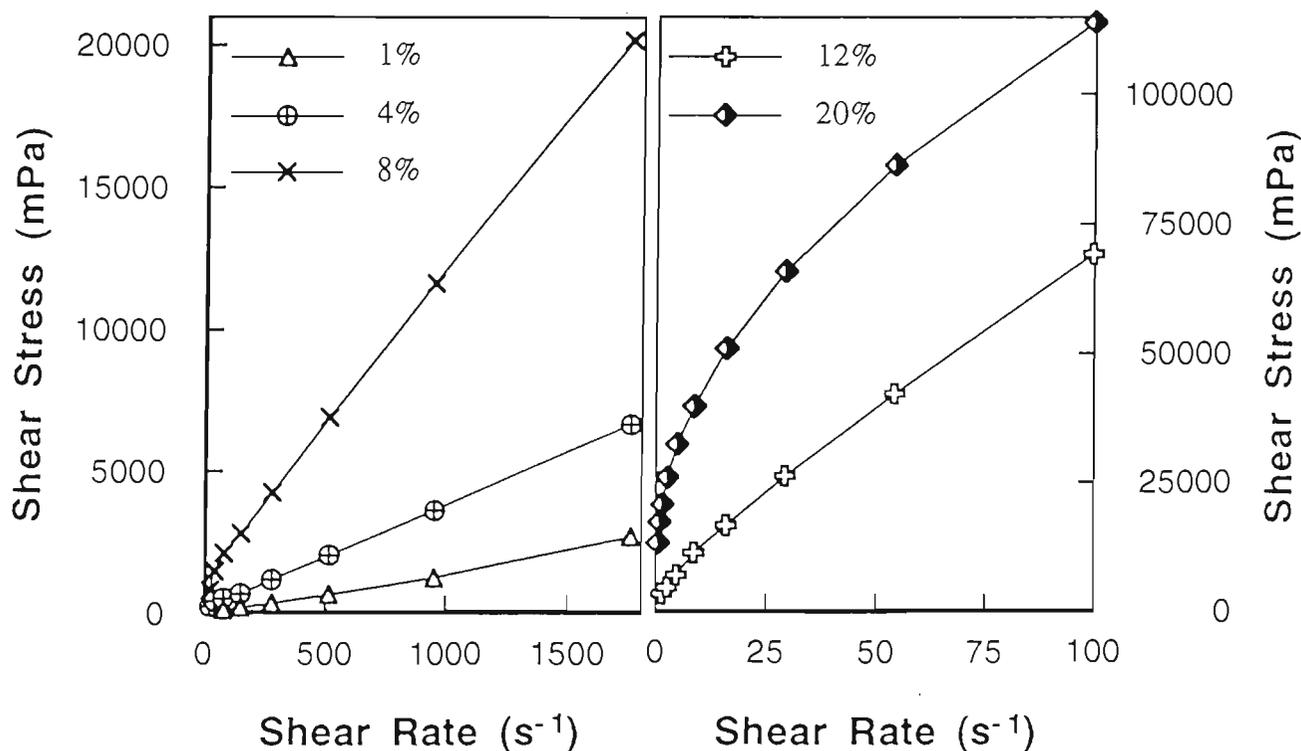


Fig 6.2.1 Flow curves of acetylated chickpea protein (ACP) dispersions at different concentrations (% based on dry weight), showing a great deviation from Newtonian behaviour.

Table 6.2.1 Effect of concentration (%) on the power law constants and apparent viscosities of acetylated chickpea protein (ACP) dispersions with various degrees of acetylation.⁽¹⁾

Degree of Acetylation ⁽²⁾ (%)	Concentration (%)	n ⁽³⁾	m ⁽⁴⁾ (mPa)	Casson yield stress (mPa)	Apparent viscosity (mPa.s)	
					81.4 sec ⁻¹	950 sec ⁻¹
0 (NCP)	2	1.00	1.06	0.00	1.33	1.31
	4	0.99	1.85	0.02	1.68	1.85
	8	0.99	4.68	0.17	4.75	4.65
	12	0.94	13.3	3.44	10.6	9.13
	20	0.85	82.4	80.2	40.4	31.6
5.8 (ACP6)	1	1.10	0.60	0.00	0.90	1.10
	4	0.77	18.0	40.9	6.40	3.80
	8	0.71	88.1	211	25.8	12.2
	12	0.69	2,598	1,264	743	ND ⁽⁵⁾
	20	0.38	18,586	14,633	1,298	ND
44.6 (ACP45)	8	0.60	378	671	57.9	26.9
49 (ACP49)	8	0.65	172	353	31.8	16.0

- (1) Averages of 4 determinations of acetylated protein dispersions at 25°C and pH7.
(2) Degree of acetylation based on the binding ratio of lysine ε-amino and acetic anhydride.
(3) n = flow index
(4) m = consistency coefficient
(5) ND = unable to determine

The effect of various levels of acetylation on the functional properties of food proteins has been widely reported (Cho and Thompson, 1984; Franzen and Kinsella, 1976). To obtain an apparent viscosity of 500 mPa.s at a shear rate of 145.8 sec⁻¹ required a neutral aqueous suspension of 27% unmodified field bean protein but only a 12% acetylated protein concentration. In this study, protein dispersions at the same level of protein concentration (8%) showed that the apparent viscosity increased up to 44.6% acetylation (ACP45), then decreased at 49% acetylation (ACP49). At 49% acetylation, not only the apparent viscosity but also the consistency coefficient (m) and Casson yield stress decreased.

These results suggested a conformational change in which the chickpea protein that associated at low acetylation levels (<45%) dissociated between the 45% and 49% of acetylation level. This formed a less viscous dispersion, and a decreased apparent viscosity, Casson yield stress and an increase in the flow index.

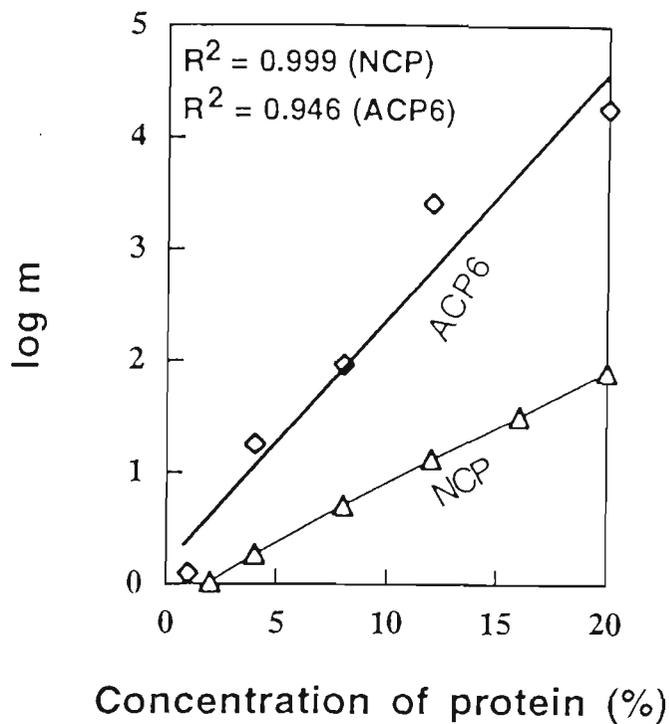
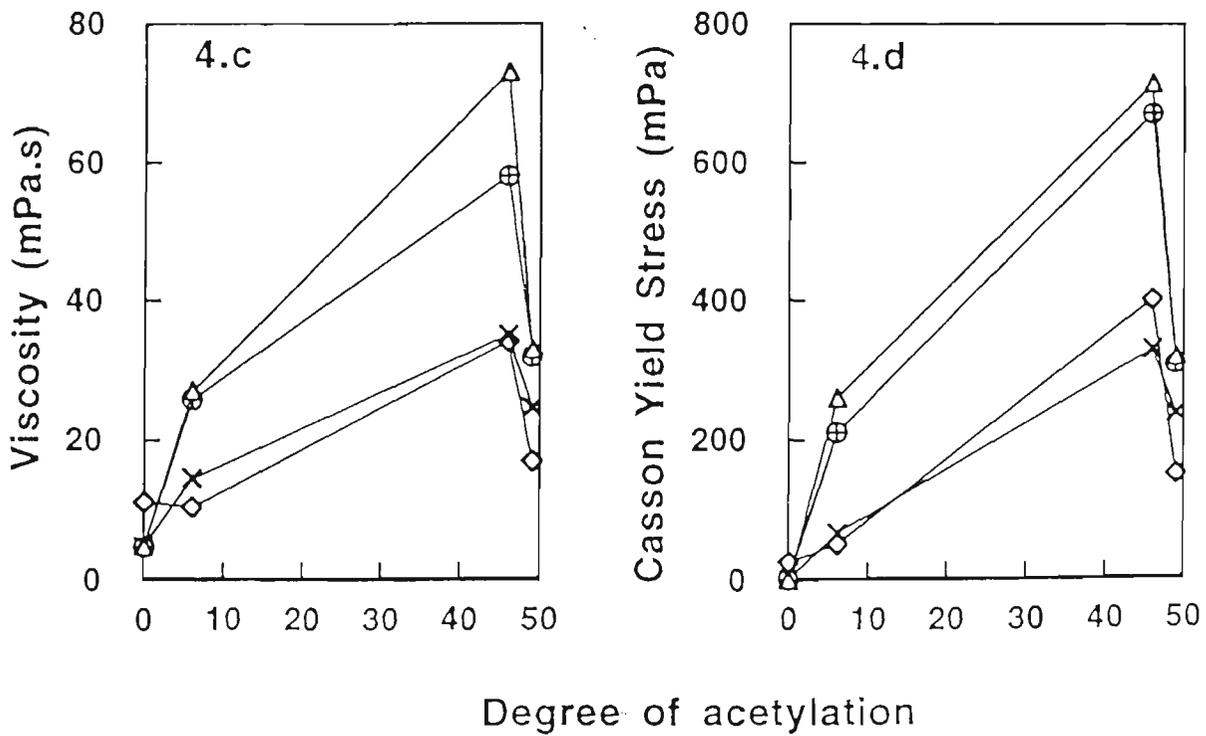
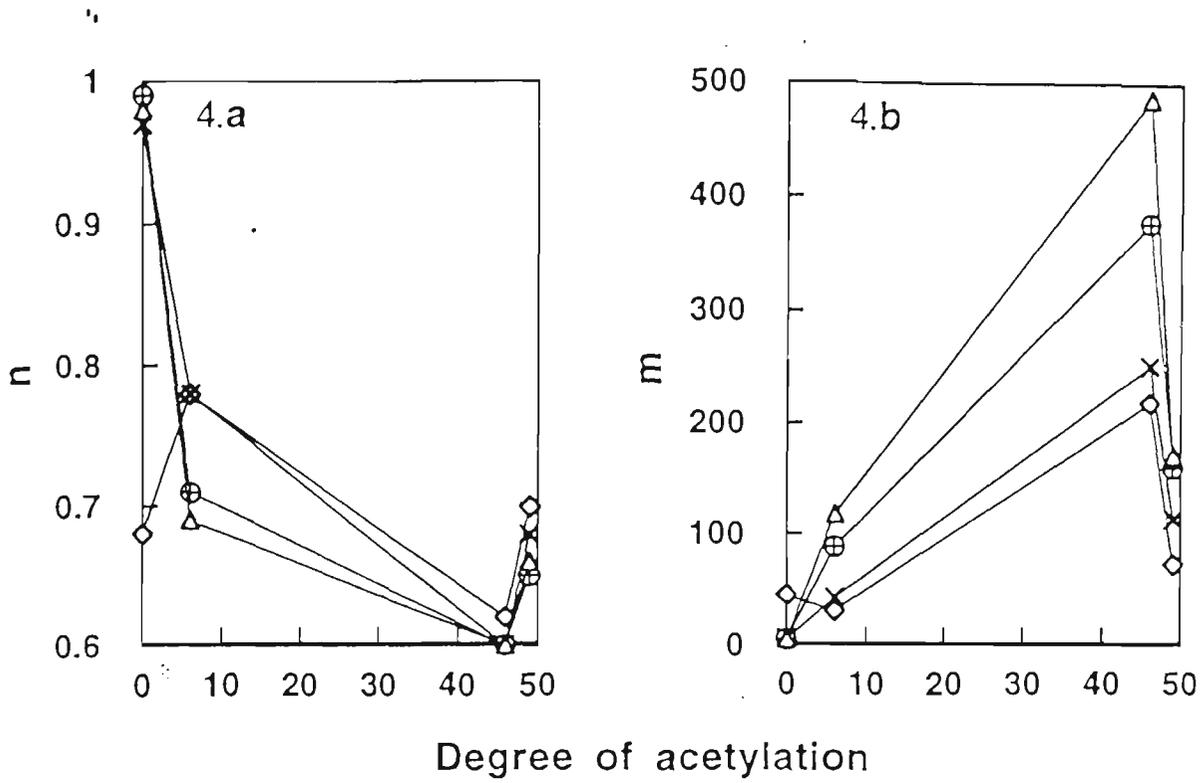


Fig 6.2.2 An index of viscosities, m , is a function of protein concentration on a semilogarithmic scale. This reflects a significant difference in the apparent viscosity of unmodified and acetylated chickpea protein dispersions, measured at the same level of protein concentration.

6.2.2 EFFECT OF TEMPERATURE

The effects of temperature on the power law constants (n , m), Casson yield stress and the apparent viscosity of protein dispersions prepared from native chickpea protein and its acetylated derivatives at several levels are given in Fig 6.2.3.a-d. For native chickpea protein dispersions, the thermal effect up to 35°C was insignificant but the consistency, yield stress and apparent viscosity increased at 55°C. The flow index remarkably reduced ($n=0.68$), indicating a great deviation from the Newtonian behaviour. In contrast, chickpea dispersions at all acetylated levels showed a gradual decrease in consistency coefficient, Casson yield stress and apparent viscosity, with increasing temperature.

The flow index of each acetylated chickpea protein dispersions was affected differently by a similar thermal treatment. The flow index (n) of the moderately acetylated ACP6 and the



—△— 15°C —⊕— 25°C —×— 35°C —◇— 55°C

Fig 6.2.3 a-d Thermal effects on the flow index (n) [a], consistency coefficient (m) [b], apparent viscosities [c] and Casson yield stress [d] of acetylated chickpea protein dispersions. Each acetylated chickpea protein dispersions was affected differently by a similar thermal treatment.

exhaustively acetylated ACP49 increased ($n=0.69-0.78$; $n=0.66-0.70$, respectively) with increasing temperature (15 to 55°C). That of ACP45 was almost constant ($n=0.60$). The flow index of acetylated chickpea proteins in this study seems to be a function of acetylation extent (Fig 6.2.3.a). Regardless of treated temperatures, ACP45 always exhibited the lowest flow index. This observation indicated that the native chickpea proteins could be associated to form large units up to 44.5% level of acetylation, but at higher levels of acetylation the associated proteins could be gradually dissociated, resulting in an increase in the flow index.

All acetylated chickpea protein dispersions showed a decrease in the consistency coefficient (Fig 6.2.3.b), Casson yield stress (Fig 6.2.3.d) and their subsequent apparent viscosities (Fig 6.2.3.c), with those of ACP6 and ACP49 being much lower than that of ACP45. Apparent viscosities of all acetylated chickpea proteins decreased with increasing temperatures (Fig 6.2.3.c). Similar observations were reported with gluten, gluten methyl ester (Mita and Matsumoto, 1980), egg albumen (Tung *et al.*, 1971) and succinylated chickpea proteins (Liu and Hung, 1995). However, Howell and Lawrie (1987) found that the viscosity of blood plasma proteins increased remarkably when the temperature increased from 20 to 76°C.

Apparent viscosities of faba bean proteins and field pea proteins increased exponentially with an increase in temperature and the degree of acetylation (Schmidt *et al.*, 1986, 1987). Flow behaviour of these proteins indicated that thermal treatments do not uniformly affect their consistency coefficient and also their apparent viscosities. Protein type and the level of chemical modification influenced their flow characteristics. Viscosity changes in the partially acetylated protein isolates from pollack muscle were almost identical to those of unacetylated isolates (Pavlova *et al.*, 1991).

Viscosity changes under the same thermal treatment in this study reflected the effect of degree of acetylation. High viscosities of ACP45 over a wide range of temperatures followed by lower viscosities of ACP49 once again suggested a maximum for the acetylated state. Above it, the associated proteins were dissociated. In the present study, the different behaviour of acetylated chickpea protein dispersions under thermal treatments indicated the great effect of acetylation on these protein conformations and their viscosities. As seen with emulsifying properties (Voutsinas *et al.*, 1983; Zhu and Damodaran, 1994), flow properties are remarkably influenced by the type of protein.

6.2.3 EFFECT OF SALT CONCENTRATION

The effect of adding salt on the power law constants and apparent viscosities of native and acetylated chickpea protein dispersions is illustrated in Table 6.2.2. Salt addition affects

native and modified protein dispersions differently. The flow index of native protein dispersions decreased with increasing ionic strength up to 1 M and then increased slightly. For slightly and extensively acetylated (ACP6 and ACP49) chickpea proteins, the flow index increased at dilute salt concentrations (0.2M), indicating an increasing Newtonian behaviour but decreased at higher concentrations (1.0M) while the flow index of ACP45 still increased at 1M salt concentration. Generally, it is expected that salt addition would produce a similar effect on power law constants and apparent viscosities of native and slightly acetylated chickpea proteins. The remarkable effect of salt addition, even at very dilute concentrations (0.2M), is reflected in a drastic reduction in the consistency coefficient (m), Casson yield stress and apparent viscosities of all acetylated chickpea protein dispersions. The salt effect was most profound with ACP45.

Table 6.2.2 Effect of salt concentration (NaCl/M) on the power law constants and apparent viscosities of 8% acetylated chickpea protein dispersions.⁽¹⁾

Degree of acetylation (2) (%)	Concentration (NaCl/M)	n ⁽³⁾	m ⁽⁴⁾ (mPa)	Casson yield stress (mPa)	Apparent viscosity (mPa.s)	
					81.4sec ⁻¹	950 sec ⁻¹
0 (NCP)	0	0.99	4.68	0.17	4.75	4.65
	0.2	0.98	3.02	0.05	1.71	0.21
	0.5	0.84	9.33	0.25	1.87	0.22
	1.0	0.73	22.1	55.6	1.98	0.22
	2.0	0.78	16.2	36.3	1.95	0.22
5.8 (ACP6)	0	0.71	85.1	274	25.8	12.2
	0.2	0.98	4.80	0.58	4.40	4.10
	0.5	0.94	7.60	2.90	5.80	5.20
	1.0	0.85	11.8	12.4	5.40	4.40
	2.0	0.89	8.10	4.20	4.20	3.90
44.6 (ACP45)	0.0	0.62	331	800	57.9	26.9
	0.2	0.73	34.7	59.0	8.60	6.10
	1.0	0.77	18.6	20.6	5.48	4.45
49 (ACP49)	0.0	0.68	138	326	31.9	16.0
	0.2	0.83	15.2	19.2	6.40	5.10
	1.0	0.77	20.0	58.0	7.20	4.00

(1) Averages of 4 measurements of 8% protein dispersions at pH 7 and 25°C.

(2) Degree of acetylation based on binding ratio of lysine ε-amino group and acetic anhydride.

(3) n = flow index

(4) m = consistency coefficient

(5) ND = samples were taken unable to determine

Flow properties of protein dispersions can be markedly influenced by salt concentrations. Urbanski *et al.* (1982) found that salt addition increased the flow index and decreased the consistency coefficients of soy proteins. Similarly, Hermansson (1975) found that the apparent viscosities of soy isolate dispersions decreased with increasing salt concentrations but had little effect on whey proteins. The consistency coefficient of succinylated fish and chickpea proteins was reduced with salt addition (Groninger, 1973).

Like all succinylated chickpea proteins, the consistency and apparent viscosities of acetylated chickpea proteins were reduced with salt addition, even at very dilute concentrations. Without salt addition, all the water in the dispersion is available for protein association and a strong network can be formed, reflected by a high consistency coefficient and Casson yield stress. When salt is added, the free water in the system becomes less available for water protein interactions because sodium chloride has a greater affinity for water. More water interacts with sodium chloride than with proteins and the flow becomes more Newtonian (n closer to 1). In a dispersion, water in the system tends to associate with small molecular weight solutes such as salt or sugar, thus the gel system becomes weaker and the viscosity of the dispersion reduces.

6.2.4 CONCLUSION

Like native protein, flow properties of acetylated protein dispersions are influenced by a number of environmental conditions and particularly by the degree of acetylation. Compared with the native proteins, acetylated chickpea protein dispersions exhibit a much stronger pseudo plastic behaviour. This is reflected in some compositional and conformational changes due to acetylation.

Salt addition reduces markedly its yield stress, consistency coefficient and apparent viscosities.

The effect of thermal treatment on the flow behaviour was influenced by the degree of acetylation.

The changes in flow properties of acetylated chickpea proteins such as the flow index (n), consistency coefficient (m), Casson yield stress and apparent viscosities suggests that chickpea proteins were associated up to 45% acetylation but the associated chickpea proteins gradually dissociated to smaller units at higher levels of acetylation.

6.3 FLOW PROPERTIES OF SUCCINYLATED CHICKPEA PROTEIN DISPERSIONS

The effects of acetylation on the flow properties of chickpea protein isolates have been presented in Section (6.2). The results clearly indicated that altering the protein structure by means of chemical modifications lead to many changes in their flow properties. Among various modification approaches, succinylation is normally found to influence functional properties of the modified protein more greatly than acetylation (Chapter 5).

Several functional properties of peanut, egg, fish, soy, casein, canola proteins were improved by succinylation (Beuchat, 1977; Sato and Nakamura, 1977; Chen *et al.*, 1975; Strange *et al.*, 1993; Paulson and Tung, 1988). Gandhi *et al.* (1968) altered the heat coagulation properties of egg white and Evans and Irons (1970) improved emulsifying properties of egg yolk by succinylation. Nakai and Li Chan (1989) reported that succinylation increased aqueous solubility, modified surfactant properties such as emulsifying and foaming and altered the viscosity of milk proteins. McElwain *et al.* (1975) found that the emulsion of a succinylated protein isolate from a single cell protein had a higher viscosity than that of a native single cell protein.

The apparent viscosity of modified canola was affected by the extent of succinylation. Succinylation of oat globulin protein markedly reduced apparent viscosity (Paulson and Tung, 1988) The pronounced effects of acetylation on the flow properties of chickpea proteins have been discussed. However, the introduction of neutral acetyl groups to the protein molecules by acetylation only neutralised some normally protonated groups.

Succinylation not only increase the net negative charge of the protein but also can convert the net positively charged molecule to a net negatively charged molecule. This dramatic change results in many changes in the functional properties of food proteins. It is likely that succinylation would give some more pronounced effects on the flow properties of the modified proteins. A number of products prepared with chickpea are fluid foods such as pastes or beverages (Fernandez and Berry, 1987). Flow properties of chickpea proteins may be modified by succinylation to meet the requirements of a specific food, once the relationship between flow properties and environmental conditions is defined.

This section reports on the flow properties of succinylated chickpea protein dispersions under various environmental conditions. The effect of succinylation at different levels was compared with those of acetylated and unmodified proteins.

6.3.1 EFFECT OF PROTEIN CONCENTRATION AND EXTENT OF SUCCINYLATION ON FLOW PROPERTIES

The flow curves of 16% succinylated chickpea protein (SCP16) dispersions at different concentrations are given in Fig 6.3.1 and the effect of concentration on the power law constants and apparent viscosity of various succinylated chickpea protein dispersions is illustrated in Table 6.3.1. For comparison the previous published data on native chickpea protein (NCP) is also given.

Table 6.3.1 Effect of protein concentration (%) on the power law constants and apparent viscosities of succinylated chickpea protein (SCP) dispersions at various degrees of succinylation.⁽¹⁾

Degree of Succinylation ⁽²⁾ %	Concentration (%)	n ⁽³⁾	m ⁽⁴⁾ (mPa)	Casson Yield Stress (mPa)	Apparent	Viscosity
					(mPa.s)	
					81.4 sec ⁻¹	950 sec ⁻¹
0 (NCP)	2	1.00	1.06	0.00	1.33	1.31
	4	0.99	1.85	0.02	1.68	1.85
	8	0.98	4.68	0.17	4.75	4.65
	12	0.94	13.3	3.44	10.6	9.13
	20	0.85	82.4	80.2	40.4	31.6
16 (SCP16)	1	1.02	1.40	0.00	1.50	1.50
	4	0.84	26.9	41.0	13.5	9.70
	8	0.57	739	810	98.2	51.1
	12	0.36	9,945	7,171	602	ND ⁽⁵⁾
	20	0.32	87,902	31,022	4,560	ND
73 (SCP73)	8	0.30	9,582	6,308	514	ND
79 (SCP79)	8	0.36	8,627	7,185	580	ND

(1) Averages of four determinations at pH7 and 25°C.

(2) Degree of succinylation was based on binding ratio of lysine ε-amino group and succinic anhydride.

(3) n = flow behaviour index

(4) m = consistency coefficient

(5) Viscosities are beyond the measurement capacity.

At a very dilute concentration (1%), the flow curve was linear whereas at higher concentrations the flow curves exhibited non-Newtonian behaviour. From a 4%

concentration, the non-Newtonian behaviour became more pronounced with increasing concentrations. Compared with unmodified chickpea proteins, the flow behaviour index (n : 0.84-0.32) decreased remarkably while the yield stress and the consistency coefficient (m) increased exponentially (26.9 to 87,902 mPa and 41 to 31,022 mPa, respectively).

The consistency coefficient of succinylated chickpea protein dispersions is a function of protein concentrations on a semilogarithmic scale (Fig 6.3.2 a, b). Succinylated chickpea protein dispersions showed a much stronger pseudo plastic tendency than those of native and acetylated proteins. The apparent viscosities also increased exponentially with increasing concentrations. At the low shear rate (81 sec^{-1}) the apparent viscosity increased from 13 to 4,560 mPa.s as the concentration increased from 4 to 20%. At the high shear rate (950 sec^{-1}) the viscosities were beyond the measurement capacity of the Rheomat. This indicated that a very strong network was formed, during shear thickening.

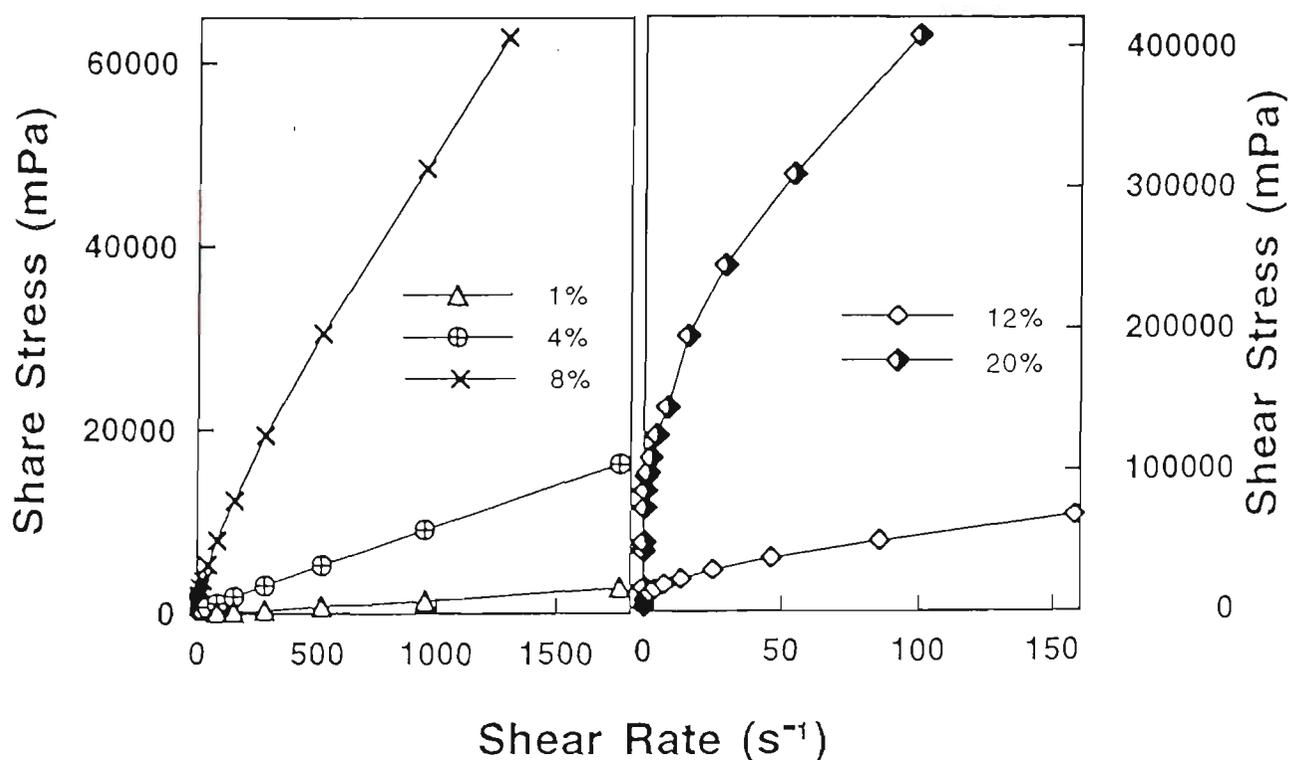


Fig 6.3.1 Flow curves of succinylated chickpea protein (SCP) dispersions at different concentrations (% based on dry weight), showing a great deviation from Newtonian behaviour.

Succinylation introduced succinyl carboxyl groups which covalently bind to the protein molecules. These carboxyl groups contributed varying negative charges to a protein, thus affected its physicochemical properties, shifting the isoelectric point to a lower pH, increasing electrostatic forces and the solubility of the protein. The increase in net negative charge and solubility of the succinylated chickpea protein contributes partly to the drastic increase in viscosity observed in this study. Also, the introduction of the bulky succinyl residues could increase the hydrodynamic volume, contributing to the increase in viscosity.

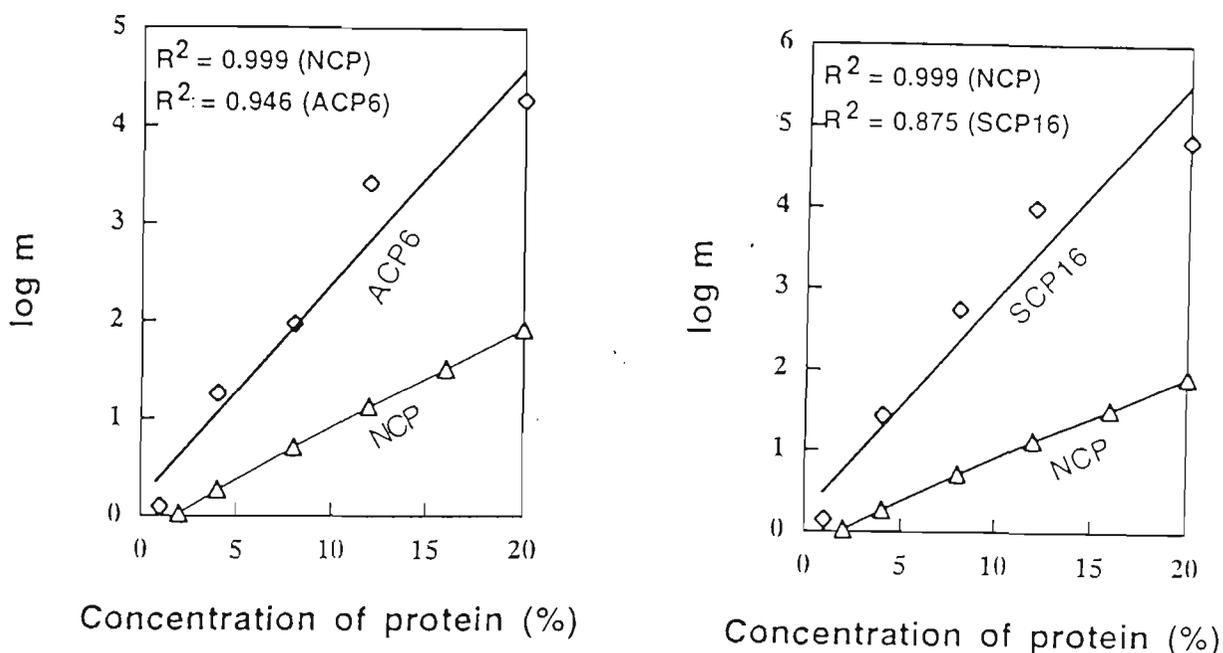


Fig 6.3.2 (a-b) Correlation of protein (%) and consistency coefficient (m) of native (NCP), acetylated (ACP) and succinylated (SCP) chickpea protein dispersions. Succinylation increased the apparent viscosities of chickpea protein dispersions.

The effect of the succinylation level on the flow properties of chickpea proteins were examined using 8% concentration dispersions of native and 16%, 73% and 79% succinylated proteins (NCP, SCP16, SCP73 and SCP79). At this concentration, the dispersion of a native chickpea protein exhibited an almost Newtonian behaviour ($n=0.99$ and viscosities were unchanged at 81 and 950 sec^{-1} shear rates) while all the succinylated chickpea protein dispersions clearly exhibited non-Newtonian behaviour (Table 6.3.1). The degree of deviation from the Newtonian behaviour was more pronounced as the n values decreased ($n=0.57-0.36$) with increasing degrees of succinylation. There was no significant difference in

all the power law constants and apparent viscosities between SCP73 and SCP79, perhaps due to their similar level of succinylation.

The influence of succinylation on the viscosity of protein dispersions is complex since it varies with different proteins and also with different degrees of succinylation. Viscosity of faba bean isolates increases gradually with increasing degrees of succinylation whereas those of sunflower and rapeseed proteins stayed almost unchanged up to 60 % succinylation, then increased sharply (Schwenke *et al.*, 1990). This observation indicated that succinylation caused a continuous conformational change in faba bean proteins but a sudden conformational change in sunflower and rapeseed proteins. Succinylation of chickpea protein caused a gradual increase in viscosity with increasing degree of succinylation (Table 6.3.1) like field pea, faba bean and canola protein (Schwenke *et al.*, 1986; Paulson and Tung, 1988). The different effect of succinylation on the apparent viscosity of these proteins reflected their different conformational structures.

6.3.2 EFFECT OF SALT CONCENTRATION

The effect of salt addition on the power law constants and apparent viscosities of succinylated chickpea proteins is illustrated in Table 6.3.2. The flow behaviour index of unmodified (NCP) protein decreased with salt concentrations up to 1M then slightly increased while those of succinylated proteins (SCP16, 73 and 79) increased proportionally with increasing salt concentrations. The increase was more pronounced at a high level of succinylation. Both the consistency coefficient and yield stress of all succinylated chickpea proteins decreased by adding salt. The effect was very pronounced even at dilute concentrations. A 0.2M salt solution decreased the *m* value and Casson yield stress of SCP73 up to 20 and 5 fold, respectively.

Unlike succinylated chickpea proteins, the effect of ionic strength on the consistency coefficient and yield stress of unmodified proteins was rather complex. At 0.2M salt, both the *m* value and yield stress decreased, then increased up to 1M, followed by a slight decrease. Salt addition decreased the apparent viscosities of both unmodified and modified chickpea proteins, even at a dilute concentration (0.2M).

The effect of salt concentrations on the apparent viscosity (AV) at 81.4 sec^{-1} was modelled by linear regressions of AV and the logarithm of AV ($\log(\text{AV})$) on the salt concentration (SC) and degree of succinylation (DS), and their logarithms. The best fitting regression equation was:

$$\log(AV) = 0.324 - 0.756 \times \log(SC + 0.1) + 0.790 \times \log(DS + 1) \quad [6.3.1]$$

The addition of constants (0.1 and 1) to the salt concentration and degree of succinylation before taking logs was required to overcome the problem of calculating the logarithm of zero. This regression equation accounted for 90.3% of the variance in $\log(AV)$, and the coefficients of the log salt and log succinylation terms were significantly different from zero at the 0.1% level. Changes in the log of apparent viscosities, for the range of conditions investigated, can be predicted by an additive model in the logs of salt concentration and degree of succinylation.

Table 6.3.2 Effect of salt concentration (NaCl/M) on the power law constants and apparent viscosities of 8% succinylated chickpea protein dispersions.⁽¹⁾

Degree of Succinylation ⁽²⁾ (%)	Salt conc. NaCl (M)	n ⁽³⁾	m ⁽⁴⁾ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
					81.4 sec ⁻¹	950 sec ⁻¹
0 (NCP)	0	0.99	4.68	0.17	4.75	4.65
	0.2	0.98	3.02	0.05	1.71	0.21
	0.5	0.84	9.33	0.25	1.87	0.22
	1.0	0.73	22.1	55.6	1.98	0.22
	2.0	0.78	16.0	36.3	1.95	0.22
16 (SCP16)	0	0.57	739	810	98.2	51.1
	0.2	0.73	63.0	187	18.1	10.2
	0.5	0.75	45.0	118	14.5	8.00
	1.0	0.75	40.0	126	14.6	7.20
	2.0	0.79	26.0	53.0	9.60	6.40
73 (SCP73)	0.0	0.36	8,709	6,308	514	ND ⁽⁵⁾
	0.2	0.55	489	1,188	59.5	23.8
	1.0	0.61	123	315	19.8	9.10
79 (SCP79)	0.0	0.36	8,630	7,186	580	ND
	0.2	0.59	758	1,413	109	55.4
	1.0	0.63	167	378	29.2	13.9

- (1) Averages of four determinations of 8% protein dispersions at pH7 and 25°C.
- (2) Degree of succinylation was based on binding ratio of lysine ε-amino group and succinic anhydride.
- (3) n = flow behaviour index
- (4) m = consistency coefficient.
- (5) Viscosities are beyond the measurement capacity.

Flow properties of the protein dispersions were markedly affected by salt concentrations. Salt addition increased the flow behaviour index (n) and decreased the consistency coefficient (m) of soy proteins (Urbanski *et al.*, 1982). Hermansson (1975) found that the apparent viscosities of soy protein dispersions decreased with increasing salt concentrations but a reverse trend was observed with caseinate, while whey proteins showed little effect. Groninger (1973) found that salts reduced the consistency of succinylated fish protein dispersions.

There was a decrease in consistency and apparent viscosities of all succinylated chickpea proteins, caused by increasing salt concentrations, even at very low ionic strength. Without adding salt, all the water in the dispersions was free to associate with chickpea proteins and the pseudo plastic behaviour was pronounced. With salt, the free water became less because sodium chloride has a great affinity for water. More water then associates with salt than with proteins, and this increases the Newtonian behaviour (Glicksman, 1969). The water in the system associates more with low molecular weight solutes, such as salt or sugar; the gel system becomes weaker and the viscosities of the dispersions are reduced.

6.3.3 EFFECT OF TEMPERATURE

The effect of temperature on the power law constants and apparent viscosities of succinylated chickpea protein dispersions are given in Table 6.3.3. Up to 35°C, flow properties of native chickpea protein dispersions remained unchanged but their n values decreased sharply while m values, yield stress and viscosities increased at 55°C. Regardless of the different degrees of succinylation, within the range of temperatures investigated (15-55°C), the flow behaviour index of all succinylated protein dispersions decreased gradually with increasing temperatures and displayed greater pseudo plasticity. A similar observation was reported with egg albumen (Tung *et al.*, 1971).

The consistency coefficient, yield stress and apparent viscosities of all succinylated chickpea proteins were also reduced with increasing temperature. The effect of temperature on the apparent viscosity (AV) at 81.4 sec⁻¹ was modelled by linear regressions of AV, the logarithm of AV (log(AV)) on temperature (T), the degree of succinylation (DS), and their logarithms. The best fitting regression equation was judged to be:

$$AV = 175.8 - 5.186 T + 6.175 DS - 0.1215 (T-32.5) (DS-42) \quad [6.3.2]$$

The cross-product term was centred on the mean values of T and DS. This regression equation accounted for 98.9% of the variance in AV and all coefficients were significantly

different from zero at the 0.1% level. The following equation fitted the data almost as well as the previous equation:

$$\log(AV) = 2.971 - 0.351 \log(T) + 0.997 \log(DS + 1) \quad [6.3.3]$$

This regression equation accounted for 96.9% of the variance in $\log(AV)$. The coefficient of the log succinylation term was significantly different from zero at the 0.1% level while the coefficient of the log temperature term was significantly different from zero at the 6.3% level. Both models fit the data very well and it is difficult to recommend one at the expense of the

Table 6.3.3 Effect of temperature ($^{\circ}C$) on the power law constants and apparent viscosities of 8% succinylated chickpea protein dispersions.⁽¹⁾

Degree of Succinylation ⁽²⁾ (%)	Temperature ($^{\circ}C$)	n ⁽³⁾	m ⁽⁴⁾ (mPa)	Casson yield stress (mPa)	Apparent Viscosity (mPa.s)	
					81.4 sec ⁻¹	950 sec ⁻¹
0 (NCP)	15	0.98	5.13	0.22	4.82	4.70
	25	0.99	4.68	0.17	4.75	4.65
	35	0.97	5.50	1.09	4.90	4.43
	55	0.68	44.7	25.4	11.2	5.02
16 (SCP16)	15	0.59	1,267	1,137	179	85.8
	25	0.57	739	810	98.2	51.1
	35	0.56	729	773	89.3	43.1
	55	0.50	803	1,102	70.3	30.7
73 (SCP73)	15	0.37	10,230	6,408	590	ND ⁽⁵⁾
	25	0.36	8,709	6,308	514	-
	35	0.28	7,961	6,081	397	-
	55	0.26	5,495	3,806	246	-
79 (SCP79)	15	0.36	10,556	7,821	706	-
	25	0.37	8,627	7,186	580	-
	35	0.35	7,345	6,187	461	-
	55	0.31	5,729	4,435	309	-

- (1) Averages of four determinations of 8% protein dispersions at pH7.
- (2) Degree of succinylation was based on binding ratio of lysine ϵ -amino group and succinic anhydride.
- (3) n = flow behaviour index
- (4) m = consistency coefficient.
- (5) Viscosities are beyond the measurement capacity.

other on statistical grounds alone. The first model achieves its slightly better fit by requiring an extra coefficient corresponding to the cross-product term involving T and DS. The difference in predictions from the two models is not great. For example, predicted AV values from both models for a temperature of 45°C are given in Table 6.3.4.

These results were in contrast with those of Schmidt and co-workers (1986). They found that the apparent viscosities of field bean proteins and faba bean proteins increased exponentially with increasing temperature (30-100°C) and degree of acetylation (Schmidt and Schmandke, 1987; Schmidt *et al.*, 1986). When blood plasma proteins were heated from 20 to 76°C, the viscosity also increased greatly (Howell and Lawrie, 1987). However the apparent viscosity of 12% gluten and gluten methyl ester was decreased markedly when the temperature was raised from 20 to 50°C (Mita and Matsumoto, 1980). The apparent viscosity of the egg albumen was also decreased as the temperature increased (Tung *et al.*, 1971).

The different behaviour of protein dispersions observed in these investigations suggested that the thermal effect on the flow properties of the protein dispersions is not uniform and is remarkably influenced by the type of protein as seen with emulsifying properties (Voutsinas *et al.*, 1983; Zhu and Damodaran, 1994).

Table 6.3.4 Predicted apparent viscosities (AV) at 81.4sec⁻¹ for 45°C

Degree of Succinylation	Predicted Values		
	AV (Cross-Product Model)	AV (Log Model)	(log(AV))
0	6.3	5.1	(1.637)
16	80.8	86.6	(4.461)
73	346.2	375.4	(5.928)
79	374.1	405.5	(6.005)

6.3.4 CONCLUSION

The effects of succinylation on the flow properties of chickpea protein dispersions were investigated over a wide range of protein concentrations (1-20% by weight), temperatures (15-55°C) and salt concentrations (0.0-2.0M NaCl). The proteins were succinylated at 3 levels: 16, 73 and 79% modification of free amino group.

Compared with the native and acetylated chickpea proteins, the succinylated protein dispersions exhibited a stronger pseudoplastic behaviour. At the same protein concentration, the flow behaviour index (n) decreased while the consistency coefficient (m), yield stress and apparent viscosities (AV) were dramatically increased by the extent of succinylation.

At 81.4 sec^{-1} of a shear rate the consistency coefficient, yield stress and the apparent viscosities of succinylated protein dispersions increased exponentially with increasing protein concentrations.

Flow properties of native chickpea protein dispersions were virtually unchanged up to 35°C and were only slightly affected at 55°C . In contrast, for highly succinylated chickpea proteins (73-79%), the consistency coefficient, yield stress and apparent viscosities dramatically decreased with increasing temperatures, even at 25°C . The effect of temperature on flow properties of these proteins varied with the extent of succinylation. A model to predict the effect of increasing temperature on the apparent viscosity of succinylated proteins was suggested.

The flow behaviour index increased while the yield stress, consistency coefficient and apparent viscosity decreased with increasing salt concentrations and also with the extent of chemical modification.

6.4 SUMMARY

Flow properties of aqueous unmodified, acetylated and succinylated chickpea protein dispersions are considerably influenced by a number of factors. These flows have some common characteristics.

At low protein concentrations (<4% by weight), chickpea protein dispersions exhibited Newtonian flow behaviour. At high concentrations (>8% by weight), non-Newtonian flow behaviour became more pronounced with increasing concentration. The extent of non-Newtonian increased as one moved from an unmodified protein system to acetylated and succinylated proteins.

The effect of salt addition on the flow behaviour varied with salt concentration, with different type of proteins and also with different levels of acylation. The Newtonian flow behaviour of unmodified chickpea protein dispersions remained intact only in a dilute salt solution (0.2M NaCl). The flow behaviour index of the unmodified protein decreased with salt

concentrations up to 1 M then increased, whilst, those of succinylated proteins increased proportionally with increasing salt concentrations. The effect of salt addition on the flow behaviour index of acetylated proteins varied greatly with the level of acetylation. The remarkable effect of salt addition on the consistency coefficient (m), Casson yield stress and apparent viscosity reflected the protein structural changes due to their chemical modification by acylation.

The effect of dissolving pHs reflected a correlation between protein solubilities and the apparent viscosities of the dispersions.

Thermal effects on the flow properties of the protein dispersions varied with different types of proteins and the levels of acylation. For unmodified proteins, the effects of thermal treatments were demonstrated at 55°C. For acetylated proteins, the flow index of each protein dispersion was affected differently by a similar thermal treatment. Apparent viscosities of unmodified protein dispersions increased but those of all acetylated protein dispersions decreased with increasing temperature. The apparent viscosities of succinylated proteins increased at 55°C. A regression equation was suggested to illustrate the effect of temperature on the apparent viscosities and degrees of succinylation.

Chemical modifications by acetylation and succinylation greatly influenced many aspects of the flow properties of the modified chickpea proteins. The effects varied with the type of acylating reagents used and the levels of acylation.

CHAPTER 7

RHEOLOGICAL PROPERTIES OF WHEAT FLOUR SUPPLEMENTED WITH CHICKPEA PROTEINS

'RHEOLOGICAL PROPERTIES OF WHEAT FLOUR SUPPLEMENTED WITH CHICKPEA PROTEINS

Numerous products can be made from wheat flours. These include breads, biscuits, cakes, pasta, noodles, breakfast cereals and other bakery products that are consumed worldwide. In Western countries, the annual consumption of various bakery products is estimated at 100 kg per capita (Pomeranz, 1988). More wheat products are also consumed in developing countries (Esuono and Bamiro, 1995). Wheat protein quality is lower than that of most cereals due to its low levels of lysine, methionine and threonine (Pomeranz, 1988). Regardless of these inferior nutritional values, demand for wheat based bakery products is increasing. The nutritional quality of these products could be improved by supplementation with non wheat proteins including those from grain legumes. Fortification with grain legume protein would increase the protein content and improve the essential amino acid balance of the bakery products. On the other hand, bakery products which have long shelf-life are easy to transport and have wide acceptance. They can serve as an effective means of promoting the utilisation of grain legume proteins for human consumption (Chavan and Kadam, 1993).

The reason for the large number and diversity of products made from wheat flour is attributed to its unique characteristics which can be demonstrated clearly by a bread making process. When mixing with water, wheat flour forms a cohesive dough with viscoelastic properties whereas other cereal flours fail to do so. The wheat flour dough, once formed, can retain gas which is an essential condition for the formation of light, leavened products. Another characteristic of wheat flour dough is its ability to transform to bread in an oven, by dramatic changes in viscosity, while retaining its shape.

The rheological properties of wheat flour dough are very complex (Matsuo *et al.*, 1972; Honesty and Rogers, 1990). They are, in fact, a result of ingredient interactions during fermentation and dough mixing. Flour, water, air and yeasts have great effects on the rheological properties of the dough formed but other minor ingredients such as fats, enzymes and emulsifiers also influence the dough's properties. The quality of bread is strongly influenced by the level and quality of protein in the dough (Dexter and Matsuo, 1977; Wasik and Bushuk, 1975). Protein is the major factor that accounts for the variation in bread's loaf volume, made from a single variety of wheat (Pomeranz *et al.*, 1976). Consequently, most studies have focussed on the effect of wheat proteins on the viscoelastic properties of wheat dough. It is believed that the viscous behaviour of wheat dough is influenced by small

molecular weight wheat protein, whereas the elastic component of wheat dough is affected by larger molecular weight wheat proteins (Andrews, 1994; Fullington *et al.*, 1987).

Information on the rheological properties of a dough will be useful for predicting the potential application of the wheat flour and also the quality of the end product. Since protein has a major role in the quality of bread, supplementation of wheat dough with chickpea or other grain legume flours and proteins certainly affects rheological properties of the fortified wheat flour dough and its subsequent finished products (Eliasson, 1990; Sathe *et al.*, 1981; Singh *et al.*, 1980). These effects can be measured by using physical dough testing devices to evaluate the bread making potential and performance characteristics of the fortified flour.

7.1 RHEOLOGICAL PROPERTIES OF DOUGH

In order to estimate the effects of supplementation of chickpea proteins on the quality of bread, the basic rheological properties of a dough, prepared from wheat flour, at different stages during the bread making process, need to be discussed. A bread making process consists of three major stages: dough mixing, dough fermentation - machining and finally, oven crumb staling. At each stage, rheological properties of a dough varies and can be measured by different devices (Fig 7.1.1).

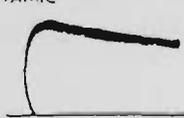
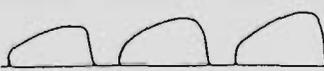
I PHASE DOUGH MIXING	II PHASE FERMENTATION + MACHINING	III PHASE OVEN CRUMB STALING
FARINOGRAPH/RESISTOGRAPH [®] DYNAMIC	EXTENSOGRAF STATIC	AMYLOGRAF DYNAMIC
FARINOGRAM 	EXTENSOGRAM 	AMYLOGRAM 
RESISTOGRAMM [®] 	RAPID EXTENSOGRAF 	RAPID AMYLO THRESHOLDTEST 
1) ABSORPTION 2) MIXING TIME 3) MIXING TOLERANCE 4) OPTIMUM	1) ENERGY 2) EXTENSIBILITY 3) $\frac{\text{RESISTENZ}}{\text{EXTENSIBILITY}} = \text{DEGREE OF MATURING}$ 4) MAXIMUM	1) CRUMB FORMATION 2) CRUMB CHARACTERISTICS 3) GELATINIZATION PROPERTIES 4) FRESHNESS OF BREAD

Fig 7.1.1 Three major stages in a bread making process: dough mixing, fermentation and oven crumb staling and the associated physical devices to examine dough rheological properties at each stage.

7.1.1 MIXING STAGE

The mixing stage consists of several important steps in which the rheological properties of a dough could be changed significantly. Firstly, it involves hydration of flour particles. Then as the wheat protein becomes hydrated, it forms fibrils as a matrix by the repeated shearing action of the mixer. A dough shows an increasing resistance to extension and reaches a maximum resistant level before its breakdown. These characteristics of a dough during this process can be measured by a Brabender farinograph. This instrument gives empirical information, illustrated on a farinogram, about mixing properties of a flour, which are reflected by the resistance of the dough to the mixing blades over a mixing time (Fig 7.1.2).

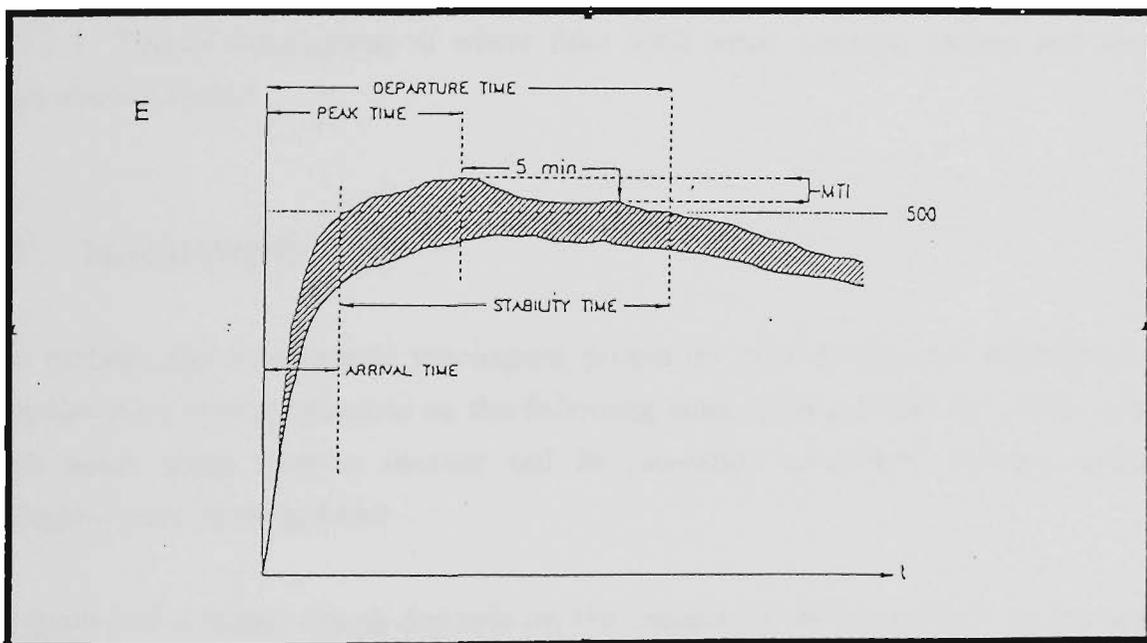


Fig 7.1.2 A farinogram of wheat dough and associated measurements: arrival time, peak time, departure time, stability time and mixing tolerance index.

A farinogram shows the amount of water required by a dough to reach its chosen consistency as recorded at 500 BU (Brabender Unit) level [*water absorption*], the time to reach its optimum mix [*peak time*] and the tolerance of a flour to over mixing by its *dough stability*, *mixing tolerance index* and *departure time*. These parameters are affected by the nature of the flour. Some flours produce doughs with strong stabilities while other flours produce weak doughs which can fall-off easier (Fig 7.1.3). The peak time correlates with the dough mixing requirement of a particular flour and the stability time can be used as an indicator for dough strength. Thus the information taken from a farinogram will show the flour strength in the process and its baking quality in the finished product.

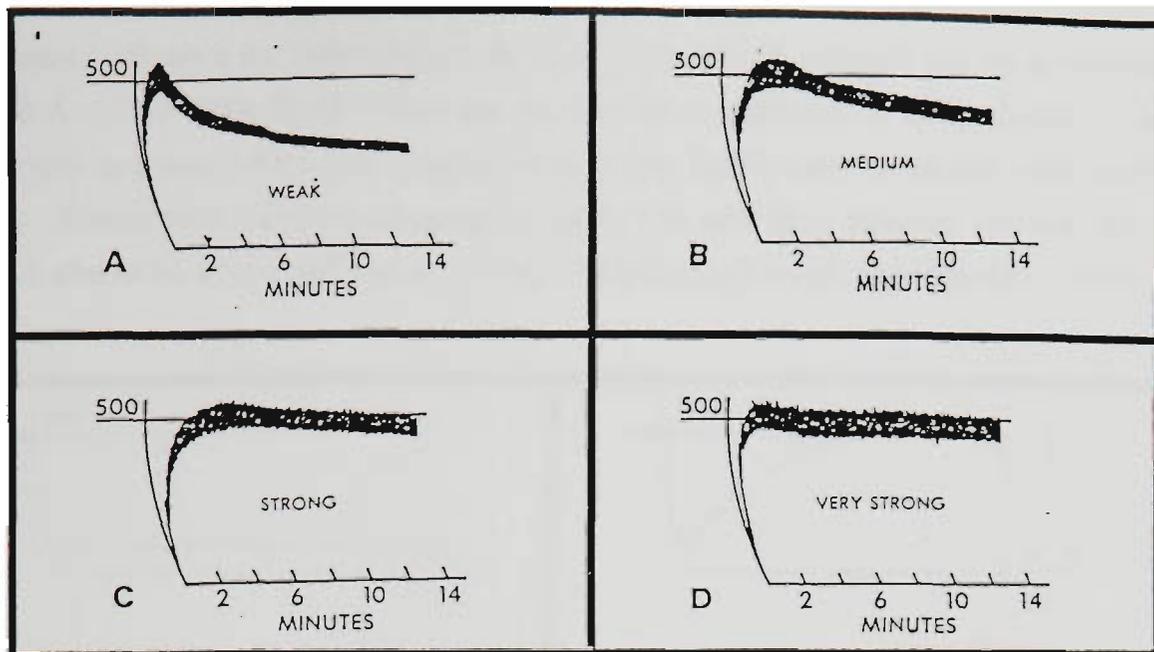


Fig 7.1.3 Typical farinograms of wheat flour with weak, medium, strong and very strong dough characteristics

7.1.2 MACHINING

After mixing, the fundamental rheological properties of a dough are established. These properties have strong influence on the following steps of bread making. After mixing, the dough needs some time to recover and its relaxation properties strongly influence the rheological state of the product.

The quality of a mixed dough depends on the balance of its viscoelastic properties for the sheeting and moulding steps. If too viscous, a dough cannot retain its desired final shape. However, if too elastic, it is difficult to form and is also hard for the dough to retain the desired shape. An excessively elastic dough will give a low volume loaf with a shape closer to the initially moulded dough. A too viscous dough will give a final product with sharp edges and flat top (Spies, 1990). Consequently the information about its rheological properties at the machining process will help to predict the final shape of the bread. This information can be obtained by the Brabender extensigraph, an instrument designed to measure stress-strain relationships of a mixed dough. An extensigram from the Brabender extensigraph provides information on the resistance to stretching and extensibility of a dough (Fig 7.1.4).

The most commonly recorded parameters include the *maximum resistance* (R_m) in extensigraph unit, *extensibility* (E , total curve length, cm), *the ratio of maximum resistance to extensibility*, called the *viscoelastic ratio* (R_m/E) and the *area under the curve* (A , cm^2). The elastic properties of a dough will affect its resistance to extension while the viscous

components influence its extensibility. In most cases, dough strength can be predicted with R_m and A values while R_m/E values can be used as an indicator of dough balance. A high R_m/E ratio is found with short doughs while a low R_m/E ratio is formed with extensible doughs. Flours with different extensigram areas (A) will have different applications. For cakes, A should be $30-45 \text{ cm}^2$ and $35-50 \text{ cm}^2$ for biscuits (Preston and Hosney, 1991).

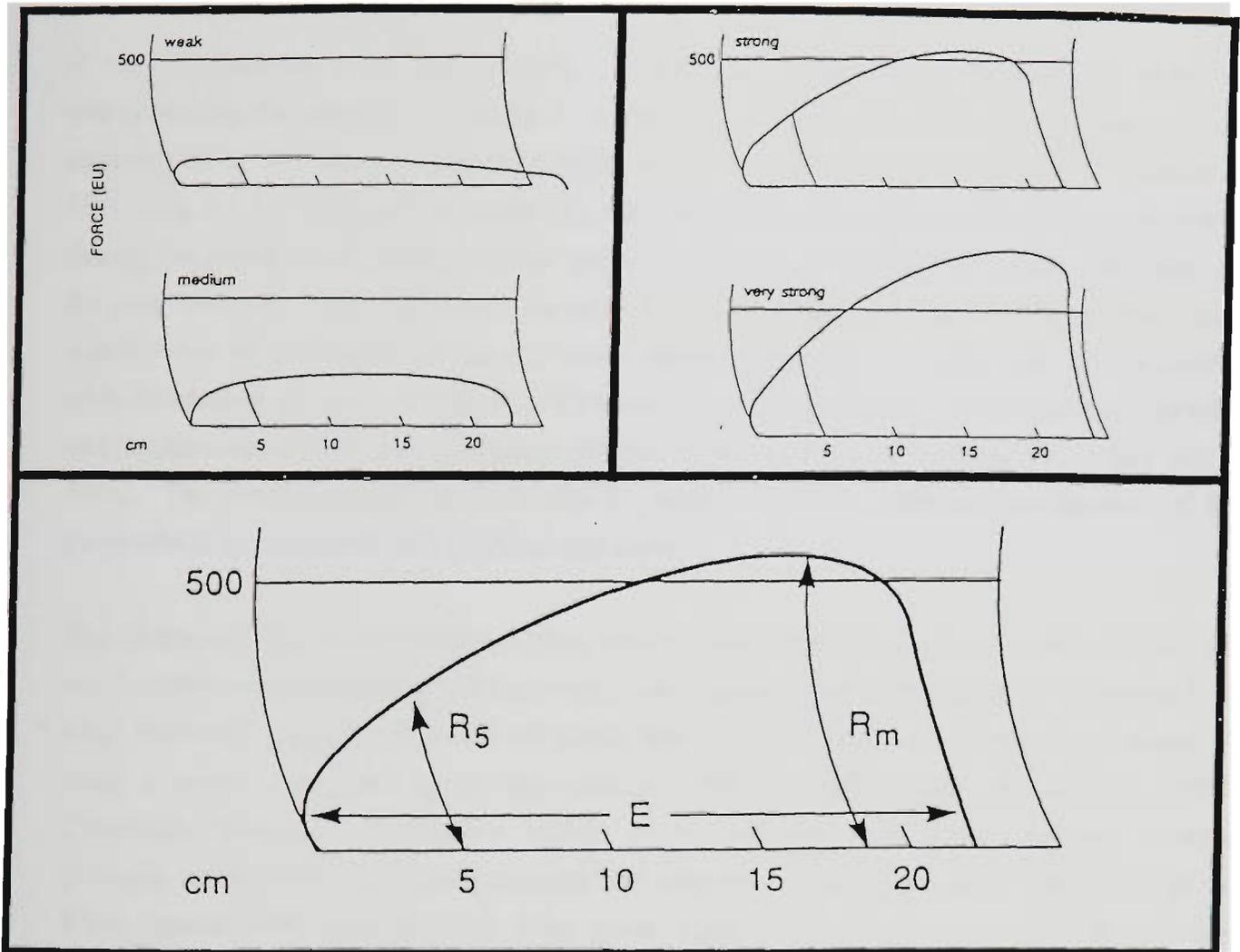


Fig 7.1.4 (A) An extensigram of wheat dough with associated measurements. E : extensibility (cm), R_m : maximum resistance to stretching (BU), N : viscoelastic ratio R_m/E (BU/cm) and A : area under curve (cm^2). (B) Extensigrams of wheat flours with weak, medium, strong and very strong dough properties.

7.1.3 PASTING PROPERTIES OF WHEAT FLOUR

An important factor in the bread making process is the pasting characteristics of the wheat flour dispersion (Rasper 1982). A starch suspension of wheat flour swells and forms a viscous paste when heated above its gelatinisation temperature. The swelling of wheat starch granules and its consequent disintegration after a prolonged heating promotes significant

changes in its paste viscosity and other rheological properties (Yasunaga *et al.*, 1968; Ring, 1985; Ollku and Rham, 1978). These pasting characteristics provide useful information on the quality of each starch and its potential applications in bakery products such as bread, roll or noodles. Some correlations between pasting properties, measured at early stages and the quality of the finished products such as bread or noodles have been established (Shuey and Gilles, 1964; D'Appolonia and MacArthur, 1974).

A viscoamylograph curve produced by a Brabender viscoamylogram provides a means of characterising the pasting properties of a starch by its *peak viscosity* (P), its viscosity on attaining 95°C, its viscosity after 30 minutes at 95°C and its final viscosity after 30 minutes at 50°C (Fig 7.1.5). The peak viscosity (P) indicates the highest viscosity yielded by the starch during the gelatinisation process under given conditions. The *peak time* is the time required for peak viscosity. This indicates how easily the starch dispersion can be cooked. The paste stability can be expressed by the difference between the peak viscosity and that measured after 30 minutes at 95°C. The ability of a heated starch dispersion to reassociate (*retrograde*) can be demonstrated by the difference between the viscosity at the cooling period and that at 50°C. The *final viscosity*, attained after 30 minutes at 50°C, indicates the stability of the paste which is most useful for practical applications.

The Brabender viscoamylograph has been widely used in bakery research and industry but the test is rather time consuming (180 minutes) and requires a substantial amount of sample (30-45g). Recently, pasting properties of cereal starches have been extensively investigated by using a newly developed rapid visco analyser (RVA) (Deffenbaugh and Walker, 1990). Commonly measured parameters include *peak viscosity*, *breakdown*, *holding strength* (trough), *set back viscosity*, *final viscosity* and *peak time*. Pasting properties measured by the RVA agreed with data obtained from other viscometers (Crosbie, 1991; Panozzo and McCormick, 1993; Walker *et al.*, 1988). The RVA requires only a small amount of sample (3.5g) and a test can be completed within 18 minutes. A RVA pasting curve shows viscosity, time and slope related parameters of a starch suspension similar to those obtained from a Brabender viscoamylograph (Fig 7.1.6). These RVA parameters will certainly be affected by protein supplementation.

The effects of supplementation of chickpea flour and several different types of proteins on the rheological properties of wheat dough at several stages were investigated by using the instruments described above. The pasting characteristic of fortified wheat flours were identified. Attempts were made to correlate the changes in these properties with the quality of the finished products by using the instruments described above. The results of these investigations are reported as follows.

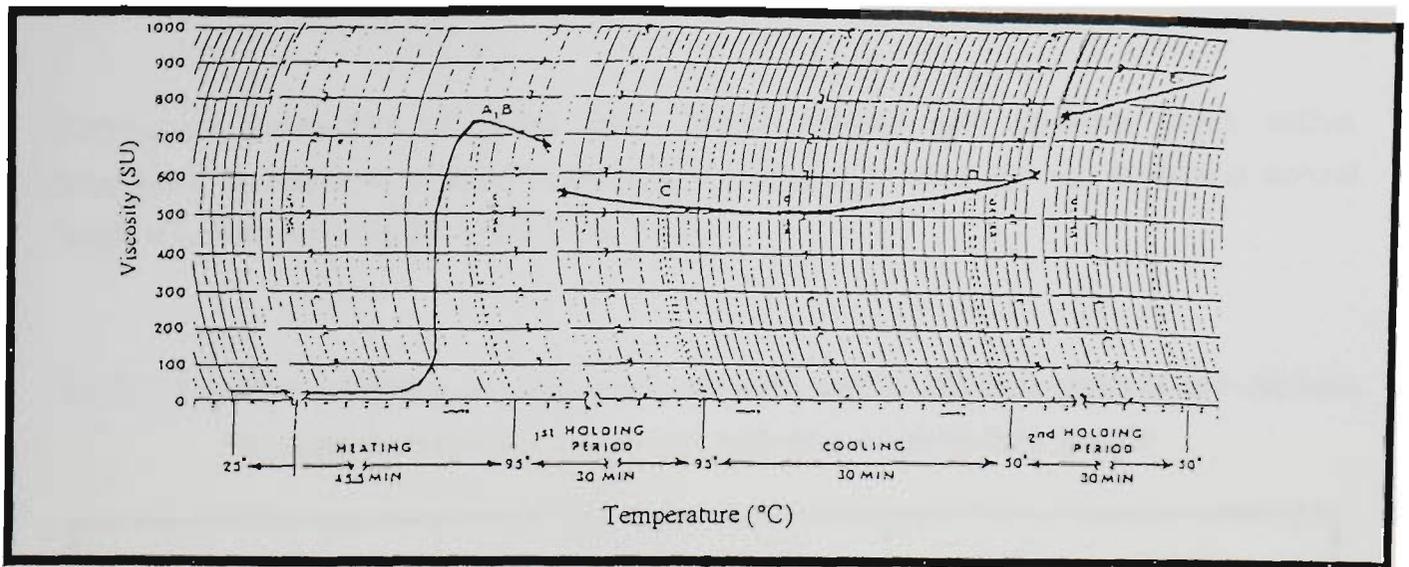


Fig 7.1.5 A Brabender amylogram with associated measurements.

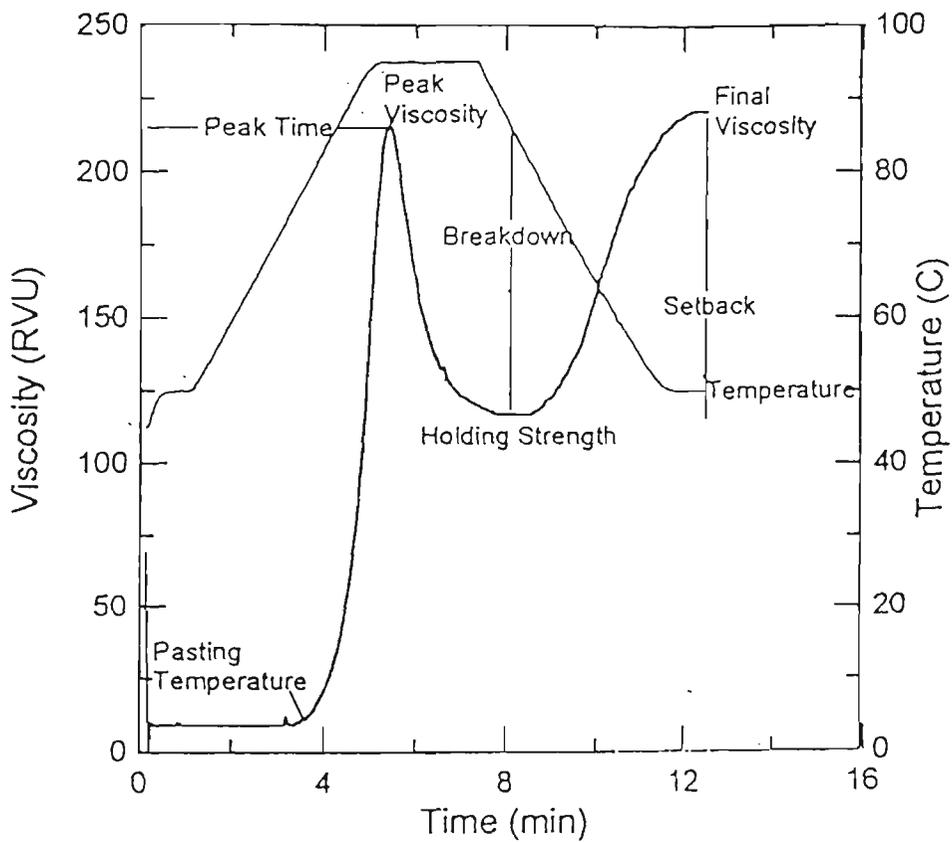


Fig 7.1.6 A typical RVA pasting curve of a wheat flour suspension with associated measurements: Peak, trough, final viscosity; peak, initial and onset time; ascending and descending slope.

7.2 FARINOGRAPH STUDY

Farinograph parameters of wheat flour doughs fortified with chickpea flours, native, acetylated, succinylated, enzymatically treated chickpea proteins and soy protein at several levels are given in Table 7.2.1 and Table 7.2.2.

Table 7.2.1 Effect of adding soy protein, native and modified chickpea proteins and chickpea flours on the water absorption and peak time of wheat flour doughs.

Dough Characteristic	Water absorption (%)				Peak time (min.)			
	1	2	4	8	1	2	4	8
Protein added %								
Soy	62.4	63.2	64.9	-	2.50	2.75	3.00	-
Chickpea protein (CP)	61.8	63.3	64.2	67.2	3.00	2.50	2.20	-
ACP6	62.9	63.2	64.2	-	2.00	2.35	2.40	-
ACP45	62.3	63.4	64.5	-	2.75	2.50	2.40	-
ACP49	62.3	63.0	64.0	66.2	3.00	2.50	2.30	2.20
SCP16	62.9	63.6	64.3	-	2.50	2.75	3.00	-
SCP73	62.9	63.5	64.6	-	2.75	2.75	2.75	-
SCP79	62.3	63.5	64.5	64.0	2.75	2.75	2.50	2.00-
Papain treated CP	61.6	61.2	60.7	-	3.75	5.90	6.00	-
Trypsin treated CP	62.0	62.3	63.2	-	3.75	3.00	2.75	-
Chickpea flour	8	15	25		8	15	25	
	60.7	60.4	60.1		5.50	5.50	6.00	
Wheat flour	62.5				3.00			

7.2.1 WATER ABSORPTION

a. Flour Substitution

Substituting wheat flour with 8%, 15% and 25% chickpea flour reduced water absorption about 2%. Water absorption decreased with increasing amount of chickpea flour added. This observation is in contrast with a similar study by Dodok *et al.* (1993) who reported that

replacement of wheat flour by 20% chickpea flour increased water absorption about 2%. However, in an earlier study, Yousseff *et al.* (1976) found that substitution of wheat flour with 5% chickpea flour resulted in a 1% reduction in water absorption on average. Similar result was also reported by Fernandez and Berry (1989).

A reduction in water absorption in this study can be attributed to two possible factors: composition and physical difference. Replacing wheat flour with 15-25% chickpea flour did not reduce the total protein content in the system but it did replace wheat protein by a non wheat protein. Firstly, if it was replaced by a protein with less imbibition or absorption power, the water absorption of fortified wheat dough would be reduced. As a major constituent of flour, starch may also have similar influences on water absorption. Secondly, chickpea flour with particle size larger than those of wheat flour would have reduced surface area, thus, reducing its water absorption capacity (Wong and Lelievre, 1982).

Supplementation of wheat flour with flours from oil seeds or grain legumes could either increase or decrease water absorption of the fortified dough. Flours obtained from oilseeds such as cottonseed, peanut, safflower, soy (Matthews *et al.*, 1970), sunflower (Zimmermann *et al.*, 1984; Fleming and Sosulski, 1977) or from grain legumes such as lupin (Campos and El Dash, 1978; Ballester *et al.*, 1984), Great northern bean (Sathe *et al.*, 1981) and navy bean (D'Appolonia, 1978) did increase the farinograph absorption. In contrast, adding flours obtained from faba bean, pinto bean, mung bean, lentils and field pea (D'Appolonia, 1977; Fleming and Sosulski, 1977) did reduce the farinograph absorption. Interestingly, adding horsebean flour did not affect water absorption of supplemented wheat flour (Patel and Johnson, 1975). In addition, faba bean flour and protein isolates reduced water absorption of Neepa wheat flour but showed no effect on that of glenlea flour which has very strong dough mixing properties (Yousseff and Bushuk, 1986). These different effects on the water absorption of composite flours could be attributed to several factors: diverse nature of starches in supplemented proteins, different particle sizes and the type of protein investigated and also the nature of the wheat used.

b. Protein Substitution

Replacing wheat flour with native, acetylated, succinylated, trypsin treated chickpea proteins and soy protein isolate increased farinograph absorption of wheat flour. Water absorption increased with the increasing level of protein substitution. In general, protein supplementation at 2% resulted in a 1% absolute increase in water absorption. Chickpea protein with different levels of acetylation or succinylation showed no difference on the effect of water absorption. Unlike other chickpea proteins, papain hydrolysed chickpea protein decreased the water absorption of the fortified dough. Overall, the change in water

absorption of protein supplemented doughs could likely be caused by the level of protein added rather than the replacement of wheat starch. Similar increase in water absorption was obtained with cottonseed (Khan *et al.*, 1976), Great northern bean (Sathe *et al.*, 1981), soy (Zimmermann and Mieth, 1986), sunflower (Zimmermann *et al.*, 1984) and lupin protein (Ballester *et al.*, 1984) fortified wheat flour.

7.2.2 PEAK TIME

a. Flour Substitution

In an earlier study on rheological properties of wheat dough, Markley and Bailey (1938) found a correlation between water absorption and peak time. As water absorption increased, the peak time increased. This observation was further supported by Pulkki (1938), who noted when particle size of the flour was reduced, the peak time was increased. An inverse relationship between peak time and water absorption was found with fortified wheat flour in this study. As the water absorption decreased (62.05% - 60.1%) with increasing level of chickpea flour added (0 - 25%), the peak time accordingly increased (3 - 6 minutes), indicating an inhibition of starch hydration and delayed swelling. This observation was in contrast to the results reported by Dodok *et al.* (1993), done also on wheat dough fortified with chickpea flour.

Deshpande *et al.* (1983) found that as the level of navy bean flour in composite doughs increased, farinograph absorption increased but peak time decreased. While using the same navy bean flour, Silaula *et al.* (1989) found a contrasting result. In a study on the effect of flour fraction and bean grade on rheological parameters of wheat dough, Lorimer *et al.* (1991) showed that although the water absorption of dough fortified with navy bean flour increased, the peak time could either be increased (prime fines 5-10%) or decreased (prime cotyledon 5-10%), depending on the flour composition. Prime fine flour contained 46% protein, double the protein level in the cotyledon flour.

b. Protein Substitution

The positive correlation between water absorption and peak time observed by Markley and Bailey (1938) was found with some, but not with all, proteins investigated. When wheat flour was replaced with soy protein isolate (1-4%), both water absorption (62.4-64.9%) and peak time (2.50-3.00 minutes) increased. Similar results were also obtained with slightly acylated chickpea proteins (ACP16 and SCP26). For unmodified, extensively acetylated and succinylated chickpea proteins, as the water absorption increased with increasing level of

proteins added, the corresponding peak times reduced. Different levels of protein acylation had no significant effect on water absorption and peak time of fortified doughs.

Chickpea proteins hydrolysed by papain and trypsin showed different effects. Replacing flour with papain hydrolysed protein (1-4%) decreased the water absorption (61.6% to 60.7%) but increased the peak time (3.75 to 6 minutes). In contrast, replacing wheat flour with trypsin hydrolysed chickpea protein (1-4%) increased water absorption but decreased the peak time (3.75-2.75). Peak time showed a strong relationship with high molecular weight glutelin subunit content (Andrews *et al.*, 1994).

A positive water absorption and peak time relationship was found with wheat doughs fortified with some oilseed proteins and flour such as cotton seed, peanut, safflower and soybean (Matthews *et al.*, 1970). In contrast, a negative relationship was observed with horse bean flour and protein (Patel and Johnson, 1975). In an extreme case, both negative and positive relationships may exist, depending on different fractions of the seed and the level of added flour.

7.2.3 DOUGH BREAKDOWN

a. Flour Substitution

Dough breakdown is the difference between the peak curve and the top of the curve measured after 5 minutes from the peak, using Brabender units. Flours with high tolerance to mixing have low dough breakdown values while the weak flours have high dough breakdown values. The high molecular weight subunits of glutenin were the major contributors to dough breakdown (Lagudah *et al.*, 1988; Fullington *et al.*, 1987). Wheat flour supplemented with chickpea flour (0-25%) became weaker. Its dough breakdown value increased from 24 to 60 BU. Similar results were obtained with wheat flour fortified with faba bean (Finney *et al.*, 1980), winged bean (Okezie and Dobo, 1980) and navy bean flour (Sathe *et al.*, 1981).

b. Protein Substitution

Supplementation with acetylated and succinylated proteins made the fortified dough much weaker (98 and 141 BU at 4% level of substitution, respectively). At the same level of substitution, succinylated proteins weakened the fortified dough more than acetylated proteins. As the level of acetylation and succinylation increased, the modified proteins had more pronounced weakening effect on the dough's strength (40 and 98 BU at 6 and 49% of acetylation and 69 and 141 BU at 16 and 141% of succinylation).

Chickpea proteins hydrolysed by papain and trypsin showed a contrasting effect on the dough's strength. While dough fortified with papain hydrolysate became weaker, supplemented with trypsin hydrolysate strengthened the fortified dough.

Table 7.2.2 Effect of adding soy protein, native and modified chickpea proteins and chickpea flours on the strength and stability of wheat flour doughs.

Dough Characteristic	Dough Breakdown (BU)				Dough Stability (min.)			
	1	2	4	8	1	2	4	8
Protein								
Soy	32	28	41	-	8.75	8.50	6.50	-
Chickpea Protein (CP)	18	29	38	-	10.75	10.5	9.50	-
ACP16	47	40	30	-	3.75	4.50	5.75	-
ACP45	44	78	78	-	3.50	2.50	2.30	-
ACP49	40	70	98	100	7.00	2.50	2.35	2.10
SCP16	43	66	69	-	5.40	2.50	2.50	-
SCP73	93	101	114	-	1.75	2.10	2.20	-
SCP79	77	101	141	154	3.00	2.45	1.90	1.90
Papain treated CP	29	32	50	-	10	8.75	8.50	-
Trypsin treated CP	23	20	20	-	11.5	11.2	8.50	-
Chickpea flour	8	15	25	-	8	15	25	-
	50	55	60	-	7.10	6.50	6.00	-
Wheat flour	24.5				10.9			

7.2.4 DOUGH STABILITY

a. Flour Substitution

Dough stability is measured as the difference in time (minutes) at the point when the curve first intercepts (arrival time) the 500 BU line and the curve departs from the line (departure time).

b. Protein Substitution

With the exception of the unmodified chickpea protein, supplementation with either flours or modified proteins reduced the dough stability of all fortified wheat flours. Substitution at a 4% level of chickpea protein showed less effect on dough stability (9.5 minutes) than soy protein (6.5 minutes) when compared with wheat flour (10.9 minutes). As the level of flour and protein substitution increased, dough stability progressively reduced. Fortified dough became very unstable by adding acetylated and succinylated chickpea proteins (2.35 and 1.90 minutes, respectively). The destabilising effect increased with increasing levels of protein acetylation (3.75 to 2.35 minutes) and protein succinylation (2.50 to 1.90 minutes). Interestingly, adding papain hydrolysed chickpea protein weakened the dough strength (dough breakdown increased from 24.5 to 50 BU) and reduced the dough stability (from 10.90 to 8.5 minutes). While fortifying with trypsin hydrolysed chickpea made the dough less stable (reduced to 8.5 minutes) its strength was increased (dough breakdown decreased from 24.5 to 20 BU).

Using a similar standard applied for wheat flour classification and based on measured farinograph parameters, the effects of protein fortification on wheat flour can be summarised as follows:

1. Supplementation of wheat flour with native, slightly acetylated and enzymatically treated chickpea proteins and soy proteins at a 4% level had some influences on its farinograph parameters but the fortified wheat flours still can be classified as medium flours.
2. Fortification with chickpea flour, extensively acetylated and succinylated chickpea proteins strongly influenced all farinograph parameters of the wheat flour and the fortified wheat flour could be considered as a weak flour.

7.3 EXTENSIGRAPH STUDY

The effects of flour and protein supplementation on the rheological properties of fortified doughs have been reflected in many farinograph measurements. However, the changes in structural parameters of the fortified doughs could only be illustrated clearly by typical extensigraph measurements. Some representative proteins were selected and examined by using the extensigraph. The effects of supplementation with native and some highly modified chickpea proteins at three levels are given in Table 7.3.1. Data on soy protein and wheat flour were also included, as reference.

As observed with farinograph absorption, salt-water absorption by extensigraph was also increased (2-4%) as the level of protein incorporation increased (1-4%). There was no significant difference in water absorption among native, acetylated and succinylated proteins but soy protein supplementation gave the highest water absorption (65.8%). The dough strength (A), initially 95.5 cm², gradually increased with increasing levels of chickpea proteins added. (115.9 cm², 109 cm² and 110 cm² for native, acetylated and succinylated chickpea proteins at 4% level, respectively). Supplementation with soy protein did not significantly alter dough strength (96.5 cm²) when compared with wheat flour (95.5 cm²). The Rm / E ratio, a measure of dough shortness, is a balance between viscous and elastic properties of a dough. Changes in dough shortness reflected changes in dough extensibility (E) and / or the resistance to extension (Rm). These two properties are related to wheat protein content.

Table 7.3.1 Extensigraph measurements of wheat flour dough fortified with native, acetylated and succinylated chickpea proteins at three levels.

Dough		Water added (%)	AR (cm ²)	Rm (BU)	E (cm)	N (BU/cm)	Loaf (cm ³)
Flour	Protein added						
Wheat only	-	60.3	95.5	440	17.5	25.1	1,360
Chickpea	-	58.5	70.3				
99%	NCP (1%)	62.4	98.0	424	16.7	25.4	
98%	NCP (2%)	62.8	109	474	17.0	27.7	
96%	NCP (4%)	63.1	116	510	17.0	30.1	1,300
99%	ACP49 (1%)	62.0	97.0	469	15.5	30.6	
98%	ACP49 (2%)	63.0	109	537	15.5	34.6	
96%	ACP49 (4%)	63.9	109	580	14.6	39.5	1,185
99%	SCP79 (1%)	61.9	110	511	15.6	32.7	
98%	SCP79 (2%)	63.0	112	564	14.7	38.4	
96%	SCP79 (4%)	63.7	110	820	10.8	76.0	1,170
96%	SPI (4%)	65.8	96.5	460	15.5	29.6	1,220

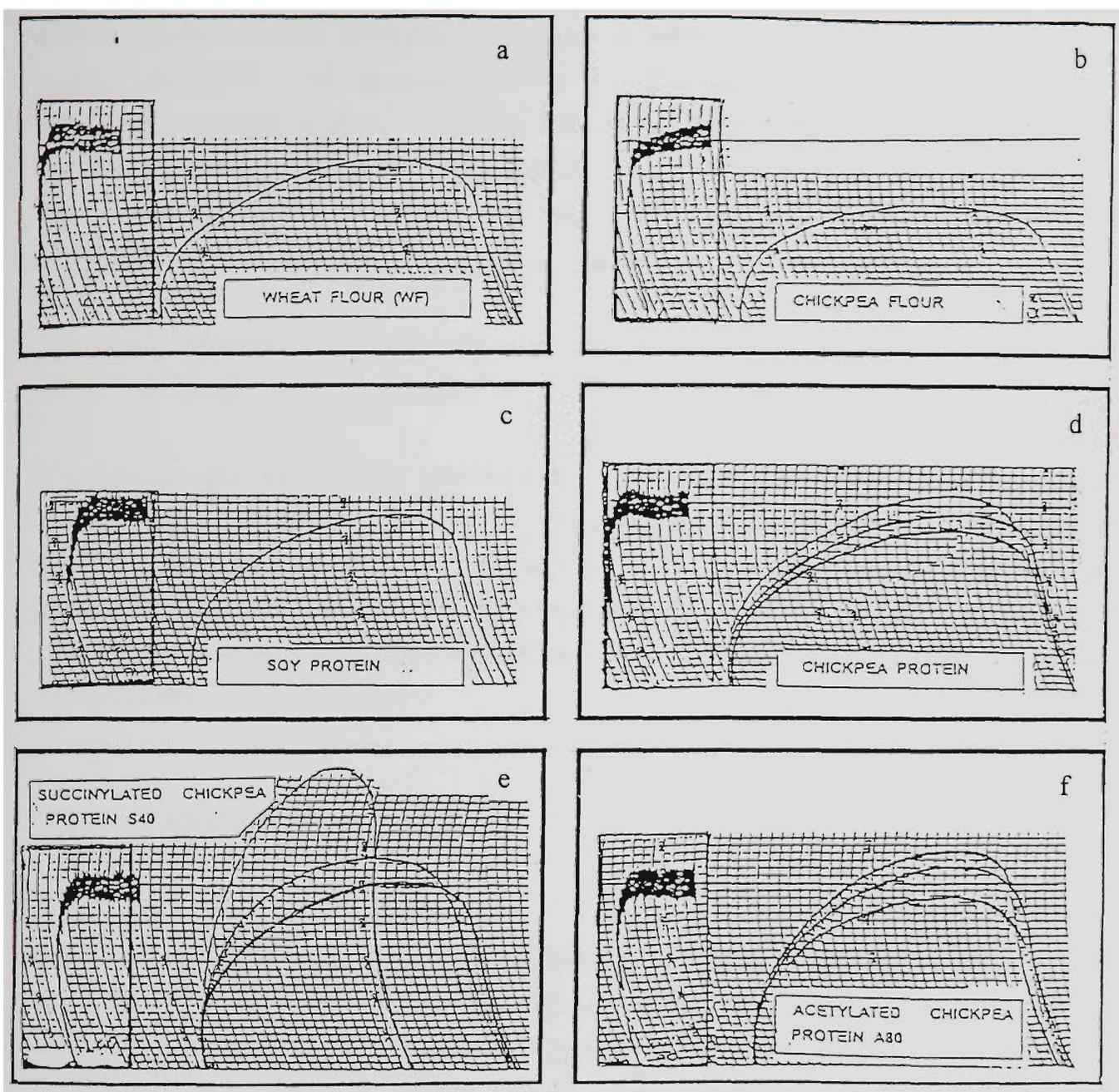


Fig 7.3.1 Extensigrams of (a) wheat flour, (b) chickpea flour, (c) wheat flour supplemented with 45 soy protein isolate, (d) wheat flour supplemented with 1, 2, 4% native chickpea protein, (e) wheat flour supplemented with 1, 2, 4% succinylated chickpea protein and (f) wheat flour supplemented with 1, 2, 4% acetylated chickpea protein.

Extensibility tended to be related to the total amount of glutenin subunits and the amount of low molecular weight glutenin subunits. The viscoelastic ratio was found to be positively related to amount of high molecular weight wheat proteins (Fullington *et al.*, 1987). The extensigram of wheat flour and chickpea flour is given in Fig 7.3.1 a-b. Soy and native chickpea protein showed only a small effect on dough resistance (460 and 510 BU at 4% compared 440 BU for wheat flour) and extensibility (almost unchanged for native chickpea and slightly reduced for soy protein) (Fig 7.3.1 c-d). Therefore, soy and native chickpea

protein only had a slight effect on dough shortness of fortified wheat flours (29.6 and 30.1, respectively, at 4% level compared with 25.1 for wheat flour). In contrast, acetylated and succinylated chickpea proteins showed a more striking effect on both dough resistance (580 and 820 BU at 4% level) and dough extensibility (reduced from 17.5 cm to 14.6 cm and 10.8 cm, respectively, at 4% level) (Fig 7.3.1 e-f). Consequently, acetylated and succinylated protein had a more significant effect on dough shortness.

The effects of adding proteins on the maximum resistance and extensibility of the fortified wheat flour doughs are clearly demonstrated with acylated chickpea proteins.

The extensigraph measurements indicated that substitution of wheat flour with chickpea flour, native, modified chickpea proteins or soy proteins changed the structural parameters of all fortified doughs at different levels. Substitution with non wheat components changed the balance between elastic and viscous properties of wheat flour. The resulting doughs have generally become more elastic and less viscous. These changes would certainly affect many aspects of the finished products.

7.4 PASTING PROPERTIES

Pasting properties of wheat flour was supplemented with chickpea flour at 15 and 30% level and with native, acetylated, succinylated, papain, trypsin hydrolysed chickpea protein at 4% level were examined. These properties are presented as viscosity (Table 7.4.1), time (Table 7.4.2) and slope related parameters (Table 7.4.3). Pasting properties of wheat flour supplemented with soy isolate are also included. These properties are illustrated in Fig 7.4.1.

7.4.1 EFFECT ON VISCOSITY RELATED PARAMETERS

Supplementation of wheat flour with either chickpea flour (15-30%) or proteins (4%) affected all its viscosity related parameters, including peak (P), trough (H) and breakdown viscosity (P-H) (Fig 7.4.1). Peak and trough viscosity was reduced about 10% (native protein) or 20% (chickpea flour). Succinylated chickpea proteins reduced peak and trough viscosity of fortified wheat flour more than acetylated proteins. As the level of succinylation increased, its effects on peak and trough viscosity decreased. Acetylated chickpea proteins did not exhibit such an effect. Soy protein did not influence any viscosity parameters (Table 7.4.1). Statistical analysis showed that there were only significant differences between peak viscosity of wheat flour and those supplemented with chickpea flour and acetylated proteins

($P < 0.05$). The overall results showed the effects of different types of proteins. Chen and Rasper (1982) found that soy supplementation at 8% not only reduced the hot paste viscosity of wheat flour but also showed that the extent of reduction was dependent on the type of soy protein.

Table 7.4.1 Effect of protein supplementation (3%) on the RVA viscosity related parameters of wheat flour. Chickpea flours were supplemented at 15 and 30% levels.

Sample	Viscosity-related parameters (RVU)		
	P	H	P-H
Wheat flour	380	279	101
NCP (Native Chickpea Protein)	342	250	92
ACP6 (Acetylated Chickpea Protein)	330	242	88
ACP45	350	255	95
ACP80	339	245	94
SCP16 (Succinylated Chickpea Protein)	348	250	98
SCP73	355	260	95
SCP79	370	265	105
Soy protein	365	265	100
Chickpea flour	342	260	82
Chickpea flour	310	237	73
Papain hydrolysate	310	238	72
Trypsin hydrolysate	350	255	95

P: Peak viscosity; H: Holding strength; P-H: Break down

Peak viscosity was dependent on starch swelling, exudation and fragmentation in a wheat flour suspension (Dengate and Meredith, 1984). Some complexes could be formed between wheat starch and other components in supplemented chickpea flour and proteins. These could inhibit starch swelling and amylose exudation, leading to the reduction of peak viscosity. Ryu *et al.* (1993) found that peak viscosity of wheat flour, supplemented with non fat dry milk powder and whole milk powder was greatly reduced at 10% level. Peak viscosity was also affected by sucrose (Deffenbaugh and Walker, 1989; Kim and Walker, 1992). The extent of starch breakdown can be measured by the difference between peak viscosity and holding strength (trough viscosity) which reflected the degree of starch dissociation into smaller units by gelatinisation. There was almost no reduction in breakdown viscosity of

wheat flour supplemented with soy or highly succinylated chickpea proteins. However, other proteins, particular chickpea flour and papain treated protein significantly decreased the breakdown viscosity ($P < 0.05$). A reduction in breakdown viscosity reflected less degradation of wheat starch. This could be a result of some inhibition of hydration due to the competition of starch for water in the system or the formation of complexes between starch and other components. Ryu *et al.* (1993) found that not only protein and sugar but also lipids could contribute to the reduction of breakdown viscosity.

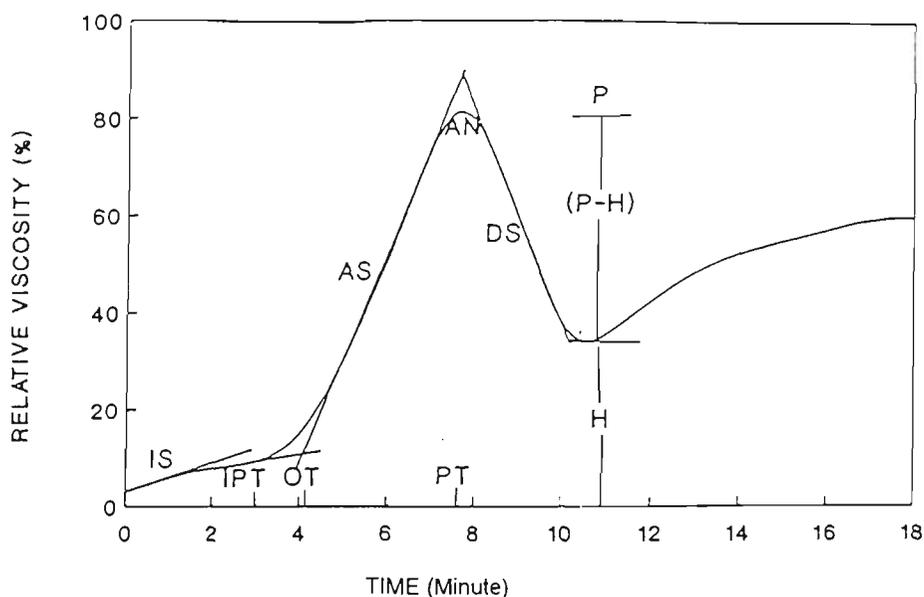


Fig. 7.4.1 Pasting parameters generated from RVA curves: time-related parameter, initial peak time (IPT), onset time (OT) and peak time (PT); viscosity-related parameters, peak viscosity (P), breakdown viscosity (H) and difference between peak and breakdown viscosity (P-H); slope-related parameters, initial slope (IS), ascending slope (AS), descending slope (DS), and angle between ascending and descending lines (AN). (Ryu *et al.*, 1993).

7.4.2 EFFECTS ON TIME RELATED PARAMETERS

Supplementation with chickpea flour and several proteins or soy protein reduced the peak time of all fortified wheat flours. Statistical analysis, however, indicated that the reduction was insignificant ($P > 0.05$) (Table 7.4.2). Ryu *et al.* (1993) found that non fat dry milk powder or egg powder did not affect the peak time of the fortified wheat flours but adding sucrose, lipids or glyceryl monostearate delayed its gelatinisation and increased its peak time.

Similar results were also obtained by other workers (Deffenbaugh and Walker, 1989; Spies and Hosney, 1982). An increase in peak time could be attributed to the formation of complexes between starch with either sucrose or lipids, to the reduction in water activity by

sugars in a starch water system, to the competition of starch for water and also to the reduction in starch retrogradation (Colonna and Mercier, 1983; Bean and Yamazaki, 1978; Ryu *et al.*, 1993). In contrast, a decrease in peak time could be attributed to the increasing concentration of starch in the suspension. The viscosity peak moved towards the lower temperatures as the starch concentration in the system increased (Rasper, 1982).

Table 7.4.2 Effects of protein supplementation (3%) on the RVA time related parameters of wheat flours. Chickpea flours were supplemented at 15 and 30% levels.

Sample	Time-related parameters (minute)		
	OT	IPT	PT
Wheat flour	7.2	6.1	9.9
NCP (Native Chickpea Protein)	8.2	6.8	9.6
ACP6 (Acetylated Chickpea Protein)	8.5	7.0	9.6
ACP45	8.3	7.0	9.6
ACP49	8.4	7.2	9.6
SCP16 (Succinylated Chickpea Protein)	8.3	7.0	9.6
SCP73	8.4	7.1	9.7
SCP79	8.3	7.2	9.6
Soy protein	8.4	7.2	9.6
Chickpea flour	7.1	8.0	9.7
Chickpea flour	7.8	7.8	9.4
Papain hydrolysate	8.0	7.1	9.3
Trypsin hydrolysate	8.2	7.0	9.8

OT: Onset time; IPT: Initial peak time; PT: Peak time

Supplementation with non wheat flour or proteins did not affect the initial peak time of the fortified flours but its onset time was influenced. Supplementation with either soy or unmodified chickpea protein increased the onset time of the fortified flours significantly ($P < 0.05$). Similar tendencies was also obtained with acetylated, succinylated and papain

treated chickpea proteins. The onset time of wheat flour fortified with acetylated, succinylated and enzymatically treated chickpea proteins was significantly higher ($P < 0.05$) than that of wheat flour, blended with chickpea flour.

7.4.3 Effect on slope related parameters

Fortification with chickpea flour or proteins did not affect the descending slope but increased the ascending slope and altered the initial slope of the fortified wheat flours. These slopes reflected the rate of gelatinisation, swelling and breakdown of starch.

Table 7.4.3 Effects of protein supplementation on the RVA slope related parameter of wheat flour dispersions.

Sample	Slope-related parameters		
	IS	AS	DS
Wheat flour	0.13	6.6	0.41
NCP (Native Chickpea Protein)	0.11	7.3	0.39
ACP6 (Acetylated Chickpea Protein)	0.13	9.3	0.38
ACP45	0.15	8.3	0.33
ACP49	0.10	9.0	0.38
SCP16 (Succinylated Chickpea Protein)	0.15	9.6	0.33
SCP73	0.13	11.2	0.34
SCP79	0.16	9.2	0.29
Soy protein	0.13	10.3	0.31
Chickpea flour	0.07	7.3	0.40
Chickpea flour	0.06	7.1	0.47
Papain hydrolysate	0.08	7.5	0.49
Trypsin hydrolysate	0.10	7.1	0.34

IS: Initial slope; AS: Ascending slope; DS: Descending slope

Adding chickpea flours (15-30%) significantly decreased ($P < 0.05$) the initial slope of the fortified flours. The initial slope of chickpea flour fortified wheat was significantly ($P < 0.05$) lower than that of wheat flour fortified with acetylated, succinylated chickpea and soybean proteins. Statistical analysis showed that adding chickpea flours, hydrolysates or native proteins did not significantly affect the ascending slope of the fortified flours. However acetylated, succinylated chickpea and soy protein significantly ($P < 0.01$) increased the ascending slope of fortified wheat flours.

7.5 FACTORS AFFECTING THE PEAK VISCOSITY

Information gained from either Brabender viscoamylograph or Rapid visco analyser is useful for practical applications in the cereal industry to assess the quality of wheat flours (Meredith, 1970; Patterson and Crandall, 1967; Bason *et al.*, 1993) and also for more basic research applications (Deffenbaugh and Walker, 1989).

Information on the peak viscosity is particularly important since several correlations have been established between the maximum viscosity and flour's baking quality as well as the characteristics of finished products. For example, Brown and Harrel (1944) showed a strong relationship between the amylograph peak viscosity and the baking quality of rye flours. The effects of peak viscosity on the quality of bread texture (Yasunaga *et al.*, 1968), pasta (Shuey and Gilles, 1964) and Japanese noodles (Bean *et al.*, 1974) have been investigated. Recently, Panozzo and McCormick (1993) showed that peak viscosity, measured by the RVA, was highly correlated with sensory eating quality of Japanese and Korean style white salted noodles.

Peak viscosity increased when starch formed complexes with hydrocolloids such as guar, xanthan, locust bean gums (Bahnassey and Breene, 1994), or with sugars (Ryu *et al.*, 1993; Deffenbaugh and Walker, 1990). The effects of adding protein on the amylo viscosity have not been adequately investigated. In an earlier investigation, Anker and Geddes (1944) found that peak viscosity increased in the presence of gluten. Consequently, more experiments were conducted in this investigation to elucidate the effects of adding non wheat proteins, either as flour or concentrates, on the peak viscosity of the fortified wheat flours.

As presented in Table 7.4.1, supplementing wheat flour with chickpea proteins at a 3% level reduced the peak viscosity of all fortified flours. Similarly, Chen and Rasper (1982) found that the hot paste peak viscosity of wheat flour was reduced when supplemented with 8% soy proteins. The peak viscosity is the highest viscosity yielded by the starch during the

gelatinisation process. When replacing 3% wheat with protein, some starch in wheat flour was removed. Such a small reduction in the starch concentration could lead to a substantially lower peak viscosity since the peak viscosity increases with the cube of starch concentration. Thus, the effect due to starch removal had to be first examined. Indeed, reducing wheat flour by 3% resulted in a decrease in peak viscosity by 9% (from 380 RVU to 343 RVU) (Fig 7.5.1). This result confirmed the pronounced effect of starch concentration on pasting characteristics of wheat flours and peak viscosity (Sanstedt and Abbot, 1964).

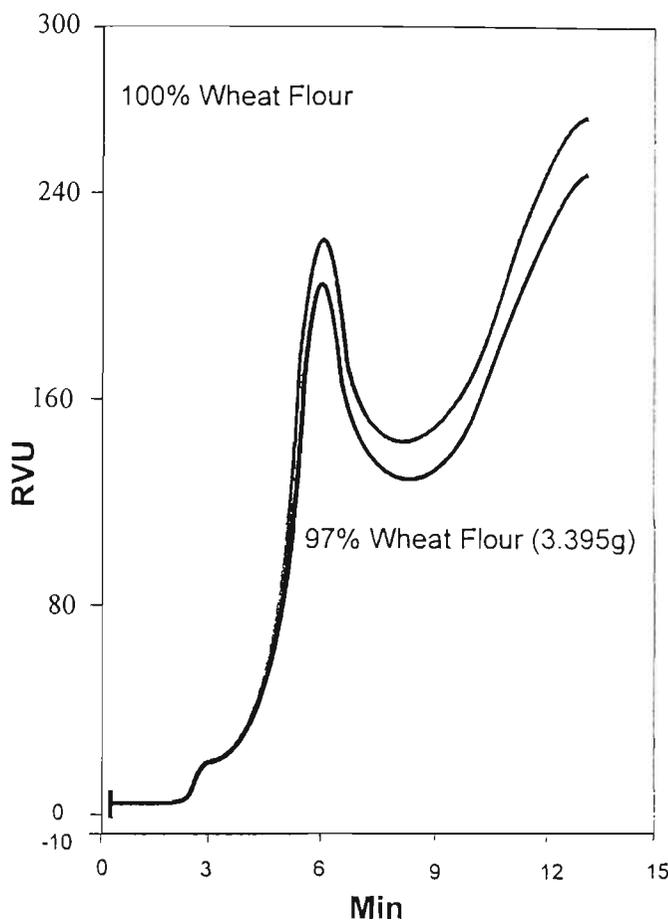


Fig 7.5.1 The RVA pasting curve of 100% (3.395g/0.97g) and 97% (3.395g) whole wheat flour dispersions.

The second effect that needs to be examined is the possible reduction in the peak viscosity due to the formation of complexes between wheat starch and lipids (Kim and Walker, 1992). Subjected to identical conditions, wheat flour showed a lower RVA peak viscosity than that of defatted wheat flour (343 RVU compared with 365 RVU). A similar result was also obtained when whole and defatted wheat flour was added with 3% bovine serum albumin (407 RVU compared with 427 RVU). The decrease in peak viscosity, again, could be

attributed merely to a lower starch level in the whole fat flour compared with a higher starch level in the defatted wheat flour, at the same flour concentration. Consequently, one should firstly take into account the effect due to starch reduction by other component such as lipids, sugar or proteins when examining their effects on peak viscosity of supplemented wheat flours.

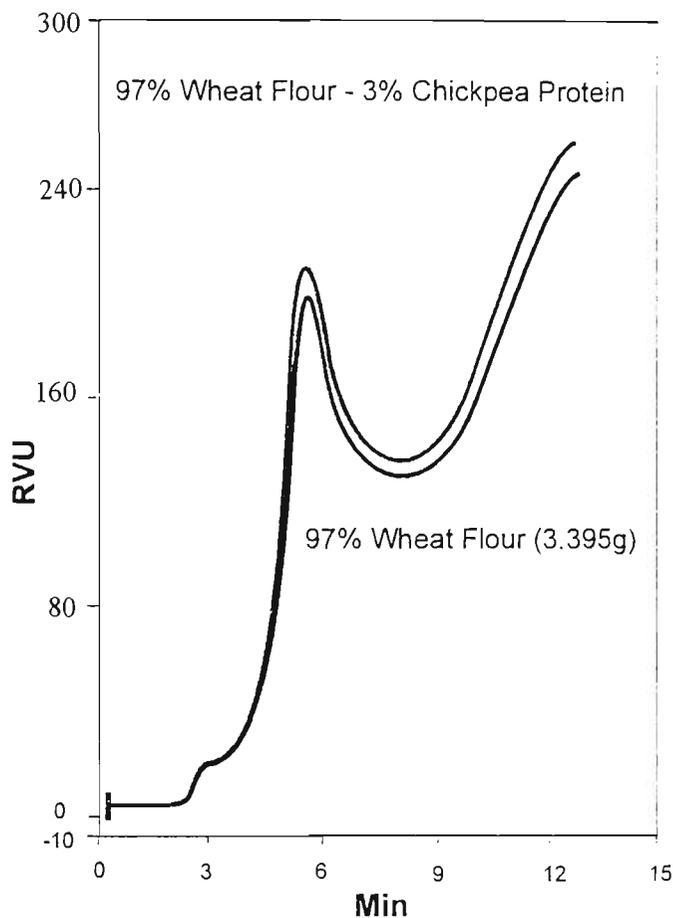


Fig 7.5.2. The RVA pasting curve of wheat flour supplemented with native chickpea protein at 3% level.

To eliminate any possible effect on the RVA peak viscosity which may be due to an unequal amount of starch in the test system, several proteins were selected and mixed with the same amount of starch. When supplemented with native, acetylated and succinylated chickpea protein at a 3% level, the peak viscosity of the fortified flours increased by about 2 to 8% (359, 350 and 370 RVU, respectively) (Fig 7.5.2). Soy, bovine serum albumin (BSA) or betalactoglobulin (3% level) further increased the peak viscosity of all 3% supplemented wheat flours by about 8-18% (370, 389 RVU and 408 RVU, respectively, compared with 343 RVU of wheat flour control) (Fig 7.5.3 and Fig 7.5.4). Since the starch in fortified wheat flour in this study was constant, the increase of peak viscosity had to be due to the proteins added.

To confirm this suggestion, 3% aqueous suspensions of soy protein and BSA were examined. Interestingly, no peak viscosity was found with their RVA curves. Several soy proteins at 8% failed to give any measurable viscosity response when used without wheat flour. Therefore, the addition of these proteins *per se* at 3% level failed to show any effect on peak viscosity or that the effect was so small as to be undetectable. However, when these were added to wheat flour suspension, the RVA peak viscosity of fortified flours was substantially increased. This suggests that some complexes might be formed between wheat starch and the added proteins, leading to an increase in the RVA peak viscosity. Bahnassey and Breene (1994) indicated that the increase in RVA peak viscosity of gum fortified wheat flour was due to the formation of starch gum complexes. An increase in the RVA of wheat flours fortified with sugar, lipids or other food ingredients was normally attributed to the formation of complexes between wheat starch and these added components (Ryu *et al.*, 1993; Kim and Walker, 1992).

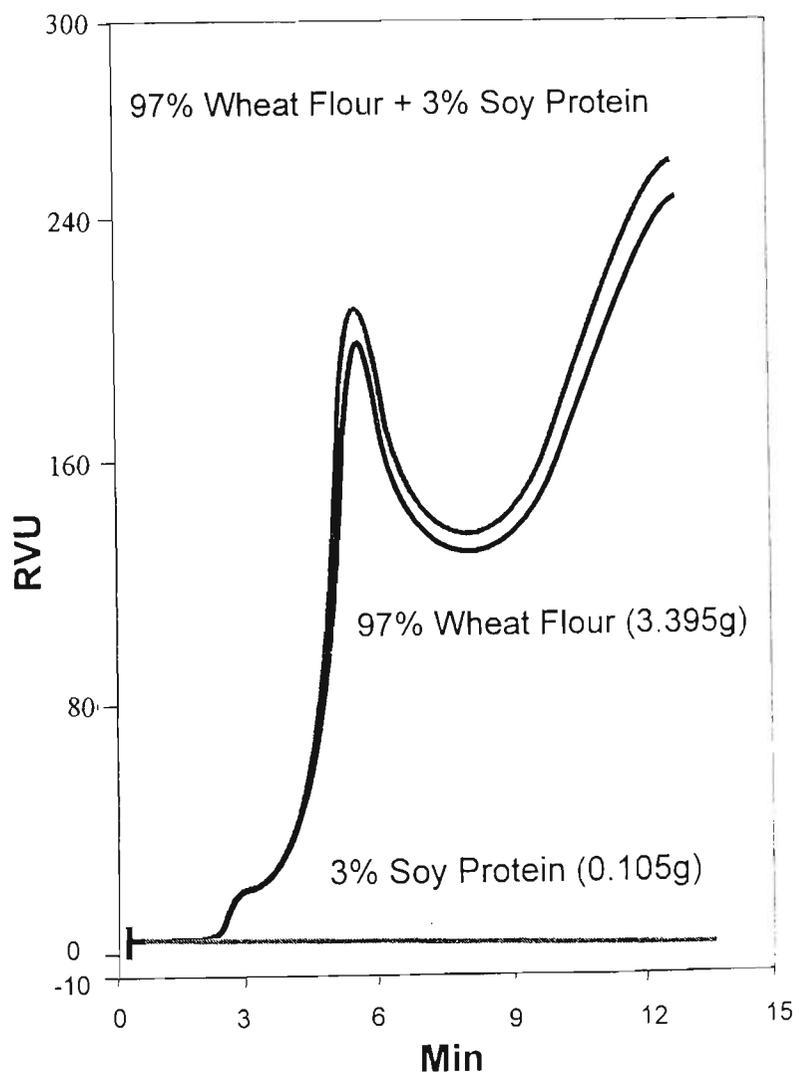


Fig 7.5.3 The RVA pasting curve of wheat flour supplemented with soy protein at 3% level.

In this study, the RVA peak viscosity of fortified wheat flours was affected to different degrees by different types of added proteins. Lactoglobulin or bovine serum albumin had more effect than soy or native chickpea proteins. The different effects on RVA peak viscosity of the added BSA and lactoglobulin suggested strongly that the viscosity changes in this study may be ultimately due to the different solubility, hydration or swelling capacity of the added proteins. Calcium soy proteinase reduced hot paste peak viscosity less than many soy proteins. This was attributed to its low hydration capacity which also reduced the competition for water between the gelatinising starch and supplemented protein (Chen and Rasper, 1982). However, the RVA curves of 14% native chickpea proteins and BSA suspensions clearly showed the different swelling capacity of these two proteins (Fig 7.5.5). Consequently, it was likely that the different swelling capacity of each type protein could contribute to the different peak viscosity of wheat flours fortified with chickpea protein and with BSA. Thus, the RVA peak viscosity could be affected not only by different type of protein but also by the same type of protein but prepared under different conditions.

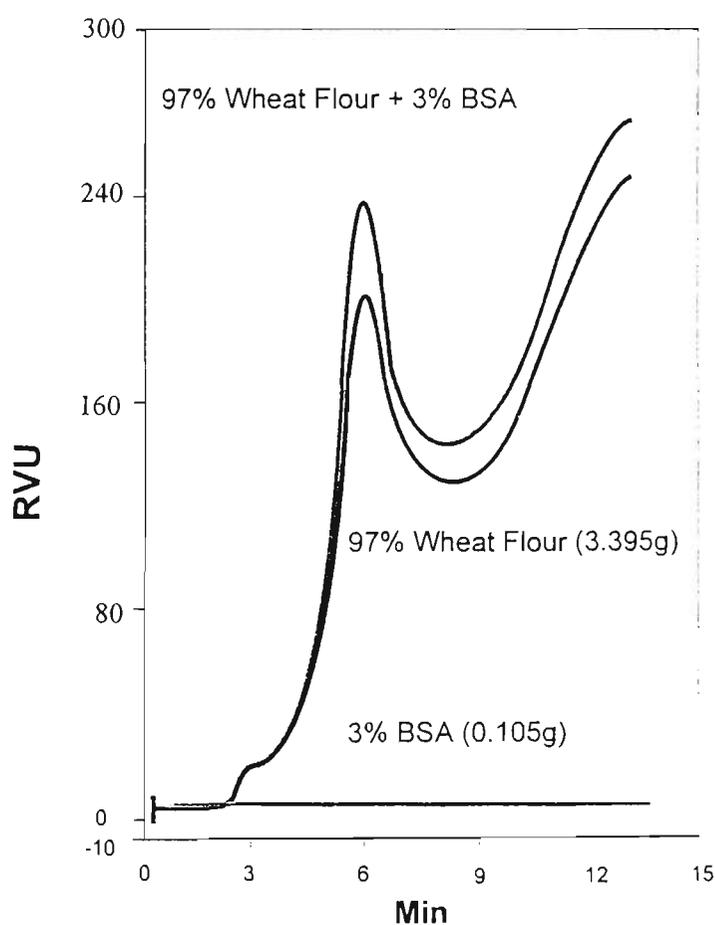


Fig 7.5.4 The RVA pasting curve of wheat and bovine serum albumin dispersions at 3% concentration.

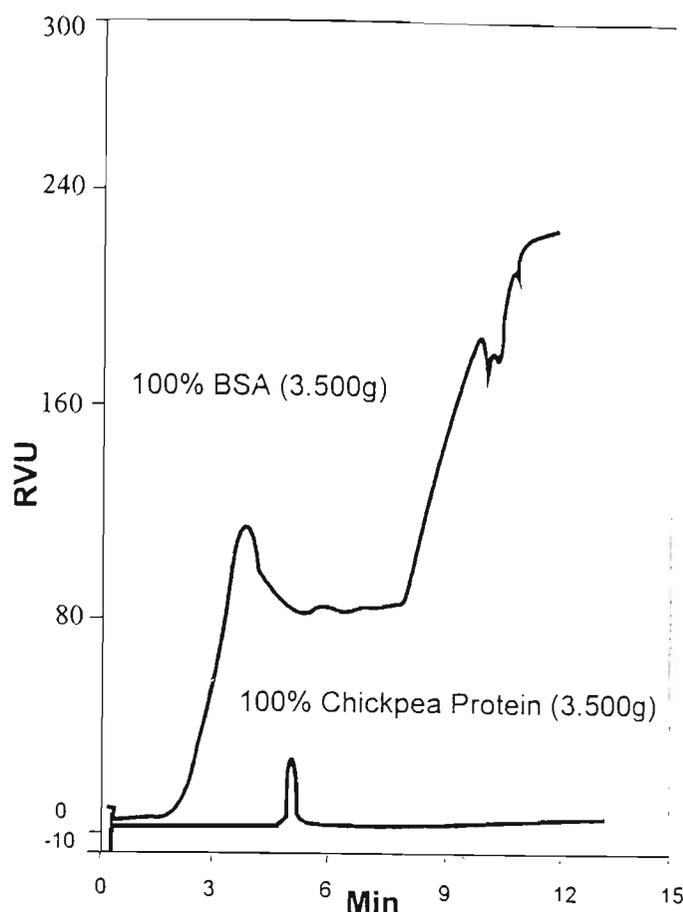


Fig 7.5.5 The RVA pasting curves of native chickpea protein and BSA dispersions (showing the different swelling capacity of each type of protein).

7.6 BAKING PROPERTIES OF WHEAT FLOUR SUPPLEMENTED WITH CHICKPEA FLOUR AND PROTEIN ISOLATES

Supplementation of wheat flour with either chickpea flour or several types of protein isolates have shown clear influences on the farinograph, extensigraph and the rapid visco analyser parameters of the fortified flours. The rheological changes of a wheat dough or pasting changes of a wheat starch suspension due to the addition of non wheat components have normally lead to some changes in the quality of the finished products. To investigate further the effect of different type of chickpea proteins and possibly to relate these rheological changes to the quality of the end products, breads were made from wheat flours supplemented with either chickpea flour (15-30%) or with isolates (1-5%) and their qualities were assessed (Fig 7.6.1 and Fig 7.6.2).

Approximate composition of breads supplemented with chickpea flour, unmodified, acetylated, succinylated chickpea proteins, protein hydrolysates and soy protein is given in Table 7.6.1. As expected, breads supplemented with either chickpea flour or proteins had higher protein content than the control bread. Adding more chickpea flour increased protein and fat levels in the fortified breads. As the level of protein substitution increased, the protein

levels in supplemented breads increased but the fat level decreased. The effect of different level of acetylation was recognised at 3% substitution. The extensively acetylated chickpea did not increase the protein level in the fortified bread as much as the mildly acetylated protein. Such an effect was not observed with succinylated protein. The same protein level was found in breads fortified at 3% with either chickpea proteins or with soy protein.

Table 7.6.1 Approximate composition of bread supplemented with a) Soy Protein b) Native, Acetylated, Succinylated and Enzymatically treated Chickpea Protein and c) Chickpea Flour.

Protein added	% Protein added level	% Bread Composition			
		Protein	Fat	Moisture	Carbohydrate
Wheat flour Control	Nil	12	0.63	10.1	76.9
Soy protein	3.0	14.3	0.79	10.2	74.6
Chickpea protein (CP)	1.5	13.3	0.29	10.3	76.4
	3.0	14.1	0.41	9.9	75.6
	5.0	15.6	0.39	9.3	74.7
Acetylated CP					
A6	3.0	14.5	0.40	9.6	75.5
A43	1.5	13.3	0.34	10.0	76.2
A49	3.0	14.2	0.40	10.9	74.5
	5.0	15.5	0.31	9.9	74.3
	3.0	14.0	0.48	10.6	74.7
Succinylated CP					
S16	3.0	14.2	0.36	10.9	74.5
S73	3.0	14.1	0.34	11.9	73.7
S79	1.5	13.1	0.38	10.9	75.6
	3.0	14.1	0.34	10.8	74.8
	5.0	15.3	0.36	9.8	74.5
Papain treated CP	3.0	13.8	0.32	11.0	74.9
Trypsin treated CP	3.0	13.9	0.36	10.8	74.9
Chickpea Flour 1	15	13.3	0.68	9.5	76.5
	30	14.0	1.1	10.4	74.5
Chickpea Flour 2	8.0	12.8	0.38	9.8	76.0
	15	13.4	0.82	9.3	76.4
	25	14.5	1.1	8.7	65.7

The incorporation of non wheat components at high levels in bread doughs can negatively affect appearance, loaf volume and some qualities of the fortified breads. Loaf volume, colour, strength and texture of crust and crumb of breads fortified with chickpea flour and proteins were subjectively evaluated and also objectively measured (Table 7.6.2).

All breads made with fortified wheat flours had lower loaf volume than bread made with only wheat flour. As the level of protein supplementation increased (1.5 to 5%), the loaf volume of the corresponding fortified breads gradually decreased. Also, the loaf volume of breads fortified at a given level gradually decreased as the level of acetylation (16 to 49%) or succinylation (19 to 79%) increased. Adding 3% native chickpea protein or protein hydrolysed by trypsin or papain only reduced the loaf volume about 4%.

Depending on the level of acetylation and succinylation, at 3% substitution, loaf volume of breads fortified with acylated proteins was reduced about 8 to 14% while at 3% supplementation, the loaf volume of soy fortified bread was reduced up to 12%. The adverse effect of chickpea flour on loaf volume of fortified breads was more pronounced. The loaf volume was reduced by 12, 31 and 33% at 8, 25 and 30% substitution, respectively.

Reduction in loaf volume of breads fortified with non wheat flours or proteins has been reported with soybean (Mathews *et al.*, 1970), broad bean (Hussein *et al.*, 1974), faba bean (Finney *et al.*, 1980), navy beans (D'Appolonia, 1977), Great Northern bean (Sathe *et al.*, 1981) and lupin (Ballester *et al.*, 1984). In a very few cases, an increase in loaf volume of bread fortified with grain legume was observed. Sathe *et al.* (1981) found that adding 5% of Great Northern bean protein to wheat flour increased the loaf volume about 8%. Adding the same protein (5%), but prepared by a different procedure, did not improve the loaf volume.

A reduction in loaf volume was normally attributed to a reduction in wheat gluten, by dilution with the fortifying agent. As mentioned previously, the gluten consists by monomeric (gliadin) and by polymeric protein units (glutenin) which contribute to the viscous and elastic properties of wheat dough. Due to the gluten, dough can stretch and rise as it holds the small CO₂ bubbles during the fermentation process and retains its characteristic sponge texture during the baking. With the introduction of non wheat flour or proteins, the glutenous matrix was reduced or modified, thus the formed dough lost its capacity to trap carbon dioxide bubbles, leading to a hard and granular product (Schofield and Booth, 1983; Wrigley and Bietz, 1988). The more gluten replaced by non wheat protein, the greater the loaf volume reduction.

Reduction in loaf volume of the fortified breads was normally attributed to the loss of some gluten in wheat flour. The volume differences found in this work, when supplemented at the

same concentration with different types of proteins found in this investigation, suggested other factors could also influence the volume of fortified breads. An aspect which needs examination is the change in the ratio of maximum resistance to the extensibility (R_m/E). At 4% supplementation with either native chickpea or soy protein, the R_m/E ratio of the fortified flours increased to a similar level (30.1 and 29.6 BU/cm, respectively). As the level of acetylated and succinylated protein supplementation increased (1% to 4%), a striking increase in the R_m/E ratio of fortified wheat flour was observed. While R_m/E of whole wheat flour was only 25.1 BU/cm, adding only 1% of acetylated chickpea protein (ACP49) increased the ratio of the fortified flour to 30.6 BU/cm. When the level of substituted acetylated protein increased to 4%, the ratio increased to 39.5 BU/cm. Replacing wheat flour with 4% succinylated protein (SCP79) strikingly increased the ratio of the fortified flour to 76 BU/cm. Adding non wheat proteins changed the balance of viscous and elastic portion of wheat flour. Consequently, loaf volume of breads fortified with either acetylated or succinylated protein was gradually reduced with an accompanying increase in R_m/E ratio.

The change in the viscoelastic ratio of fortified flours also influenced some physical qualities of their corresponding breads. Compared with the wheat bread, crust and crumb of all fortified breads were harder. The assessment was based on the force (Newton) required to press a whole slice (crust) or the centre of a slice of bread (crumb) to a final depth of 4mm. It is difficult to define precisely which properties (elastic or viscous) had a more pronounced effect on the hardness of the fortified breads. Wheat flours fortified with 4% acetylated and succinylated chickpea proteins had similar dough strength (extensigraph area: 109 and 110 cm^2 , respectively) but different elastic (R_m) and viscous (E) properties. Dough fortified with succinylated chickpea protein was more elastic and less viscous than that fortified with acetylated protein. The extensibility of the former was reduced from 17.5 to 10.8 cm while its maximum resistance increased from 440 BU to 820 BU. Crust and crumb of the dough fortified with succinylated chickpea protein was softer than that of the bread fortified with acetylated proteins. Therefore, besides the viscoelastic ratio, the solubility or water absorption capacity of the supplemented protein may have some influence on the hardness of fortified bread.

Replacing wheat flour with soy or chickpea proteins influenced the fortified breads in terms of loaf volume and textures. The influence depends on the type of protein used and the level of supplementation. Native chickpea protein or enzymatic hydrolysates showed a very mild effect on the quality of their fortified breads and compared favourably with soy protein as a source of protein enrichment for bakery products. The different changes in farinograph, extensigraph and the RVA measurements of wheat flours supplemented with acetylated and succinylated proteins, together with their functional effects on bread quality, provided a useful means of investigating the rheological properties of wheat flour dough.

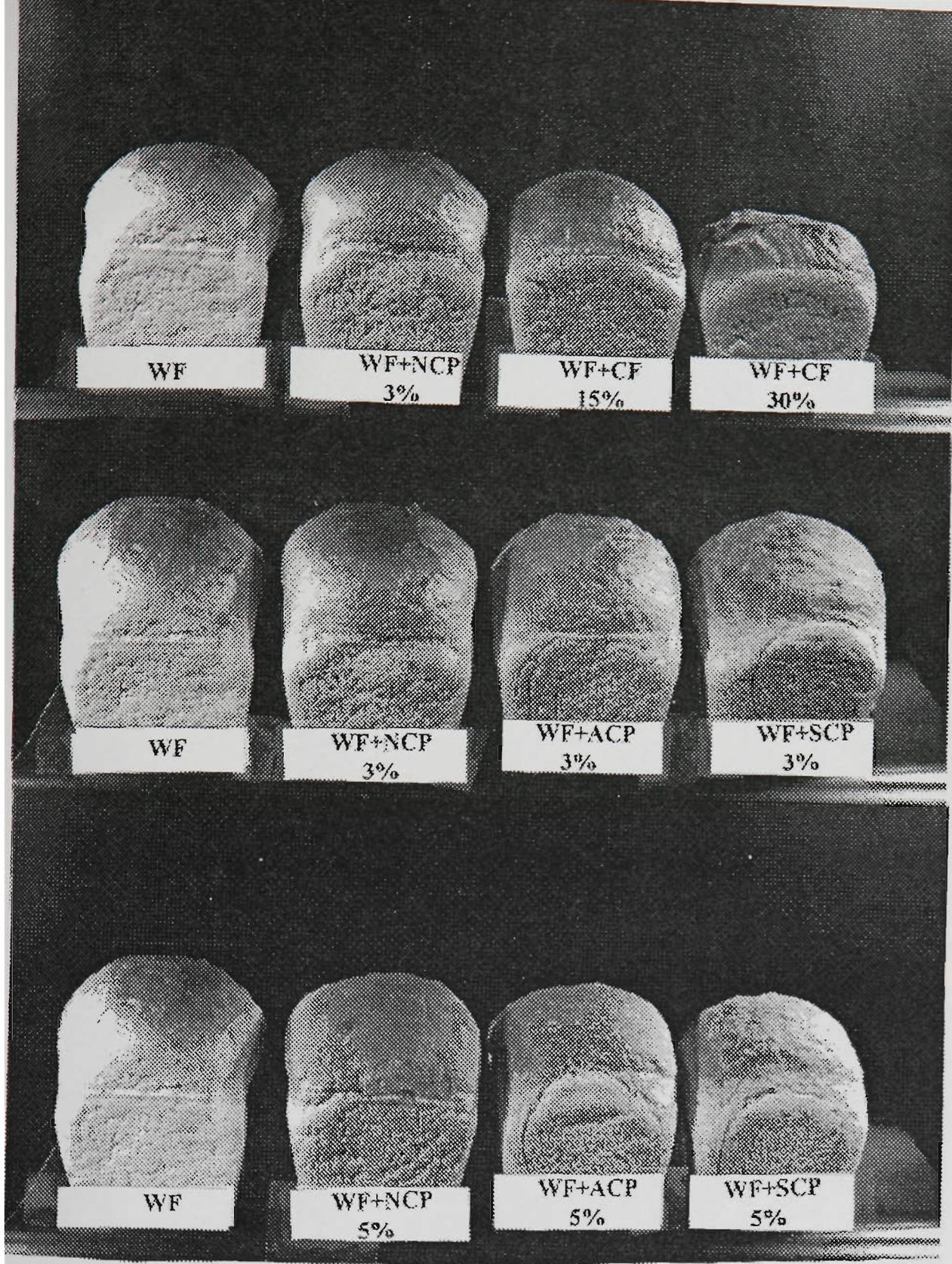


Fig 7.6.1 Wheat flour bread and breads supplemented with chickpea flour or chickpea proteins at different levels. **First row** from left to right: Wheat flour bread only (WFB, control); with 3% of native chickpea protein added (3% NCP+WFB); with 15% of chickpea flour added (15% CF+WFB) and with 30% of chickpea flour added (30% CF+WFB). **Second row** (L to R) WFB; with 3% of native chickpea protein (3% NCP+WFB); with 3% of acetylated chickpea protein (3% ACP+WFB) and with 3% of succinylated chickpea protein (3% SCP+WFB). **Third row**; (L to R) WFB; with 5% of native chickpea protein (5% NCP+WFB); with 5% of acetylated chickpea protein (5% ACP+WFB) and with 5% of succinylated chickpea protein (5% SCP+WFB).

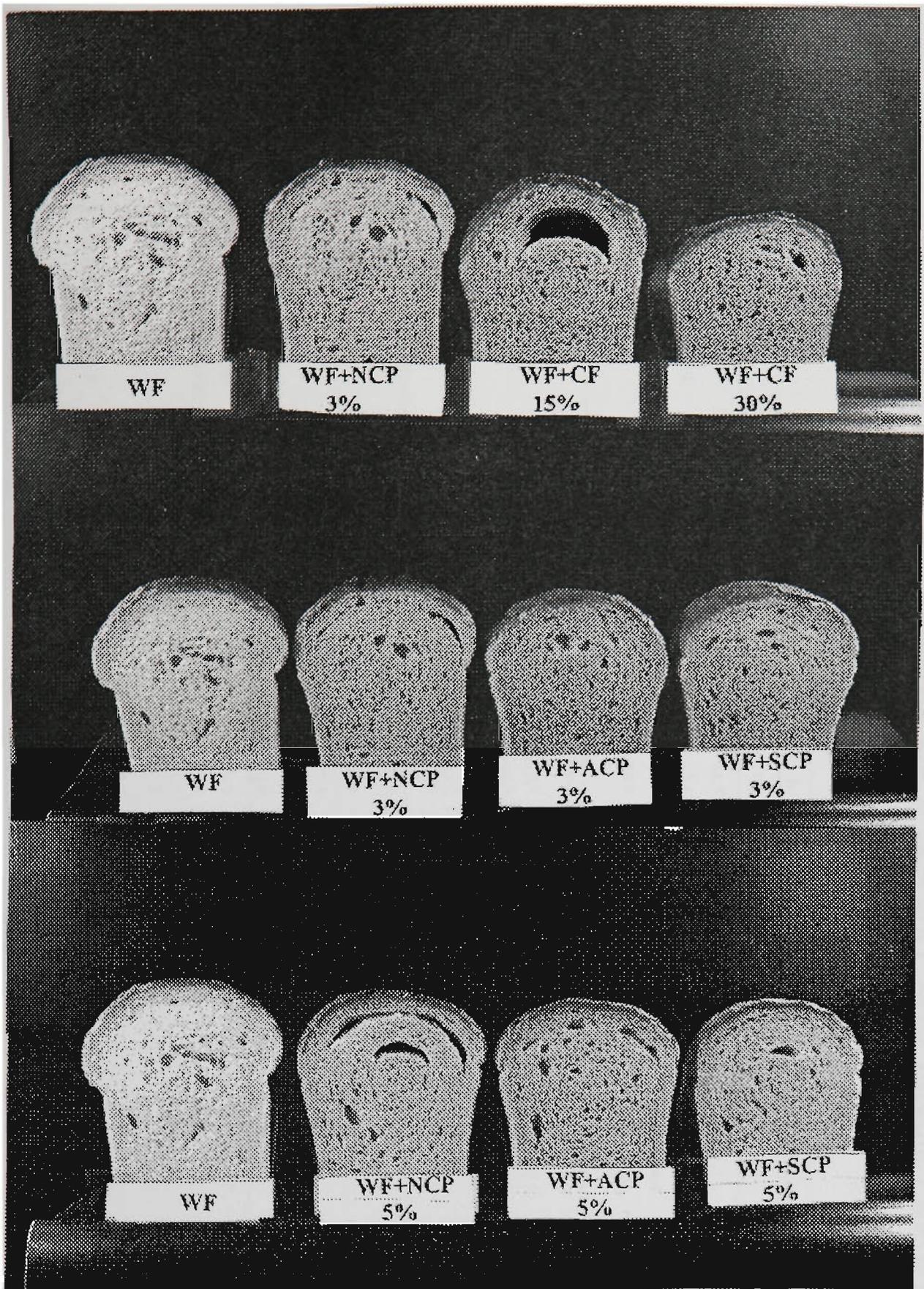


Fig 7.6.2 The effect of non wheat protein supplementation on the texture of wheat flour bread was shown in breads supplemented at 3% of protein levels. **First row** from left to right: Wheat flour bread (WFB, control); 3% of native chickpea protein added bread (3% NCP+WFB); 15% of chickpea flour added bread (15% CF+WFB) and 30% of chickpea flour added bread (30% CF+WFB). **Second row** (L to R) WFB; 3% of NCP+WFB; 3% of ACP+WFB and 3% of SCP+WFB. **Third row**; (L to R) WFB; 5% of NCP+WFB; 5% of ACP+WFB and 5% of SCP+WFB.

Table 7.6.2 Characteristics of bread supplemented with a) Soy protein b) Native, acetylated, succinylated and enzymatically treated chickpea proteins and c) Chickpea flours.

Supplemented Protein	% Protein added	Loaf Vol. cm ³	Colour (Minolta) ^a			Observation	Lloyd		Subjective Evaluation	
			L	a	b		Crust	Crumb	Crumb (softness)	Crumb (visual obs.)
Wheat control	-	1,360	76.2	-1.6	14.2	6.5	273	12.7	15.0	13.0
Soy Protein Chickpea Protein	3.0	1,220	76.4	-1.1	15.7	7.0	327	18.2	12.0	13.0
	1.5	1,270	77.9	-1.3	15.4	7.0	268	10.7	14.0	11.0
	3.0	1,300	75.7	-0.7	16.9	6.5	295	14.7	14.0	11.5
	5.0	1,265	75.5	-0.4	17.7	6.5	292	-	12.0	11.0
ACP6	3.0	1,200	75.1	-0.9	16.0	5.5	299	10.9	14.0	10.0
ACP45	1.5	1,270	76.2	-1.2	15.5	7.0	231	13.3	13.5	11.0
	3.0	1,230	76.5	-0.9	16.2	5.5	321	15.1	13.5	11.0
	5.0	1,135	75.3	-0.5	16.7	6.0	311	-	11.5	11.0
ACP49	3.0	1,185	76.9	-0.8	16.0	5.5	327	16.0	13.0	11.0
SCP16	3.0	1,265	75.7	-0.7	16.2	6.5	282	13.1	13.0	11.0
SCP73	3.0	1,325	76.0	-0.8	16.6	6.0	248	17.7	13.0	11.0
SCP79	1.5	1,315	77.0	-1.2	15.8	6.5	230	13.2	13.5	11.5
	3.0	1,170	76.6	-0.9	16.2	6.0	286	13.2	13.0	11.5
	5.0	1,030	74.5	-0.9	16.8	5.5	287	-	11.0	11.0
Papain hydrolysate	3.0	1,295	74.9	-1.0	16.5	7.5	252	14.0	14.0	12.0
Trypsin hydrolysate	3.0	1,295	76.0	-1.0	16.4	7.5	270	15.5	13.5	11.5
Chickpea Flour 1	15.0	1,185	74.9	-1.7	20.4	7.0	355	22.4	11.0	12.0
	30.0	910	72.1	-1.5	25.6	7.0	373	35.9	9.0	11.0
Chickpea Flour 2	8.0	1,270	76.6	-1.6	7.5	7.0	274	-	13.5	11.5
	15.0	1,200	75.2	-1.5	7.5	7.5	347	-	10.5	11.5
	25.0	945	73.5	-1.3	22.3	7.5	406	-	10.0	12.0

a. Average of 8 readings.

CHAPTER 8

SUMMARY OF RESULTS AND CONCLUSIONS

SUMMARY OF RESULTS AND CONCLUSIONS

The potential applications of chickpea proteins (*Cicer arietinum* L. cv Kaniva) as food ingredients have been investigated and reported in three major parts.

The first part was to identify different extraction conditions and their effects on the extraction yield and characteristics of the resulting proteins. The second part was to define the functional properties of these isolated proteins and relevant factors affecting their behaviour. The third part was to investigate the effects of chemical and to some extent enzymatic modifications on the functional properties of the modified proteins, particularly their flow and rheological properties in wheat flour dough systems.

Chickpea protein extractabilities were substantially similar to those of other grain legumes and oil seeds, being basically pH dependent. More proteins were extracted at alkaline pHs (>8) or at acidic pHs (<3) and the extracted proteins could be precipitated at about pH 4.

Several factors affecting the extraction yield were investigated. Since the extraction involved is normally multifactorial, the combined effect of two or more factors was examined. The result of forty eight combinations were analysed with a fractional analysis of variance. For the main effects investigated, extraction pH and particle size of the grits were the most statistically significant factors. The highest yield was obtained at pH>9 or pH<3. When the particle size was reduced from 26 to 52 mesh, yield could be increased up to 50%, from 37% to 73%. There was no significant advantage in increasing the extraction time to more than 45 minutes in a single extraction process or extracting the protein at low (15°C) or high (35°C) temperature. Salt solution did not improve the extraction yield compared to the use of aqueous solutions.

To confirm the concepts developed in the laboratory, several pilot scale extractions (500 kg) were conducted with commercial equipment and many aspects of the isolated proteins were further investigated.

Extraction at different conditions would not only give different extraction yields but also different type of proteins. Gel filtration patterns, electrophoretic and densitometric analysis, and enzymatic *in vitro* digestion studies indicated that water extracted proteins (CPI-2) are characteristically more similar to those extracted at alkaline pH (CPI-3) than proteins extracted at acidic pH (CPI-1).

Another result of the present study suggested that the relative amount of albumin and globulin in chickpea depend on extraction conditions. Their extractable yields varied with extraction medium and also with different variety. Depending on the procedure employed, albumin could be a major protein fraction. This result casts doubt on the perception that the albumin fraction of chickpea only accounted for a minor proportion of the total proteins. Fractionation with K_2SO_4 solutions produced highly purified albumins. The structural differences of Kabuli and Desi chickpea albumins and globulins could be identified by their corresponding distinct electrophoretic patterns as the albumins were comprised of subunits with high molecular weight and globulin comprised of subunits with low molecular weight. The level of essential amino acids in albumin is always found to be higher than that of globulin. Regardless of the structural similarity found among globulins prepared under different conditions, the effect of processing conditions on some of their functionality could be detected by their *in vitro* digestibility.

The second part was to examine the functional properties of the extracted proteins. Chickpea proteins extracted at pH 2, pH 7 and pH 9 exhibited different functional properties. Like soy protein, CPI-3 showed a characteristic shaped V nitrogen soluble index at the isoelectric point region (pH 4.4-4.8), while those of CPI-1 and CPI-2 were broader. CPI-3, CPI-2 were more soluble than CPI-1, over a range of pH 2-10. At certain pHs they were more soluble than soy protein isolate (SPI).

CPI-3 and CPI-2 also absorbed more oil and water than CPI-1. Emulsion capacity (EC) of three chickpea protein isolates increased with increasing protein concentration and showed a good relationship between their solubility and EC. Their pH/EC curves resembled those of their pH/solubility.

Thermal effect on EC of CPI-3 and CPI-2 was greater than that of CPI-1, indicating that CPI-3 and CPI-2 were more sensitive to heat treatment than CPI-1. Depend on the concentration, salt could decrease or increase the EC of CPI-1, CPI-3 and SPI. The salt effect was less with ES of CPI-2. All emulsions were stable at pH 9 and EC of CPI-3 was more stable than that of SPI at pH 9. With salt addition, all chickpea protein isolates were less stable than soy protein isolate. Foaming capacity (FC) of all chickpea protein isolates, particularly, CPI-3 was higher than that of soy protein isolate. The difference in these functional properties reflected the difference in their composition and structures .

Solubility is an important functional property of all food proteins. To examine the protein solubility, the Kjeldahl procedure is widely used to determine the nitrogen in extracts of the protein but this chemical procedure is still a time consuming and rather laborious assay. Infrared spectroscopy provided a quick, non-destructive and easy to perform alternative

procedure. When protein contents in an aqueous solution are measured there is a significant difference between the values obtained by the Milkoscan, an infrared spectrometer and the Kjeldahl procedure at certain pHs. The Milkoscan, however, could still provide a rapid approach to obtain the solubility of different grain legume proteins over pH 7 - pH 9, a most practical range in food applications. A correction factor derived from each grain family needs to be introduced to compensate the Milkoscan readings at low and high pHs (pH<3 and pH>9). The different response of the three chickpea protein isolates toward infrared measurement reflected their different amino acid composition and also conformation, due to the effects of different extraction conditions.

The third part was to investigate the effect of protein modifications on their functional and rheological properties. To investigate the effects of modification on their functional properties, chickpea proteins were acylated by succinic and acetic anhydride and also partly hydrolysed by papain and trypsin. For acylation, the levels of modification varied with the type of reagent, its concentration and also with different types of proteins involved in the system. The lysines were slightly reduced but the proportion of essential amino acids of acylated proteins was unchanged and still met the required level for children and adults. Acylation did not alter significantly the gel filtration profile of the modified proteins but the SDS-PAGE patterns showed their electrophoretic mobility was retarded. Densitometric scanning of SDS-PAGE confirmed that some structural changes occurred in chickpea proteins due to protein aggregations. Acetylation improved the *in vitro* digestibility of the modified proteins by papain, pepsin and chymotrypsin while succinylation only improved the protein hydrolysate produced by pepsin digestion.

Acetylation improved solubility of chickpea proteins at high alkaline pHs and also their water and oil absorption capacities. The emulsion capacity of acetylated chickpea proteins was higher than that of the native protein but these emulsions were less stable than those of unmodified chickpea proteins. Compared with the unmodified proteins, succinylated proteins were more soluble at pHs above its pI and less soluble at pHs below its pI (pH 4.8). Dilute salt solutions (0.2M NaCl) depressed the solubility of all succinylated proteins and shifted its pI to pH 4.0. Succinylation greatly increased their water and oil absorption compared with unmodified and acetylated proteins. Similarly, succinylation *per se* also greatly improved their emulsifying capacities and stabilities. The extent of succinylation showed very moderate influence on these properties.

Flow properties and the relevant factors affecting the behaviours of aqueous unmodified, acetylated and succinylated chickpea protein dispersions were investigated. These flow characteristics were considerably influenced by a number of factors but they exhibited some common features.

At low protein concentrations (<4% by weight), chickpea protein dispersions possessed Newtonian flow behaviour. At high concentrations (>8%), non-Newtonian flow behaviour became more pronounced with increasing concentration. The non Newtonian behaviour as one moved from unmodified to acetylated and succinylated proteins.

The effect of salt addition on the flow behaviour varied with salt concentration, type of proteins and also with different levels of acylation. The Newtonian flow behaviour of unmodified chickpea protein dispersions remained intact only in a dilute salt solution (0.2M NaCl). The flow behaviour index of the unmodified protein decreased with salt concentrations up to 1 M then increased. Those of succinylated proteins increased proportionally with increasing salt concentrations. The effect of salt addition on the flow behaviour index of acetylated proteins varied greatly with the level of acetylation. The remarkable effect of salt addition, even at a very low concentration (0.2M) on the consistency coefficient (m), Casson yield stress and apparent viscosities of the acylated proteins reflected the structural changes due to the chemical modification.

The effect of dissolving pHs reflected a correlation between protein solubilities and the apparent viscosities. Thermal effects on the flow properties of the protein dispersions varied with different types of proteins and the levels of acylation. For unmodified proteins, the effects of thermal treatments were demonstrated at 55°C. For acetylated proteins, the flow index of each protein dispersion was affected differently by a similar thermal treatment. Apparent viscosities of unmodified protein dispersions increased but those of all acetylated protein dispersions decreased with increasing temperature. The apparent viscosities of succinylated proteins increased at 55°C. A correlation equation to illustrate the effect of temperature on the apparent viscosities and degrees of succinylation was suggested .

Chemical modifications by acetylation and succinylation greatly influenced many aspects of the flow properties of the modified chickpea proteins. The effects varied with the type of acylating reagents and the levels of acylation.

Wheat flour doughs supplemented with chickpea proteins were to examine the behaviour of chickpea proteins as food ingredients in a food system . This also allowed an evaluation of the effects of chemical and enzymatic modifications on the protein's functional and rheological properties. The different changes in farinograph, extensigraph and the RVA measurements of wheat flours, supplemented with acetylated and succinylated proteins, together with their effects on bread quality, provided a useful means to investigate the rheological properties of wheat flour dough. Replacing wheat flour with soy or chickpea proteins influenced the fortified breads in terms of loaf volume and textures. The influence depends on the type of protein used and the level of supplementation. Native chickpea protein or enzymatic

hydrolysates showed a very mild effect on the quality of their fortified breads and can be compared favourably with soy protein as a source of protein enrichment for bakery products.

CHAPTER 9

EXPERIMENTAL METHOD

EXPERIMENTAL METHOD

9.1 WATER ABSORPTION AND PROTEIN EXTRACTION PROCEDURE - LABORATORY SCALE

9.1.1 MATERIAL AND METHODS OF ANALYSIS

9.1.1.1 Material

a. Seed

Seven chickpea (*Cicer arietinum*) and three field pea (*Pisum sativum*) cultivars were studied for water absorption characteristics. All chickpeas, consisting of three Desi (small grain) cultivars (Tyson, Dooen and Amethyst), three Kabuli (large grain) cultivars (Kaniva, Macareena and Garnet) and a cultivar intermediate between Desi and Kabuli (Sensen) were grown in Kaniva, Victoria, Australia. Three cultivars of field pea (Dun, Dundale and Progetta) were grown in Dooen, Victoria. The chemical composition and seed size of these varieties are given in Table 3.1.1 (p.42).

b. Defatted flour

For protein extraction, the seed of the popular Kaniva was selected for investigation. Certified seeds of chickpea (*Cicer aritenium*. cv Kaniva) grown in 1990 were ground in a laboratory mill (3303). The chickpea flour was classified to pass through a 26 mesh (600 μ m) and 52 mesh (300 μ m) screen and be retained on a 100 mesh (150 μ m) screen, which enables them to be collected separately. Chickpea flours with two different particle sizes (150-300 μ m and 300-600 μ m) were defatted by extractions with hexane (flour, solvent :1, 10[w/v] twice) and then air dried (24 hr) at room temperature (25°). The defatted flours were stored in a cool room (4°C) while awaiting further extraction. The defatted flour contains less than 1% fat.

9.1.1.2 Method of Analysis

The protein content was determined in duplicates of 0.2g of (<300 μ m and 300-600 μ m) chickpea flour and defatted chickpea flours, measured by LECO (FP-228, U.S.A.), and

calculated as % N x 6.25. Analysis in duplicates of fat (Soxhlet extraction), ash and moisture were carried out in the chickpea meal, following the standard method (AOAC 1960). Carbohydrates were calculated by difference. The size of each seed variety was determined by the number of seeds counted from 50 g of dried seed sample.

9.1.2 WATER ABSORPTION

9.1.2.1 Water absorption assay

For water absorption, seeds (10 g) of each cultivar were randomly chosen and placed in an incubator for 30 min at 5, 15, 25 and 42°C before soaking in distilled water (75 mL) which was preadjusted to the soaking temperature. Preliminary experiments showed that some cultivars of chickpea disintegrated after prolonged soaking at 42°C. Those cultivars were then soaked at 25°C only. Also, field peas did not absorb notable amounts of water at 5°C and so were studied at 15, 25 and 42°C.

Weight gains were measured after 2, 6, 12, 18 and 24 hour soaking by weighing soaked beans after centrifuging at 1,500 g for 10 min. Initial moisture was determined by heating 5 g of each freshly ground flour in a forced circulation drying oven at 105°C to constant weight (2 hours). All measurements were performed in duplicate.

9.1.2.2 Measurement of water absorption by the Peleg equation

The predicted water absorption was calculated using the following equation proposed by Peleg:

$$M(t) = M_0 + t / [K_1 + K_2 t] \quad [3.1]$$

where $M(t)$ is the moisture content at time t ,
 M_0 is the initial moisture content,
 K_1, K_2 are constants

and $M_{eq} = M_0 + 1/K_2 \quad [3.2]$

where M_{eq} is the equilibrium moisture content as $t \rightarrow \infty$

Equation [1] can also be transformed to the linear relationship:

$$t/[M(t) - M_0] = K_1 + K_2 t \quad [3.3]$$

where $1/K_1$ is the initial rate of absorption.

The unit of K_1 is hr/% weight and that of K_2 is the reciprocal of % weight.

A constant K_3 was developed to describe the temperature effect on water absorption rate by plotting K_1 against temperature as illustrated in the following equation.

$$K_1 = K_3T + K_4 \quad [3.4]$$

Using equation [3.4], a straight line with K_4 as the ordinate intercept and K_3 as the gradient was obtained. The unit of K_3 is hr/% weight.

The fit of equation [3.3] to experimental data on water absorption of seven chickpea cultivars was shown in Fig 3.1.1 (p.43). Peleg's constants K_1 and K_3 , derived from the linear fit and initial absorption rate ($1/K_1$) of these chickpea cultivars are tabulated in Table 3.1.2 (p.44).

Peleg's constant K_2 and calculated equilibrium moisture content (Meq) are presented in Table 3.1.3 (p.45). Fig 3.1.2 showed the experimental and predicted water absorption characteristics of two chickpea cultivars, Dooen and Macareena (p.46).

The effect of temperature on the Peleg's constant K_1 of two types of chickpeas- Kabuli type and Desi type was calculated by using equation [3.4]. The result is presented in Fig 3.1.3 (p.48). A correlation between the seed size of chickpeas and constant K_3 was established (Fig 3.1.4) (p.48).

9.1.3 PROTEIN EXTRACTION

9.1.3.1 Protein Extractability Profile

Defatted chickpea flour (2g) of two different particle sizes was suspended in 50 ml of distilled water (4% suspension) in a 100 ml beaker under continuous agitation by a magnetic stirrer. A series of pH extractions were carried out between pH 2-12 for one hour at constant temperature (25°C). The extraction pHs were adjusted with 0.1M and 1M solution of NaOH or HCl. The pH was continuously monitored to the second decimal place using a JENCO EEL microcomputer pH-Vision 6071. The protein dispersions were then centrifuged (20 minutes, 20°C, 7000 g) in a centrifuge (IEC centra-4R, England). The resulting supernatants were filtered under vacuum in a filter flask through a filter funnel (with a sintered disc of grade 1). The filtrate samples were refrigerated at 4°C.

Processing of results was carried out for averages and standard deviations. The protein content was measured by LECO and calculated as %N x 6.25 and all samples were assayed in

duplicate. The result is given in Fig 3.2.1 as protein extraction curves against extraction pH (p.50).

9.1.3.2 Factors affecting protein extraction yield

Factors that influence the protein extractability, such as extraction pH, particle size, temperature, duration of extraction and solvents were investigated. The combined factors of these parameters were examined with factorial design ($2^4 \times 3$) as shown in Table 3.2.1 (p.54). A typical extraction is presented as follows.

Defatted flour (20g) with different particle sizes was suspended in 140 ml of extraction medium (meal:solvent = 1:7) at different pHs. The suspension was stirred for 30, 45 and 90 minutes at 15°C, 35°C and 55°C. The pH adjustment, centrifugation and filtration were conducted as in Expt. 9.1.2.1. A total of 48 extractions according to the design were conducted.

9.1.3.2.1 Effect of grit particle size and extraction pH

Chickpea seeds as described in 9.1.1.1 were ground in a laboratory mill to grits having the following mesh numbers: 14, 26 and 52. The sieved grit samples were collected on the corresponding mesh screens and used to extract the proteins at pH 2, pH 7 and pH 9 according to Expt. 1.1.2.2. The protein concentration was determined in duplicates of 0.2g as described above. The results are given in Fig 3.2.2 a-c (p.52).

9.1.3.2.2 Effect of Temperature

The effect of temperature on the protein extractability was examined at three different temperatures 15°C, 35°C and 55°C. The protein extraction was conducted at a constant temperature for one hour in a water bath. Protein in the filtrate samples was determined according to Expt. 9.1.2.1. Grits with three particle sizes (14, 26 and 52 mesh) were used to extract the proteins at 15°C and 35°C but only two grits (26 and 52 mesh) were extracted at 50°C. The results are illustrated in Fig 3.2.3 a-b (p.53).

9.1.3.2.3 Effects of Extraction Time

The effect of the extraction time was examined by extracting the protein at two different sets of extraction duration. Grits with a particle size of 52 mesh were extracted over 30, 45 and 90 minutes at pH 2, pH 7 and pH 9 over a range of temperatures 15°C and 35°C. Grits of a particle size of 26 mesh. The results are shown in Fig 3.2.4 a-d (p.54).

9.1.3.2.4 Effects of Extraction Medium

The effects of the extraction medium on the extraction yield of proteins were investigated by extracting the protein with 0.1M NaCl solutions under different conditions. Grits of two particle sizes (26 and 52 mesh) were used to extract the proteins according to the methods of Expt. 9.1.2.1 at three pHs (pH 2, pH 7 and pH 9) and two temperatures (15°C and 35°C). The results are shown in Fig 3.2.5 a-b (p.55).

A total of 48 extractions, based on the major factors which could affect the extraction yield were conducted and the results are tabulated in Table 3.2.2 (p.58).

9.2 PROTEIN ISOLATION

Based on the results gained from the study on the protein extractability of chickpea grits as described previously, a procedure for the isolation of these proteins was developed. This procedure is mainly aimed at producing different types of proteins which would be used for further investigation of the effects of processing conditions on the characteristics and functional properties of these proteins. Only limited efforts were devoted to improving or optimising the extraction or isolation yield.

9.2.1 ISOLATION OF CHICKPEA PROTEINS EXTRACTED AT ACIDIC, NEUTRAL AND ALKALINE pH

The isolation of chickpea protein in laboratory scale experiments consisted of four main steps, involving protein extraction, precipitation, purification and drying. Raw materials used for protein isolation were supplied according to details given in Expt. 9.1.1.1. Sample for analysis were taken during the protein isolation according to recommended procedures in Expt.9.1.3.1. Description of the main steps involved in protein isolation is now presented as follows:

9.2.1.1 Extraction

Chickpea defatted meal (1 kg) was extracted with tap water (5:1 = water:solid) under constant stirring by a stirrer. Protein extractions were carried out at alkaline (pH 9), neutral (pH 7) or acidic (pH 2) conditions using double extraction steps at the same pHs. The protein extraction pH was conducted at a constant pH for one hour at room temperature (25°C) by

using 1N and 3N of NaOH and HCl. The resulted slurry was centrifuged (3,500 g, 20 min., 15°C) by using 750 ml centrifuge bottles of a cool spin LP-784 (MES) centrifuge to yield a residue and a supernatant. The residue was then extracted further with water (1:1.5 = solid:water [w/v]) for 30 minutes and separated as previously described. The combined supernatant was vacuum filtered to form a soluble protein fraction or filtrate sample.

9.2.1.2 Precipitation

The supernatant contained the soluble protein from the extraction step at alkaline (pH 9), acid (pH 2) or neutral (pH 7) conditions. The extract was adjusted to the isoelectric point (pH 4.6-5) with 3M NaOH or HCl, kept for 30 min to yield a curd or precipitate after centrifuging (9,500 g, 20 min., 4°C).

9.2.1.3 Purification

The protein curd from the protein precipitation step was subjected to three washings (10:1= water:solid) using distilled water, pH adjusted to the protein isoelectric point (pH 4.6-4.8) with subsequent centrifuging (9,000 g, 20 min., 15°C) to yield finally the purified protein curd.

9.2.1.4 Drying

The protein curd from the protein purification step was freeze dried (Freezer Drier DynaVac, Model FD-5, Australia) to yield the protein isolate which was ground in a coffee grinder and kept for further tests.

The final yields of proteins extracted at pH 2 (CPI-1), pH 7 (CPI-2) and pH 9 (CPI-3) were 66.8, 54.8 and 73.7 %, respectively (Table 3.2 1). The purity of these proteins was over 91% These three protein isolates were further characterised.

9.2.2 ALBUMIN AND GLOBULIN EXTRACTION

9.2.2.1 Material

Defatted chickpea flours from the seeds of three varieties (Dooen, Kaniva and Garnet) were prepared as described in 9.1.1.1. These seeds were collected from Horsham, Victoria in 1992. The three following methods were employed to extract and fractionate chickpea proteins into albumin and globulin fractions.

The fractionation of chickpea proteins to albumin and globulin fractions was also extended to four more varieties (Tyson, Amethyst, Semsen and Macareena) of two major chickpea groups.

9.2.2.2 Albumin and Globulin Fractionation

Method 1

This procedure was suggested by Bhatta (1982) and used in this investigation with some minor modifications. The slurries were extracted twice and the extraction duration was longer than 45 minutes. Defatted flour (150-600 μ m) (5g) was stirred for 1 hour at 5°C in a 5% K₂SO₄ buffer of pH 7.1 (100 ml). The resulting slurry was centrifuged at 12,000 g, 20 min. and the supernatant was collected. The residue was extracted again with 80 ml of the same solution for 20 min. at 5°C and centrifuged as above. The combined supernatants were dialysed at 4°C for 48 hours against three changes of deionised water, then centrifuged at 13,000 g for 20 min. to give a supernatant fraction (albumin) and a precipitated fraction (globulin). These dialysed fractions were freeze dried and the resulted proteins were used to further characterise each corresponding protein.

Method 2

Singh et al (1980) used this procedure to extract chickpea and other grain legume albumins. Like Method 1, the extraction was conducted twice and longer than 45 minutes. Defatted flour (150-600 μ m) (5g) was extracted at 5°C with K₂HPO₄ buffer, adjusted at pH 7.1 and containing 0.5M NaCl (100 ml). The residue was extracted with a further 80 ml buffer for 20 minutes. The following steps for separation and purification of albumin and globulin were carried out as Method 1.

Method 3

Defatted flour (150-600 μ m) was extracted twice with distilled water (100 ml and, then, with 80 ml). All the steps were followed Method 1.

The experiments were initially conducted at a small scale (5-10g of defatted flours) then, were also conducted at a larger scale (100g of defatted flours). The overall results were tabulated in Table 3.4.1 (p.70).

9.3 ' PROTEIN CHARACTERISATION

The proteins isolated at pH 2 (CPI-1), at pH 7 (CPI-2) and at pH 9 (CPI-3), described in 9.1.3.1 and also the proteins fractionated as globulins and albumins as described in 9.2.2 were subjected to a detailed characterisation based on their amino acid profiles, gel filtration patterns, SDS-PAGE behaviour, densitometric scanning and also *in vitro* digestibility.

9.3.1 AMINO ACID ANALYSIS

Amino acid content was determined as described by Fox *et al.* (1985), except for tryptophan. Amino acids were analysed by ion exchange chromatography on a Waters HPLC system using post column derivatization with ninhydrins. Tryptophan was measured following a HPLC method described by Delhaye and Landry (1986). Each amino acid was presented as % of the total protein.

The amino acid profile of each investigated protein was characterised by the total amount of the essential amino acids, by the sulphur containing and aromatic amino acids as well as by the highest individual acid (s) found in both essential and non essential amino acid fractions. For a nutritional comparison, the recommended amino acid guideline suggested by WHO was employed.

The amino acid profile of CPI-1, CPI-2 and CPI-3 is presented in Table 3.3.1 (p.59) and that of both albumin and globulin fraction isolated from Kaniva, Dooen and Garnet is shown in Table 3.4.2 a, b and c (p.71-72).

9.3.2 GEL FILTRATION

9.3.2.1 Column Packing

Sephadex G-200 (Pharmacia Fine Chemicals, Sweden) was used to pack a Pharmacia column (1.6 x 30 cm). The general procedure for column packing was followed as suggested by the supplier. Dry Sephadex G-200 (30-40 ml/g bed volume per g of dry gel) was mixed with distilled water, placed under microwave oven for 30 seconds and allowed to stand at 25°C for 5 hours. The swollen gel was drained to remove any excess water and placed in a working buffer (0.1M Tris·HCl buffer pH 8.3) then degassed. The degassed gel was poured carefully into the column. The gel was allowed to stand until it settled. The column was connected to a

UV detector (LKB). Before applying the sample, the packed gel was further stabilised by eluting with the working buffer for 2 hours with a control pump at a constant rate (40 ml/hr). The pre mix Sepharose 6 B (Pharmacia LKB Biotechnology AB Uppsala, Sweden) was also used to pack the same column (1.6 x 30 cm). The pre mix gel was washed with an excess amount of the working buffer and degassed before being packed into the column as described previously.

9.3.2.2 Markers

To establish a standard calibration curve for protein molecular determination, a number of markers with known molecular weight were used. These markers were thyroglobulin (670 kD), gamma globulin (158 kD), ovalbumin (44 kD), myoglobin (17 kD) and vitamin B12 (1.35 kD). These markers were diluted with distilled water at a concentration of 5-10 mg/ml before being applied to the gel. The column void volume (V_0) was determined by using Blue dextran ($MW 2 \times 10^6$) and an elution was completed by observing the complete elution of the sharp pink vitamin B12.

9.3.2.3 Sample Preparation and Elution

Protein samples (0.3 g) were mixed with 1 ml of Tris buffer (pH 8.3) or 1 ml of sample buffer under constant stirring (2 hours) and then centrifuged for 1 minute. The soluble protein (0.6 ml) was loaded into the top of the column with a pipette and eluted at a constant flow rate by a control pump. The eluant was passed through a UV detector and a gel filtration chromatogram was established by a recorder. The eluant volume of each peak was recorded and the elutant of each peak was collected, dialysed against distilled water (1:20, v/v) at 4°C for 48 hours before being freeze dried. The peak area was calculated by tracing the peaks onto tracing paper, cutting out and weighing to establish a ratio between different peaks.

9.3.2.4 Estimation of Protein Molecular Weight

Following the method suggested by Pharmacia and using a standard molecular weight curve constructed from a set of makers (9.3.2.2) of known molecular weight, the estimated molecular weight of the protein eluted at several major fractions was calculated.

The gel filtration pattern of CPI-1, CPI-2 and CPI-3, using Sepharose B6 is presented in Fig 3.3.1 (p.61) and the estimated molecular weight of the proteins eluded at different fractions was determined.

The Sepharose B6 revolving pattern of 9 albumin fractions with the estimated molecular weights of the constituted proteins (three varieties: Kaniva, Dooen and Garnet, extracted by three different solvents) are given in Fig 3.4.1 (p.76).

9.3.3 ELECTROPHORESIS

9.3.3.1 SDS Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) was based upon the method of McPherson and Kitchen (1981).

Gel preparation

a. Separating gel

Separating gels were prepared according to Table 9.3.3.1. Acrylamide solution (38g of acrylamide and 2g of methylenebisacrylamide), stocking buffer solution (Tris-HCl buffer, pH 8.8) and water were mixed in a 100 ml beaker and degassed under vacuum for 10 minutes. Ammonium persulphate and TEEMED (N,N,N',N'-tetramethylenediamine), were added and swirled gently to mix, then used immediately. The above amount was sufficient to prepare 2 X 1 mm thick gels in the Hoefer SE 600 gel electrophoresis kit.

b. Stacking gel

In a 30 ml beaker, the acrylamide solution, Tris-HCl buffer (pH 6.8) and distilled water were mixed and degassed under vacuum for 10 minutes, 2% SDS and TEMED were added and stirred gently to mix, then used immediately.

The glass plate sandwich was assembled using two clear plates and two 1.0 mm spacers. The sandwich was locked to the casting stand. The separating gel solution was poured into the sandwich along an edge of one of the spacers until the height of the solution in the sandwich was 12 cm. The top of the gel was slowly covered with a cm layer of water. The gel was allowed to polymerise for 60 minutes at room temperature. The water layer was removed and a 1 mm teflon comb was inserted. The stacking gel solution was poured onto the separating gel in the sandwich and allowed to polymerise for 40 minutes at room temperature.

Table 9.3.1 Recipe for separating gel and stacking gel

Chemical Solution	Separating Gel(ml)		Stacking Gel (ml)
	12%	15%	5%
40% acrylamide	18.0	22.5	3.00
buffer	10.01	27.0	3.25
Distilled water	25.0	1.5	20.50
2% SDS	3.0	3.0	1.25
TEMED (15 μ l/ml)	2.0	3.0	0.50
(NH ₄) ₂ SO ₄ (30 μ g/ml)	2.0	3.0	1.0

9.3.3.2 Loading and Running the gel

The protein samples were dissolved in a sample buffer (stacking buffer, SDS, glycerol, H₂O and mercaptoethanol). Protein standards were dissolved in 0.5 ml distilled water, according to the supplier's instructions and used as molecular weight markers. A set of pre stained standards was used (Bio Rad Catalogue 161-0305 Low range MW). They include phosphorylase B (106 kD), bovine serum albumin (80 kD), ovalbumin (49.5 kD), carbonic anhydrase (32.5 kD), soybean trypsin inhibitor (27.5) and lysozyme (18.5). A standard calibration curve was established with these proteins and was used to estimate the molecular weight of the major subunits of the screened gel.

For loading, the comb was carefully removed from the stacking gel without tearing the edges of the polyacrylamide wells. A 20 μ l auto pipette was used to load the protein samples (15-25 μ l of the soluble protein solution or 1-2 mg protein per well) into the wells by carefully applying the sample as a thin layer at the bottom of the wells.

The gel sandwich was attached to the upper buffer chamber following the manufacturer's instructions. The lower buffer chamber was filled with four litres of electrophoresis running buffer prepared as follows: Tris base (15.1g), glycine (72g) and SDS (5g) were dissolved in distilled water to a final volume of five litres. The sandwich, attached to the upper buffer chamber, was placed into the lower buffer chamber. The upper buffer chamber was filled slowly with electrophoresis running buffer so that the platinum electrode was completely covered.

The power supply applied 15 mA constant current on a slab gel of 1 mm thick, until the Bromophenol Blue tracking dye entered the separating gel. Then, the current was increased

to 25-35 mA. Once the blue dye reached 0.5 cm above the bottom of the separating gel, the power was shut down. The gels were removed and stained for 45 minutes in a dye solution (0.3g Brilliant blue; 150 ml methanol; 50 ml ethanol; water to 500 ml). Destaining was carried out overnight in the destaining buffer (62.5 ml methanol, 125 ml ethanol, water to 1L). After destaining, the gel was photographed and a densitometric measurement of the stained gel was made at 650 nm using a Shimadzu CS-910 TLC scanner (Shimatsu Seisakuso Ltd, Japan).

The SDS-PAGE pattern of CPI-1, CPI-2 and CPI-3 is given in Fig. 3.2.2 (p.62) and that of 9 albumin and globulin fractions isolated from 3 varieties (Kaniva, Dooen and Garnet) with 3 different solvents) is illustrated in Fig 3.4.2 (p.77).

9.3.4 DENSITOMETRIC SCANNING

After destaining, the gel was photographed and densitometric measurement of the stained gel was made at 650 nm using a Shimadzu CS-910 TLC scanner (Shimatsu Seisakuso Ltd, Japan). A standard curve for estimation of protein's molecular weight was established with six markers (9.3.3.2). The molecular weight of the major subunits were estimated by using the standard curve.

The densitometric scanning profile of CPI-1, CPI-2 and CPI-3 is given in Fig 3.2.3 (p.53) together with the estimated molecular weight of each major subunit. Also the major subunits of 9 albumins and 9 globulins, described in 3.3.5 are given in Fig 3.4.3 (p.78).

9.3.5 *IN VITRO* DIGESTIBILITY

The *in vitro* digestibility of chickpea protein was measured by using the method of Tran and Ishii (1975) with some modifications. The hydrolysis of the proteins was conducted at a smaller scale and only the amount of small proteins and large peptides soluble in TCA solutions was determined.

9.3.5.1 Enzymes

Four proteolytic enzymes were employed to examine the *in vitro* digestibility of proteins. Papain (EC 3.4.22.2), porcine pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were purchased from Sigma.

9.3.5.2 *In vitro* digestion Assay

Protein samples were dissolved in a sample buffer (0.05M Tris-HCl) to form a 2% protein solution and the enzymes were dissolved in distilled water. The pH of the samples was adjusted to maximise the reactivity of each enzyme. The optimal pH for pepsin treatment was 2 and other enzymes was 7.5. Protein solution (0.5 ml) was mixed with enzyme solution in each Eppendorf centrifuge tube at a concentration of 2% (Protein:enzyme = 98:2). The protein enzyme mixture was incubated at 37°C for various periods of incubation (1-24 hrs). At scheduled times the digestion was terminated by adding 0.4M trichloroacetic acid (TCA) (1 ml) to the digested mixture and centrifuged for 5 minutes at 15,000 g. Part of the supernatant (0.7 ml) was mixed with 20% (v/v) aqueous Folin Ciocalteau reagent (0.7 ml), 0.4M Na₂CO₃ (2.3 ml) and held for 15 minutes. A control protein-enzyme solution was subjected to the same treatment and the absorbance of the control and mixture was measured at 650 nm with a Hitachi UV spectroscope.

9.3.5.3 *In vitro* digestibility

The *in vitro* digestibility of each protein was presented by the relative activity (RA) of each enzyme employed by measuring the amount of small proteins or large peptides soluble in TCA solutions after the digestion of each enzyme. The relative activity was calculated as follows:

$$RA (\%) = [OD_t - OD_i / OD_i] \times 100$$

Where OD_t is absorbance measured at t time
 OD_i is absorbance measured at initial time

The *in vitro* digestibility of CPI-1, CPI-2 and CPI-3 by papain, pepsin and trypsin is presented in Fig 3.3.6. Similarly, the *in vitro* digestibility of 9 albumins and 9 globulins by papain, pepsin, trypsin and chymotrypsin is presented in Fig 3.4.4 a-d and in Fig. 3.4.5 a-d, respectively (p.80-81).

9.3.5.4 Gel filtration profiles of chickpea protein hydrolysates by papain and trypsin

Three protein isolates (CPI-1, CPI-2 and CPI-3) were hydrolysed with papain and trypsin as described in 9.3.5.2. After 30 minutes, 3, 6 and 24 hours of digestion, the reaction was terminated and the resulting hydrolysates of these proteins by papain and by trypsin were subjected to Sepharose B6 filtration as described in 9.3.2.

The Sepharose B6 resolving pattern of the hydrolysates of these proteins by trypsin is given in Fig. 3.3.4 (p.65) and that by papain was given in Fig. 3.3.5 (p.66). The gradual break down of

each protein over a period of digestion was reflected by the formation of several peaks of smaller molecular weight proteins. The estimated molecular weight of proteins in each major peak was calculated.

9.3.6 PROTEIN EXTRACTION AT PILOT PLANT SCALE

Based on the information gained from previous chapter, chickpea and field pea protein isolation was conducted at a pilot scale level by using the most suitable extraction parameters found with laboratory experiments. The processing equipment used in the wet process is available in Australia. The aims of the experiments at a pilot scale level were:

- a. To investigate the technical aspects of the wet process at conditions closer to an industrial operation.
- b. To obtain sufficient material for further studies, particularly on the functional properties of the extracted proteins under industrial conditions.

9.3.6.1 Materials

500 kg of Kabuli-type chickpeas Kaniva were obtained from a grain merchant and milled to flour. Particle size analysis was done on a Malvern Instruments SB.0D laser particle analyser. Approximate 90% of the chickpea flour were smaller than 374 micron.

9.3.6.2 Processing Equipment

Centrifuges	Westfalia Decanter Model SD 230 Westfalia desludging Clarifier SA-1 and SA-12
Vats	4 x 300L mixing vats fitted with mechanical agitators
Pasteuriser	Spiraflo shell-and -tube, monotube
Ancillary	Pumps, pipes, high-speed Silverson Mixer
Ultrafiltration	DDS pilot plant fitted with diafiltration facility Module Type 37, 6.6m ² , membrane was polysulfone GR 61PP with a molecular weight cut-off of 20 000

9.3.6.3 Extraction Process

40 kg of the flours were mixed with 200 L of filtered water in a 300L vat, with vigorous agitation. The pH was adjusted to either pH 7 or 9 and the mixture agitated for 50 mins. The mixture was pumped through the decanter centrifuge where the protein solution and starch-fibre solids were separated. The residue of starch-fibre was washed with water at the extraction pH and re-separated to recover more of the protein. The two liquid streams were combined and called the "protein extract".

9.3.6.4 Concentration

Concentration of the protein, then, followed one of two processes: (a) Iso-electric precipitation and (b) Ultra-filtration.

a. Isoelectric precipitation

At pH 4.5-4.6, proteins isolated from chickpeas are largely insoluble. The process therefore is to adjust the pH of the protein extract with hydrochloric acid to cause the proteins to be precipitated, followed by centrifugal separation to remove the soluble material (largely soluble carbohydrate), leaving a concentrated protein sludge. Separation was done in a desludging clarifier where the insoluble protein (sludge) is collected in the centrifuge bowl until the bowl is filled, then discharged (desludged). The liquid stream is continuously removed. The protein can be purified by washing the sludge in acid water and then re-separated.

The insoluble protein was then adjusted to pH 7 to make it soluble, pasteurised by heating to 73-75°C, cooled to 5°C and spray-dried. Four batches of chickpea proteins were prepared with this process.

b. Ultrafiltration

In this process, the mixture of soluble protein, carbohydrate and minerals is passed into a selective membrane which has pores only large enough for dissolved salts and sugars to pass through, leaving the larger protein molecules inside the membrane. The filtrate passing through is called the permeate.

The protein extract was adjusted to pH 7. When necessary, it was warmed to 50°C and recirculated in a 6 m² membrane until the solids content (estimated from a refractometer) rose

from 4% to 12.5-13%. The protein concentrate was pasteurised by heating to 73-75°C, cooled and spray dried as described above. This process was successfully applied for field pea extract but it was not suitable for chickpea protein, perhaps due to the high fat content and high foaming character of the chickpea extract.

9.3.6.5 Spray Drying

The two critical parameters in spray drying are the temperature of the hot inlet air (Inlet Temperature) and the temperature of the outlet air (Outlet Temperature). The feed solid was about 12%. Initially, an inlet temperature of 200°C and outlet temperature of 100°C were selected. The resulting protein powder had an overheated odour and dark appearance. When the spray drying was conducted with a 180°C Inlet and 80°C outlet the obtained protein powder had a better odour and lighter appearance.

9.3.6.6 Process control

Solids in the supernatant from each centrifuging step was monitored by centrifuging (10,500 g, 3 min) a 10 ml sample in a calibrated test tube on a laboratory centrifuge. The volume of "sludge" in the bottom of the tube was recorded. From the Decanter this was typically 1-2% at optimum feed rate and < 0.5% from the Clarifier. The test was used to decide on the appropriate feed rate. Samples from each stage were taken and analysed for total solids (drying oven method) and protein (IR Transmission for liquids, Leco Nitrogen analyser for solids).

a. Separation of soluble protein

Separation of the starch and fibre solids from the soluble protein was achieved efficiently in the Decanter. The moisture content of this residue was typically 63%. In the liquid stream from the Decanter, a typical sludge content was 1% which could be reduced by slowing the feed rate. After clarification in the desludger the sludge content of the liquid stream was typically 0.2%, so the crude protein extract before isoelectric precipitation contained less than 0.1% entrained solids.

Separation of the precipitated protein from the liquid stream in the desludger was rather tedious. While flow rates of 200 L/hr were used, the configuration of the centrifuge required much of the liquid stream to be re-circulated into the feed so the process was still very slow and laborious. Desludging every 4 mins. required skill in operating the desludge valve to obtain a solids content over 10%. The solids content of this sludge were typically 9% and were considered too low for efficient spray drying. The liquid entrained in this sludge was not

just water but included other soluble components. Washing with acidified water was done to purify the material.

Pasteurisation of the concentrate was found essential when microbiological analyses of preliminary batches found serious contamination, likely to have come from residual material in the decanter.

b. Ultrafiltration

The solid content in the extracts was rather low (10%) which was not ideal for spray drying. Ultrafiltration appeared to be a suitable mean to concentrate it for a more economic feed solid content when the spray drying approach was considered. For milk powder, a feed solid content was normally expected at the level of 40% or higher for an economic spray drying process. The ultrafiltration process proved to be very successful with field pea extracts but unfortunately difficulties were experienced with chickpea extracts. The possible reasons were the high foaming capacity of chickpea protein and also the fat level.

The protein isolation procedures on pilot plant scale experiments were carried out as an extension of bench scale experiments previously described in Expt. 1.3.1 which were basically followed and adapted to the needs of the pilot plant experiments. When transferring the knowledge obtained with bench scale experiments to pilot scale production, several difficulties were experienced. For a commercial production, further work needs to be done in order to identify the maximum conditions to concentrate the protein level in the extract before being spray dried. Also difficulties experienced with ultrafiltration suggested that defatting of the raw material was a critical step and defoaming of chickpea protein extract was also an important aspect for an effective concentration.

The protein isolates obtained from the pilot scale production were further purified and were used to study the effects of modification on the functional properties, the flow properties as well as the rheological properties of chickpea proteins.

9.4 FUNCTIONAL PROPERTIES OF PROTEINS ISOLATED AT ACIDIC, NEUTRAL AND ALKALINE pH

Chickpea flour protein extracts (CFPE 1, CFPE 2, CFPE 3 extracted at pH 2, pH 7 and pH 9, respectively) and chickpea protein isolates (CPI-1, CPI-2, CPI-3 extracted at pH 2, pH 7 and pH 9, respectively), prepared by the method described in 9.1.3 and 9.2.1 were used to study

the functional properties of chickpea proteins isolated under different conditions. A commercial soy product (Supro 500E) produced in USA with 87.4% protein content was used as reference sample.

9.4.1 SOLUBILITY

The procedure was based upon the method of Anon (1985). 1g of protein sample was dissolved in 50 ml of water over the range of pH 2 - pH 10 by stirring for one hour at room temperature (25°C). The slurries were centrifuged at 3040 rpm for 20 minutes, filtered with a filter paper (Whatman #541) under vacuum. The resulting supernatant was kept in a cool room (4°C) for the testing.

Solubility is calculated as the ratio of soluble protein to total protein. Total protein content in the chickpea seed was measured using a modified Kjeldahl procedure (Tecator application Note 750416-RA for cow milk proteins). Each sample (5g) was added to a digestion tube containing Missouri catalyst ($\text{CuSO}_4/\text{K}_2\text{SO}_4$: 1/35) and concentrated sulphuric acid (20 ml). The digest tube was heated in a heating block at 400°C until the digestion solution was clear. The ammonia formed by adding 40% NaOH to the cooled digest was removed by steam distillation into 4% boric acid and quantitated against standard sulphuric acid. Protein was calculated as %N x 6.25 and all samples were assayed duplicate. Recovery was checked using tryptophan (AJAX, 98%).

The solubility of chickpea proteins isolated at pH 2, pH 7 and pH 9 (Sample CPI-1, CPI-2 and CPI3) and that of the reference soy protein isolate (SPI) is given in Fig 4.1.1 (p.86).

9.4.2 OIL ABSORPTION

Assay on oil absorption was based on the method of Sosulski and McCurdy (1987) and Lin et al (1974). Vegetable bland oil (5 ml) was added to the sample (0.5g) in a 10 ml graduated centrifuged tube. The mixture was allowed to stand for 30 minutes at 25°C. Every 10 minutes it was mixed with a wire or stirring rod for 1 minute and then centrifuged at 3,000 rpm for 15 minutes. Free oil was decanted and the percentage of absorbed oil was determined by volume difference.

The oil absorption capacity of chickpea proteins isolated at pH 2, pH 7 and pH 9 (Sample CPI-1, CPI-2 and CPI-3) and that of the reference soy protein isolate (SPI) is given in Fig 4.1.2 (p.87).

9.4.3 WATER ABSORPTION

Water absorption was determined by the method of Sosulski (1962) with some minor modifications. The protein dispersions were separated at a lower centrifuging force and shorter time (3000 rpm/10 minutes) since at the reported centrifuging conditions (5000 rpm/30 min) no difference in water absorption was observed (Sathe *et al.*, 1982). Distilled water (5 ml) was added to the sample (0.5g) in a 10 ml graduated centrifuge tube and mixed with a glass stirring rod. The mixture was allowed to stand for 30 minutes after each of three mixings and then centrifuged. The amount of water released was measured. The water absorption per gram of sample was measured as follows:

$$WA = \text{water absorption (g)} / \text{total sample used (g)}$$

The water absorption capacity of chickpea proteins isolated at pH 2, pH 7 and pH 9 (Sample CPI-1, CPI-2 and CPI-3) and that of the reference soy protein isolate (SPI) is given in Fig 4.1.2 (p.87).

9.4.4 EMULSIFYING CAPACITY (EC)

Emulsion capacity was measured by the method of Webb *et al.* (1970), modified by Satterlee *et al.* (1973) based on electrical conductivity. EC was expressed as the maximum volume of oil (gr) that could be emulsified by a protein without phase inversion or collapse of the emulsion. The EC was recorded when the electrical conductivity suddenly decreased as the result of the emulsion breaking because the system was beyond maximum emulsified capacity of the oil.

Effect of protein concentration was measured with aqueous suspensions prepared at pH 9 with four concentration levels (0.025, 0.05, 0.075 and 0.125%). Effect of pH was measured with 0.05% aqueous suspensions at pH 5, pH 7 and pH 9. Effect of salt concentration was measured with 0.05% aqueous dispersions prepared at pH 9 with 0.125M, 0.25M, 0.5M and 1M NaCl. Effect of temperature was examined with 0.05% aqueous dispersions prepared at pH 9 and measured at 15, 45 and 75°C. All suspensions (300 ml) were prepared by stirring the protein isolate with solvents for 3 hours at 25°C. A portion of the suspensions (100 ml) was placed in the water bath for thermal equilibrium (30 min) before testing. The dispersion was then homogenised at high speed (Ultra-Turrax PT, 20,000 rpm) for 30 sec. The vegetable oil was then added at a rate of 1 ml/sec into the stirred mixture. EC was recorded by the increase in electrical resistance at the inversion point. The total volume of added oil was recorded and calculated as EC.

$$EC = \text{ml oil} / 100 \text{ ml sample solution}$$

All suspensions were thermally equilibrated at 15°C, except the ones used for the effect of temperature.

The effect of protein concentration on the emulsifying capacity of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) is presented in Fig 4.1.3 (p.87).

The effect of solubilising pH on the emulsifying capacity of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) is illustrated in Fig 4.1.4 (p.88).

The effect of solubilising temperature on the emulsifying capacity of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) is given in Fig 4.1.5 (p.89).

The effect of solubilising salt on the emulsifying capacity of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) is given in Fig 4.1.6 (p.90).

9.4.5 EMULSION STABILITY (ES)

Emulsion stability (ES) was measured by the Method of Acton and Saffle (1970) with some minor modifications. The emulsified suspension as described above was transferred to 250 or 500 ml measuring cylinders and the volume of water released at 0, 30, 60, 180 minutes and 1, 2 and 3 days was recorded. ES was calculated as follow

$$ES = (V_i - V_t) / V_i \times 100\%$$

The effect of protein concentration on the emulsifying stability within 3 hours of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) was given in Fig 4.1.7 a-d (p.91).

The effect of protein concentration on the emulsifying stability in 3 days of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) is given in Fig 4.1.7 e-f (p.92).

The effect of solubilising pH on the emulsifying stability of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) in 3 hours and 3 days is given in Fig 4.1.8 a-d and 4.1.8 e-f (p.93).

The effect of solubilising salt on the emulsifying stability of chickpea proteins CPI-1, CPI-2 and CPI-3 and that of the reference soy protein isolate (SPI) in 3 hours and 3 days is given in Fig 4.1.9 a-d and 4.1.9 e-f (p.94).

9.4.6 FOAMING PROPERTIES

Foaming properties were determined by the method of Mohanty *et al.* (1988). A 3% aqueous protein dispersion (100 ml) at pH 7 was prepared by stirring the sample vigorously with a magnetic stirrer for 30 minutes followed by mixing with a Bamix at setting 4 for 10 seconds. The sample was then transferred to a Kenwood mixing bowl, whipped with a wire whisk at setting 2 for 30 seconds and on maximum speed for 5 minutes. The whipped material was immediately transferred into a graduate 100 ml measuring cylinder. Foam expansion (FE) was calculated as follows:

$$FE (\%) = (V_o - V_i) \times 100 / V_o$$

where V_o is the total volume (Foam volume + Liquid volume = FV+LV)
 V_i is the initial volume of solution
FV is the foam volume at t time

The effect of protein concentration (1-5%) on the foam expansion of chickpea proteins (CPI-1, CPI-2 and CPI-3) and that of the reference soy protein isolate (SPI) is given in Fig 4.1.10 (p.96).

Fig 4.1.11 (p.96) shows that the foam expansion vs pH curve of chickpea protein CPI-3 resembles its solubility vs pH curve while the foam expansion of soy protein isolate decreased gradually with increasing pHs (2-9).

9.4.7 SOLUBILITY OF CHICKPEA PROTEINS MEASURED BY INFRARED SPECTROSCOPY

The solubility of chickpea proteins extracted from chickpea flours and also of chickpea protein isolates were measured by an infrared spectrometer. The obtained results were

compared with those obtained from the conventional Kjeldahl method. The solubility of soy protein and field pea proteins were also examined by both methods.

9.4.7.1 Measured proteins

a. Chickpea protein extracts

Chickpea (*C. arietinum* cv Kaniva) grown at Horsham, Victoria, was dehulled, defatted and the resulting flours (100 g) were suspended in deionised water (500 ml), readjusted to pH 2, 7 or 9 with 1M NaOH or HCl and stirred for 1 hour at 20°C. The extraction pH was maintained during the extraction time by adding NaOH or HCl solutions. The suspension was centrifuged at 4500g for 20 minutes and filtered through Whatman paper #541. Portions of the filtered extracts were diluted 2-fold and 4-fold for comparative measurements. The protein contents were measured by both the Kjeldahl procedure and infrared spectroscopy.

b. Chickpea protein isolates

The filtered extracts from Fig. 4.2.3 (p.101) at pH 2, pH 7 or pH 9 were precipitated at pH 4.2 with 2M NaOH or 2M HCl and centrifuged at 8,000 g for 20 minutes at 25°C to give an acid precipitated curd with approximately 43% protein. The curds were re-extracted in a 5% suspension for 1 hour at 20°C, centrifuged at 3,500 g for 25 minutes and filtered through Whatman #541 filter paper. The filtered supernatants were precipitated with 2M HCl at pH 4.2 and centrifuged at 8,000 g for 25 minutes. The precipitated curds were washed three times with pH 4.2 water and freeze dried to give protein isolates containing 90-97% protein.

c. Field pea protein isolate

Defatted field pea flour (*P. Sativum* cv Bonzer) from seeds grown at Horsham Victoria, was extracted at pH 9 as described above. The protein content of field pea isolate was 89%.

d. Soybean protein isolate

A commercial soy protein isolate (SP-500) was used.

9.4.7.2 Protein assay

a. Kjeldahl procedure

The nitrogen content of protein extracts was measured using a Tecator Kjelttec system (Tecator application Note 750416-RA for cow milk proteins) as described in 9.4.1.

b. Infrared measurement

The protein extracts (30 ml) were measured in triplicate with the Milkoscan Model 104, (Foss Electric, Hillerod, Denmark). This instrument was calibrated with cow milk samples, having a range of protein contents from 2-4%. The milk samples were assayed by the Kjeldahl procedure, corrected to measure true protein only and protein calculated by $N \times 6.38$. The Milkoscan 104 calibration for protein measurement was based on the infrared radiation absorbed at 6.5 mm by the nitrogen hydrogen bond within the peptide skeleton of the protein molecules (Van de Voort, 1980). Consequently the instrument measured only protein nitrogen. Each sample took approximately 1 minute to analyse.

9.4.7.3 Comparison of Milkoscan and Kjeldahl methods

a. Correlation between the two methods

Initial comparison of the two methods was achieved by using chickpea flour protein extracts at three different pHs (CFPE 1, CFPE 2 and CFPE 3) and diluting to three different concentrations, each of which was assayed by both methods. The correlation between the two methods for protein estimation by linear regression analysis are presented in Fig 4.2.1 (p.99). The calculated slopes indicated that the results obtained with the Milkoscan method agreed with those of the Kjeldahl method for extracts at pH 7 and pH 9 ($r=1.011$ and 0.982) but not for pH 2 extract ($r=0.745$).

b. Solubility curve by the two methods

Suspensions of chickpea isolates (CPI-1, CPI-2 and CPI-3), field pea isolate (FPI) and soy protein isolate (SPI) (2%) were prepared over the range of pH 2-10. The filtered extracts were divided into two portions and the soluble protein content assayed by both methods. The solubility curve of chickpea proteins (CPI-1, CPI-2 and CPI-3) as a function of extraction pH, measured by the two methods are given in Fig 4.2.2 (p.100).

c. Comparison of Milkoscan readings and Kjeldahl values

A ratio between the results obtained at each pH by the two methods was calculated (Table 4.2.1) (p.102). The results indicated that the Milkoscan readings at the pHs at which the measured proteins (CPI-1, CPI-2 and CPI-3, also field pea and soy protein) were likely to

have a net negative or positive charge were lower than the Kjeldahl values. A ratio of 0.8 found with proteins measured at acidic pHs (<4) while at high alkaline pHs (>8), a ratio of approximate 1 was established regardless of the processes used.

d. Accuracy of the two methods

To evaluate the accuracy of the Milkoscan at low levels of protein content similar to those in the legume extracts, a commercial skim milk was diluted to several low concentrations (expected Kjeldahl values = 0.046-0.370%) and assayed by the two methods. The results are given in Table 4.2.3 (p.103).

e. Factor affecting the Milkoscan readings

Simple amides were used to evaluate the pH effect on the infrared reading of the N-H bond in peptides. A series of 1% N-methyl and N-ethyl acetamide aqueous solutions were adjusted to pH 2 - pH 10 by adding 0.1M NaOH or 0.1M HCl. The concentration of acetamide in the solution was kept constant by adjusting with deionised water to a constant volume. These solutions (n = 6) were measured by the Milkoscan under the same conditions as with the protein solutions (Table 4.2.2) (p.102).

f. SDS-PAGE profile of the measured proteins

The SDS-PAGE profile of the measured proteins (CPI-1, CPI-2 and CPI-3, FPI and SPI) was obtained by the method described in 9.3.3.1.

9.5 MODIFICATION OF CHICKPEA PROTEIN ISOLATES

9.5.1 CHARACTERISATION OF ACYLATED CHICKPEA PROTEINS

9.5.1.1 Acylation

a. Succinylation

Chickpea protein concentrates (CPC-2 and CPC-3) and isolates (CPI-2 and CPI-3), extracted at pH 7 and pH 9 at a pilot scale level were selected. These protein concentrates and isolates were succinylated with succinic anhydride at four levels by the method of Alford

et al. (1984) in order to establish the effects of acylation reagents (type and concentrations) on the extent of protein modification.

To a 5% aqueous protein dispersion, adjusted to pH 8.5 with 2M NaOH, solid succinic anhydride at a level of 4, 10, 20 or 40 g per 100 g of total protein in the dispersion was added gradually with continual magnetic stirring at 25°C. The reaction was completed when the pH of reaction mixture was constant. The succinylated protein was precipitated at pH 4 with 1M HCl, neutralised and dialysed at 4°C over 48 hours prior to freeze drying.

The extent of succinylation was measured by reactions of the epsilon amino acid groups of lysine and N-terminal amino groups of proteins with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Concon, 1975). The ratio of free lysine in the succinylated proteins to that of native protein is the degree of succinylation (%).

Four succinylated chickpea proteins, prepared from CPI-3 were used in this study. They were SCP 16, SCP 72, SCP 73 and SCP 79 with 16, 72, 73 and 79% succinylated level, respectively.

b. Acetylation

Similarly, the chickpea protein concentrates (CPC-2 and CPC-3) and isolates (CPI-2 and CPI-3) were acetylated with acetic anhydride at five levels (4, 10, 20, 40, 80 ml / 100g) by the same method of succinylation as of the method by Alford et al (1984). The extent of succinylation was measured by the same method as above (Concon, 1975).

Four acetylated chickpea proteins, prepared from the chickpea protein CPI-3 were used in this study. They were ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49 with 6, 26, 45, 47 and 49% acetylated level, respectively.

The extent of acylation of the chickpea protein concentrates and isolates (CPC-2 and CPC-3; CPI-2 and CPI-3) by acetic and succinic anhydride at various concentrations was illustrated in Fig 5.1.1 a-b (p.109).

9.5.1.2 Amino acid composition of acylated chickpea proteins

The amino acid composition of unmodified (CPI-3 or NCP), acetylated (ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) was measured by the method described in 9.3.1. The results are tabulated in

Table 5.1.1. and were compared with the reference proteins as recommended by WHO (p.111).

The ratio between the essential amino acids and the total amino acids, the amount of sulfur containing and aromatic amino acids were also calculated.

9.5.1.3 Gel filtration

Unmodified (CPI), acetylated ((ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79)) were subjected to Sepharose 6B gel filtration by the procedure described in 9.3.2.

The gel filtration pattern of all investigated proteins was similar with three major fractions. For the unmodified protein, the estimated molecular weight of the major protein fractions was 912, 52.5 and 20.9 kD respectively. For the acetylated proteins (ACP 6 and ACP 45) the estimated molecular weight of the major protein fractions was 923, 58.9 and 22.9 kD, respectively.

9.5.1.4 SDS-PAGE profile

SDS-PAGE analysis of the unmodified (CPI), acetylated (ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) was performed as described in 9.3.3. The SDS-PAGE pattern of these proteins is given in Fig 5.1.2 (p.113).

Three major subunits ($R_f = 0.21, 0.33$ and 0.83) was found with CPI. The succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) showed a similar SDS-PAGE pattern but a new distinct band was found at $0.32 R_f$. As the degree of succinylation increased, the number and also the intensity of several bands in the range of 0.34 to $0.83 R_f$ decreased and completely disappeared at 79% succinylation.

The SDS-PAGE pattern of CPI and acetylated chickpea proteins ((ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) was outstandingly similar. As the degree of acetylation increased, a major band at $R_f 0.32$ and $R_f 0.83$ gradually disappeared and several bands at $R_f 0.91$ were formed (p.113).

9.5.1.5 Densitometric scanning profile

The densitometric profile of the unmodified (CPI), acetylated (ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) was obtained by the method described in 9.3.5. The pattern of these proteins is given in Fig 5.1.3 (p.115).

The densitometric profile showed that the unmodified proteins consisted of seven subunits (15.8, 21.9, 24.5, 36.3, 41.7, 44.7 and 52.5 kD). Of these, those of 15.8, 44.7 and 52.5 kD were the major subunits.

Succinylation changed the densitometric patterns of the modified proteins with the disappearance of some subunits with low molecular weights (15.8-24.5 kD) and the formation of a new subunit having higher molecular weights (61-63.1 kD). As the degree of succinylation increased more subunits with low molecular weights disappeared.

Five subunits were found with acetylated chickpea protein (ACP 6) having estimated molecular weight of 16.2, 26.3, 39.8, 45.7, 46.8 and 53.7 kD. As the level of acetylation increased, an increase in retarded mobility of acetylated proteins was observed. The subunit having MW of about 53 kD at 6% acetylation increased to 61.7 kD at 49% acetylation.

9.5.1.6 *In vitro* digestibility

Unmodified (CPI), acetylated (ACP 6, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) were hydrolysed by papain, pepsin, trypsin and chymotrypsin by the procedure described in 9.3.5. The *in vitro* digestibility of each protein was presented as the relative activity (RA) (%) of each enzyme.

The *in vitro* digestibility of acetylated chickpea proteins (ACP 6, ACP 45, ACP 47 and ACP 49) is presented in Fig 5.1.4 a-d (p.118) and that of succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) is in Fig 5.1.5 a-d (p.119).

9.5.2 FUNCTIONAL PROPERTIES OF ACETYLATED AND SUCCINYLATED CHICKPEA PROTEINS

9.5.2.1 Solubility

The procedure described in 9.4.1 was used to examine the solubility of the unmodified and acetylated chickpea proteins in water and in salt solutions (NaCl and CaCl₂ at 0.25M and 0.75M concentrations)

The solubility of the unmodified (CPI) and acetylated (ACP 6 and ACP 49) in water is given in Fig 5.2.1 (p.122), in 0.25M and 0.75M NaCl solutions in Fig 5.2.2 a-b (p.123) and in 0.25M and 0.75M CaCl₂ solutions in Fig 5.2.3 a-b (p.123).

The solubility of the unmodified (CPI) and succinylated (SCP 16 and SCP 79) in water is given in Fig 5.3.1, (p.139) in 0.25M and 0.75M NaCl solutions in Fig 5.3.2 a-b (p.140) and in 0.25M and 0.75M CaCl₂ solutions in Fig 5.3.3 a-b (p.140).

9.5.2.2 Water absorption capacity

Water absorption capacity of the unmodified and acylated chickpea proteins was examined by the procedure described in 9.4.2.

Water absorption of acetylated chickpea proteins (ACP 6, ACP 45 and ACP 49) and the unmodified chickpea protein is presented in Fig 5.2.4 (p.125) and that of succinylated chickpea proteins (SCP 16, SCP 72 and SCP 79) in Fig 5.3.4 (p.141).

9.5.2.3 Oil absorption capacity

Oil absorption capacity of the unmodified and acylated chickpea proteins was examined by the procedure described in 9.4.3.

Oil absorption of acetylated chickpea proteins (ACP 6, ACP 45 and ACP 49) and the unmodified chickpea protein was presented in Fig 5.2.5 (p.126) and that of succinylated chickpea proteins (SCP 16, SCP 72 and SCP 79) in Fig 5.3.5 (p.142).

9.5.2.4 Emulsifying Capacity (EC)

Emulsion capacity of the unmodified and acylated chickpea proteins was examined by the procedure described in 9.4.4, by the method of Webb *et al.* (1970) and modified by Satterlee *et al* (1973).

The effect of protein concentration on the emulsifying capacity of the unmodified chickpea proteins NCP, acetylated (ACP 6, ACP 26, ACP 45, ACP 47 and ACP 49) and succinylated chickpea proteins (SCP 16, SCP 72, SCP 73 and SCP 79) is given in Fig 5.2.6 (p.127) and Fig.5.3.6 (p.143).

The effect of solubilising pH on the emulsifying capacity of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP

79) is given in Fig 5.2.7 and Fig 5.3.7 (p.128, 143). The pH effect on the solubility of some of these proteins is given in Fig 5.2.8 (p128).

The effect of solubilising salt on the emulsifying capacity of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP 79) is given in Fig 5.2.9 and Fig.5.3.9 (p.129, 144).

9.5.2.5 Emulsion stability

The effect of protein concentration on the emulsifying stability within 3 hours of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP 79) is given in Fig 5.2.11a (p.131) and Fig 5.3.10a (p.146).

The effect of protein concentration on the emulsifying stability in 4 days of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP 79) within 3 hours is given in Fig 5.2.11b and Fig 5.3.10 b (p123-147).

The effect of solubilising pH on the emulsifying stability of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP 79) within 3 hours was given in Fig 5.2.12a (p.133) and 5.3.11a (p.148) and after 4 days is given in Fig 5.2.12b (p.134) and Fig 5.3.11b (p.149).

The effect of solubilising salt on the emulsifying stability of the unmodified chickpea proteins NCP, acetylated (ACP 6 and ACP 49) and succinylated chickpea proteins (SCP 16 and SCP 79) within 3 hours is given in Fig 5.2.13a (p.135) and Fig 5.3.12 a (p.150) and after 4 days is given in Fig 5.2.13b (p.136) and Fig 5.3.12b (p.151).

9.6 FLOW PROPERTIES

9.6.1 FLOW PROPERTIES OF UNMODIFIED CHICKPEA PROTEIN (NCP)

9.6.1.1 Solution Preparation

Aqueous protein dispersions were prepared by mixing the protein in an ultra mixer (Tarrux) at 20,000 rpm for 5 minutes. Each dispersion was adjusted to pH 7 with 1M NaOH. To

examine the effect of salt, protein dispersions were prepared with 0.25M, 0.5M, 1M, 2M NaCl solutions

9.6.1.2 Viscosity Measurements

Viscosity determinations were carried out by using a Rheometer (Rheomat 30 Viscometer, Germany). Depending on the nature of these samples, several standard cups (stainless steel) and spindles were used. A double-gap measuring system (cup and spindle) MS-O was suitable for extremely low viscosity thin solutions while the MS-A, B, C systems were suitable for thick dispersions (temperature 0-80°C). Rheomat 30 Viscometer equipped with a viscosity range of 1-17 x 10⁶ (Pa·s), shear rate range of 12 x 10⁻³ - 36 x 10³ (sec⁻¹), shear stress range of 0.6-200 x 10³ (Pa) and 30 different speeds (30 steps subdivided in geometrical progression within a range of 350-0.048 min⁻¹ (rpm). The range of the millivolt meter for torque measurements are 0-0.49 cNm, 0-0.98 cNm, 0-1.96 cNm and 0-4.9 cNm. Measuring ranges can be changed during measurements. Viscosities were determined using the full range of speeds, the speeds being increased and decreased successively to detect any hysteresis. The viscosity readings are presented as follows.

9.6.1.3 Presentation of Data

The Rheomet 30 Viscometer measures the torque to revolve a measuring system, rotating at a constant definite speed. By the use of the torque, its reading is converted into shear rate, shear stress and viscosity ($\dot{\gamma}$ -, σ - and η - values tables).

The following power law equation was used to express flow properties of chickpea protein dispersion

$$\delta = m \dot{\gamma}^n \quad [6.1.1]$$

$$\text{or } \log \delta = n \log \dot{\gamma} + \log m \quad [6.1.2]$$

where δ is the shear stress, $\dot{\gamma}$ is the shear rate (sec⁻¹), n is the flow index and m is the consistency coefficient in the power law relation.

The Casson equation was used to calculate the yield values of the protein dispersions

$$\sigma^{1/2} = k_0 + k_1 \dot{\gamma}^{1/2} \quad [6.1.3]$$

The yield stress value ($\sigma_y = k_0^2$) was obtained by extrapolating the rheogram of $\sigma^{1/2}$ versus $\dot{\gamma}^{1/2}$ to zero shear rate.

The apparent viscosity was calculated by the following equation

$$\eta = m \dot{\gamma}^{n-1} \quad [6.1.4]$$

9.6.1.4 Parameters Affecting Flow Properties

9.6.1.4.1 Protein Concentration

The protein (NCP) solutions were made to 2, 4, 6, 8, 12 and 20% (w/v) concentrations in distilled water adjusted to pH 7 by addition of 1N NaOH. Each protein dispersion was mixed by magnet stirrer for 1 hour and an ultra mixer (Tarrux, 20,000 rpm) for 5 minutes. After being placed in the rheometer, each sample was allowed to rest at a give temperature (25°C) for 30 minutes to thermal equilibrate before 4 readings were recorded at each shear rate. The shear rate / shear stress characteristics of each protein dispersion was measured. This was a typical measurement used to establish a flow curve for all dispersions in this investigation.

A flow curve was established by plotting shear rates against shear stresses. Flow curves of the unmodified chickpea protein (NCP) dispersions at 2-20% concentration is given in Fig 6.1.1 (p.158). Shear stress as a function of shear rate on a logarithmic scale for chickpea protein dispersions at different concentrations (2-20%) was calculated and presented in Fig 6.1.2 (p.158). Effect of shear rate on the apparent viscosities of chickpea protein dispersions at different concentrations was given in Fig 6.1.3 (p.159). Shear rate had no effect on the apparent viscosities of dilute protein dispersions (<4%). Also, the consistency index m , an index of viscosities is also illustrated as a function of protein concentration on a semilogarithmic scale (Fig 6.1.4) (p.160). The effect of protein concentration on the Casson yield stress of the native protein NPI is presented in Fig 6.1.5. This demonstrates the capacity of this equation [6.1.3] to calculate the yield stress of the chickpea protein dispersions (p.160).

The effect of protein concentration on the power law constants (n , m), Casson yield stress and apparent viscosities of chickpea protein dispersions are tabulated in Table 6.1.1 (p.160).

9.6.1.4.2 Temperature

The protein samples (NCP), ACP 6, ACP 45, ACP 49, SCP 16, SCP 73, SCP 79) were made to 8% (w/v) protein concentration in distilled water adjusted to pH 7 by addition of 1N NaOH. Protein dispersions were, then, incubated in 15°C, 25°C, 35°C and 55°C water bath for 30 minutes. The effect of the dissolving temperature on the power law constants (n, m), Casson yield stress and apparent viscosities of chickpea protein dispersions are tabulated in Table 6.1.2 (p.161). At 55°C, heat treatment increased the apparent viscosities of the 8% chickpea protein dispersion (Fig 6.1.6) (p.162).

9.6.1.4.3 Ionic Strength

Chickpea protein samples (NCP, SCP 16, SCP 73, SCP 79) were made to 8% (w/v) protein concentration in 0.2M, 0.5M, 1M and 2M NaCl solutions, adjusted to PH 7 by addition of 1N NaOH. The effect of salt concentration on the power law constants (n, m), Casson yield stress and apparent viscosities of chickpea protein dispersions are tabulated in Table 6.1.3 (p.163). Flow curves of the chickpea NCP in NaCl solutions with different concentrations are given in Fig 6.1.7 (p.163).

9.6.1.4.4 pH

The protein (NCP) were made to 8% (w/v) protein dispersions in distilled water adjusted to pH 2, pH 5, pH 7 and pH 9 by addition of 1N NaOH or HCl solutions. The effect of dissolving pH on the power law constants (n, m), Casson yield stress and apparent viscosities of the chickpea protein dispersions are tabulated in Table 6.1.4 (p.164). Flow curves of the chickpea NCP dispersions with different pHs are given in Fig 6.1.8 (p.165). The solubility and apparent viscosity of the NCP dispersions at pH 2, pH 5, pH 7 and pH 9 are given in Fig 6.1.9 (p.165).

9.6.1.4.5 Effect of protein denaturing agents

8% protein dispersions (NCP) were made with 6M urea and 1% SDS solutions and the flow curves were established as in 9.6.1.4.2. The effect of protein denaturing reagents on the power law constants (n, m), Casson yield stress and apparent viscosities of these chickpea protein dispersions are tabulated in Table 6.1.5 (p.166).

9.6.2 FLOW PROPERTIES OF ACETYLATED CHICKPEA PROTEINS

Three acetylated chickpea proteins (ACP 6, ACP 45, ACP 49) were selected to investigate the effects of concentration, temperature and salt concentrations on the flow behaviour of

these proteins. The effects of different degrees of acetylation on power law constants, Casson yield stress and apparent viscosities were also considered.

9.6.2.1 Protein Concentration

The protein dispersions (ACP 6) were made to 1, 4, 6, 8, 12 and 20% (w/v) concentrations in distilled water adjusted to pH 7 by addition of 1N NaOH.

The flow curve and flow behaviour of these protein dispersion (Fig 6.2.1) (p.169) were established and characterised as in 9.6.1.4.1. Shear stress as a function of shear rate for these acetylated chickpea protein dispersions at different concentrations (1-20%) was presented. The effect of concentration on the flow properties of acetylated proteins was greater than that of the unmodified proteins.

The effect of shear rate on the apparent viscosities of acetylated chickpea protein dispersions at different concentrations is given in Fig 6.2.2 (p.171) by comparison the m values of 8% acetylated with that of the unmodified protein dispersions.

The effect of protein concentration on the power law constants (n , m), Casson yield stress and apparent viscosities of the ACP 6 chickpea protein dispersions are tabulated in Table 6.2.1. To examine the effect of acetylation at different levels, 8% dispersions of the ACP 45 and ACP 49 were also examined as in 9.6.1.4.1. and the results are illustrated in Table 6.2.1 (p.170).

9.6.2.2 Temperature

The protein samples ACP 6 were made to 8% (w/v) protein concentration in distilled water adjusted to pH 7 by addition of 1N NaOH, then, incubated in 15°C, 25°C, 35°C and 55°C water bath for 30 minutes.

The effect of heat treatment on the power law constants (n , m), apparent viscosities and Casson yield stress of chickpea protein dispersions are given in Fig 6.2.3 a-d (p.172). To demonstrate the effect of acetylation at different degrees, each of these flow behaviour parameters were plotted against the degree of acetylation.

9.6.2.3 Salt concentration

Chickpea protein dispersions ACP 16 were made to 8% (w/v) protein concentration in 0.2M, 0.5M, 1M and 2M NaCl solutions and adjusted to pH 7 by addition of 1N NaOH. The

effects of the salt concentrations on the other two proteins (ACP 45, ACP 49) were only examined at 0.2M, 1M and 2M concentrations and were compared with that of the NCP. The effect of salt concentration on the power law constants (n , m), Casson yield stress and apparent viscosities of chickpea protein dispersions are tabulated in Table 6.2.2 (p.174). Flow curves of the chickpea NCP in NaCl solutions with different concentrations are given in Fig 6.1.7 (p.163).

9.6.3 FLOW PROPERTIES OF SUCCINYLATED CHICKPEA PROTEINS

Three succinylated chickpea proteins (SCP 16, SCP 73, SCP 79) were selected to investigate the effects of concentration, temperature, salt concentrations, different degrees of acetylation on the flow behaviour of these proteins with their power law constants, Casson yield stress and apparent viscosities.

9.6.3.1 Protein Concentration

The SCP 16 protein dispersions were made to 1, 4, 8, 12 and 20% (w/v) concentrations in distilled water adjusted to pH 7 by addition of 1N NaOH.

The flow curve (Fig 6.3.1) (p.178) and flow behaviour of these protein dispersions (Table 6.3.1) (p.177) were established and characterised as in 9.6.1.4.1. Shear stress as a function of shear rate for these acetylated chickpea protein dispersions at different concentrations (1-20%) was presented.

The effect of protein concentration on the apparent viscosities of acetylated chickpea protein dispersions at different concentrations is given in Fig 6.3.2 (p.179) by comparison the m values of the succinylated protein SCP 16 and that of acetylated and unmodified protein ACP 6 and NCP, respectively, on a semi logarithmic scale.

The effect of protein concentration on the power law constants (n , m), Casson yield stress and apparent viscosities of the SCP 16 chickpea protein dispersions are tabulated in Table 6.3.1 (p.177). To examine the effect of acetylation at different levels, 8% dispersions of the SCP 73 and SCP 79 were also examined as in 9.6.1.4.1 and the results are also illustrated in Table 6.3.1.

9.6.3.2 Salt concentration

Chickpea protein (SCP 16, SCP 73, SCP 79) were made to 8% (w/v) protein dispersions in 0.2M, 0.5M, 1M and 2M NaCl solutions, adjusted to pH 7 by addition of 1N NaOH. The effects of salt concentrations on the other two proteins (SCP 73, SCP 79) were only examined at 0.2M, 1M and 2M concentrations and were compared with that of the NCP. The effect of salt concentration on the power law constants (n , m), Casson yield stress and apparent viscosities of these chickpea protein dispersions are tabulated in Table 6.3.2 (p.181).

The effect of salt concentrations on the apparent viscosity (AV) at 81.4 sec^{-1} was modelled by linear regression of AV and the logarithm of AV ($\log \text{AV}$) on the salt concentration (SC) and degree of succinylation (DS) and their logarithms. The best fitting regression equation [6.3.1] is presented in page 181.

9.6.3.3 Temperature

The protein SCP 16 dispersions were made to a 8% (w/v) protein concentration in distilled water adjusted to pH 7 by addition of 1N NaOH, then, incubated in 15°C , 25°C , 35°C and 55°C water bath for 30 minutes.

The effect of heat treatment on the power law constants (n , m), apparent viscosities and Casson yield stress of chickpea protein dispersions are given in Table 6.3.3 (p.183). To demonstrate the effect of acetylation at different degrees, each of these flow behaviour parameters were plotted against the degree of acetylation. The effect of temperature on the apparent viscosity (AV) at 81.4 sec^{-1} was modelled by linear regression of AV and the logarithm of AV ($\log \text{AV}$) on the temperature (T) and degree of succinylation (DS) and their logarithms. The best fitting regression equation [6.3.2] is presented in page 182. The apparent viscosities of the succinylated proteins at a certain temperature can be predicted by the equation [6.3.3] presented in page 183.

9.7 RHEOLOGICAL CHARACTERISTICS OF WHEAT DOUGHS SUPPLEMENTED WITH NATIVE AND MODIFIED CHICKPEA PROTEINS

The rheological characteristics of wheat flour doughs supplemented with non wheat proteins were investigated by examining the effects of supplementation with non wheat proteins on the farinograph, extensigraph and pasting characteristics of wheat flour doughs. The effects caused by the non wheat proteins on the baking characteristics were also investigated.

9.7.1 RHEOLOGICAL CHARACTERISTICS

9.7.1.1 Supplemented non wheat proteins

The following non wheat protein samples were used in this study.

a. Flour Commercial wheat flour was purchased locally. Chickpea flour was prepared from the Kaniva variety seed. The seed was cleaned and ground to pass a 100 micron sieve.

b. Chickpea proteins Unmodified, acetylated and succinylated chickpea proteins (NCP, ACP 6, ACP 45, ACP 49, SCP 16, SCP 73, SCP 79) and partially hydrolysed chickpea proteins by papain and by trypsin.

c. Hydrolysed chickpea proteins The unmodified chickpea protein (NCP) was hydrolysed with papain and trypsin for 2 h as described in 9.3.5.

d. Soy protein Commercial soy protein (SP E-500).

9.7.1.2 Rheological determination

a. Farinograph study

Farinograms (RACI 1988) were determined, using 50g of wheat flour or wheat flours supplemented with chickpea flours or other non wheat proteins. Water absorption (%), the amount of water added to sample and calculate the amount necessary to bring the centre of the peak to the 500 BU line), dough development time (min, time taken from the addition of the water to the peak time centred on the 500 BU line, measured to the nearest 0.25 minute), stability time (min) and dough breakdown (BU, difference between centre of the curve at the peak and the centre of the curve 10 minutes after the peak) were recorded. Chickpea flours were supplemented at 8, 15 and 30% while other soy and chickpea proteins were added at 1, 2, 4 and 8%. The results are tabulated in Table 7.2.1 (p.195) and 7.2.2 (p.199).

b. Extensograph study

Extensograms (RACI 1988) were produced by using 300g of wheat flour and chickpea flour as a control. Soy was supplemented at a 4% level and chickpea proteins were supplemented at a 1%, 2% and 4% level. Extensigraph measurements of fortified wheat flour doughs included water absorption (%), extensibility (E, cm), maximum resistance to stretching (R_m, cm), Viscoelastic ratio (R_m/E, BU/cm), dough strength (A, cm²). The results are presented

in Table 7.3.1 (p.201) and the extensograms of wheat, chickpea flour, wheat supplemented with unmodified, acetylated and succinylated chickpea proteins at three levels are given in Fig 7.3.1 (p.202).

c. Pasting characteristics

Pasting properties of wheat flour and wheat flour supplemented with non wheat proteins were measured by the Rapid Visco Analyser (RVA) (Newport Scientific Pty, Ltd, NSW, Australia). Samples were tested in the RVA in duplicate using the STD 1 profile by adding the total sample weight of 3.500 g as is, to 25 ml distilled water. Chickpea flour was replaced at 15 and 30% level but all proteins were supplemented at only the 3% level. The pasting characteristics were recorded over 18 minutes, initial equilibration at 50°C for 2 minutes, heating 2 minutes to maximum of 95°C, holding 6 minutes at 95°C and cooling 8 minutes to minimum of 50°C.

As illustrated in Fig 7.4.1 (p.205), the RVA curves were analysed by ten pasting parameters related to time (initial peak time, IPT; onset time, OT; and peak time, PT) to viscosity (peak viscosity, PV; breakdown viscosity, H; difference between peak and breakdown viscosity, P-H) and to slope (ascending slope, AS and descending slope, DS) (Ryu *et al.*, 1993).

The results are tabulated in Table 7.4.1, Table 7.4.2 and Table 7.4.3 (p.204, 206, 207).

7.1.2.3 Factors affecting the RVA peak viscosity

Factors may affect the RVA peak viscosity were investigated using the procedure described in 9.7.1.2 c.

a. Starch concentration A comparison was made between 100% wheat flour (3.500 g) and 97% wheat flour (3.395 g) alone. The result is given in Fig.7.5.1 (p.209).

b. Wheat flour lipids A comparison was made between wheat flour and wheat flour /chickpea protein blends. The result is given in Fig.7.5.2 (p.210).

c. Protein supplementation A comparison was made between wheat flour alone and with 3% (0.105 g) soy protein supplemented flours. The results are given in Fig. 7.5.3 (p.211).

d. Type of protein A comparison was made between wheat flour and those supplemented with bovine serum albumin. The results are given in Fig 7.5.4 (p.212).

9.7.2 BAKING PROPERTIES OF WHEAT FLOUR SUPPLEMENTED WITH CHICKPEA PROTEINS

9.7.2.1 Wheat flour-Non wheat protein blends

All blends were prepared by replacing dry solids of commercially milled bread wheat flour with non wheat proteins at different levels. Chickpea flours were supplemented at 8, 15, 25, 30% levels. Soy protein, ACP 6, ACP 49, SCP 16, SCP 73, papain and trypsin treated chickpea proteins at a 3% level while NCP, ACP 45, SCP 79 at 1.5, 3 and 5% levels.

9.7.2.2 Baking test

The baking test (Panozzo et al 1990) was carried out using the wheat flour-non wheat protein blends. Briefly, the blend was mixed with yeast, salt, malt, improver solution and water (determined by baking water absorption) for 30 min, then returned to the prover for 105 min before the first punch. The punched dough remained in the prover for 50 minutes before the second punch and was panned after 25 minutes. The panned dough was returned to the prover for 55 minutes before baking for 35 minutes.

9.7.2.3 Baking performance

Baking performance was evaluated by loaf volumes, colour, strength of crust and crumb.

a. Composition of baked products

The approximate composition of breads supplemented with chickpea and soy proteins is given in Table 7.6.1 (p.214). Protein, fat, moisture and carbohydrates (%) were measured as described in 9.1.1.2.

b. Colour measurement and observation

Colour of the breads was measured by a Minolta Chromameter (CR 200, Japan) using the L (lightness), a* (colour hue) and b* (saturation) values. A white standard tile (L: 97.86, a*: -0.43 and b*: +2.06) provided with the instrument was used to calibrate the chromameter. Observation was also made with high score for the darker bread 10 hours after baking (Table 7.6.2) (p.219).

c. Crumb softness and crumb texture

Crumb softness and texture were subjected to a subjective evaluation with a maximum of 20 points. A high score was given to a harder crumb texture (pressing on the bread surface). For an objective measurement, textural quality of the breads was measured by a Lloyd instrument (1000 R Model) using bread crust (whole slice, 12 mm thickness) and crumb (the same bread slice without crust). A 5 kN load cell and an OTMS (Ottawa Texture Measuring System) type forward extrusion test cell was used. The test cell was screwed onto the load cell and was driven downward to the bread slice at a speed of 100 mm/ min through a travel distance of 6 mm. The average extrusion force was read from the force deformation curve plotted by the computer which was connected to the Lloyd instrument's sensor panel.

d. Loaf volume

Loaf volumes were measured by rapeseed replacement between 30-90 minutes after baking.

These characteristics of breads supplemented with non wheat proteins (soy and chickpea) are illustrated in Table 7.6.2 (p.219) and the photo of these breads are given in Fig 7.6.1 and Fig 7.6.2 (p.217, p.218).

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Water Absorption in Chickpea (*C. arietinum*) and Field Pea (*P. sativum*) Cultivars using the Peleg Model

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ABSTRACT

Moisture absorption in seven chickpea cultivars and three field pea cultivars was investigated at 5, 15, 25 and/or 42°C using the Peleg model ($M(t) = M_0 + t/[K_1 + K_2t]$). The Peleg constant K_1 varied with temperature. At a given temperature, the lower the K_1 , the more water was absorbed. The Peleg constant K_2 was almost unaffected by temperature and could be used to predict the equilibrium water absorption. A constant K_3 expressing the temperature effect on water absorption ($K_1 = K_3T + K_4$) was developed to distinguish two types of chickpea — Desi and Kabuli. All chickpeas had similar composition and initial moisture. The difference in water absorption rate was probably due to thickness and structure of the seed coat. The Peleg model could be used to predict water absorption in chickpea and field pea.

Key Words: chickpea, water absorption, Peleg equation.

INTRODUCTION

GRAIN LEGUMES are important sources of protein which are potential ingredients for many processed foods such as meat products, dairy blends and infant formulae. Regardless of end use, soaking grain legumes in water is common either to facilitate cooking or to enhance protein extraction. Since soaking is a long process and conditions affect cooking, nutritional qualities and physical properties of end products, the soaking process needs to be characterised for practical applications (Sefa-Dedeh et al., 1978; Kon, 1979). Water absorption in this soaking process needs to be predictable as a function of time and temperature.

Water absorption of soybean, pigeon pea, cowpea and rice has been investigated and several rather complex models, mainly based on Fick's law of diffusion, have been suggested to describe it (Hsu, 1983; Singh and Kulshrestha, 1987; Sefa-Dedeh and Stanley, 1979; Hendrickx et al., 1987). Peleg (1988) proposed a simple, empirical equation not derived from any set of physical laws or diffusion theories, to model water absorp-

tion of food materials. The model has been used to predict long range moisture absorption from experimental data obtained over a short time. The general application of the model has been confirmed by Sopade and Obekpa (1990) with soybean, cowpea and peanut. They reported that the model could adequately describe absorption behavior of these grain legumes at 2°C to 42°C.

Our objective was to evaluate the general applicability of the Peleg model and investigate the effect of grain composition and type on water absorption in several chickpea and field pea cultivars.

MATERIALS & METHODS

SEVEN CHICKPEA (*Cicer arietinum*) and three field pea (*Pisum sativum*) cultivars were studied for water absorption characteristics. All chickpeas, consisting of three Desi (small grain) cultivars (Tyson, Dooen and Amethyst), three Kabuli (large grain) cultivars (Kaniva, Macareena and Garnet) and a cultivar intermediate between Desi and Kabuli (Semsen) were grown in Kaniva, Victoria, Australia. Three cultivars of field pea (Dun, Dundale and Progretta) were grown in Dooen, Victoria.

Official AOAC methods (1985) were used for fat, moisture and ash determinations. Protein (Nitrogen x 6.25) was measured by a Leco Nitrogen Analyzer (Model FP228) and carbohydrates were calculated by difference. The size of each seed variety was determined by the number of seeds counted from 50g of dried seed sample.

For water absorption, seeds (10g) of each cultivar were randomly chosen and placed in an incubator for 30 min at 5, 15, 25 and 42°C before soaking in distilled water (75 mL) preadjusted to soaking temperature. Preliminary experiments showed that some cultivars of chickpea disintegrated after prolonged soaking at 42°C. Those cultivars were then soaked at 25°C only. Also, field peas did not absorb notable amounts of water at 5°C so were studied at 15, 25 and 42°C. Weight gains were measured after 2, 6, 12, 18 and 24 hr soaking by weighing soaked beans after centrifuging at 1,500 g for 10 min. Initial moisture was determined by heating 5g of each freshly ground flour in a forced circulation drying oven at 105°C to constant weight (2 hr). All measurements were performed in duplicate. The predicted water absorption was calculated using the following equation proposed by Peleg:

$$M(t) = M_0 + t/[K_1 + K_2t] \quad [1]$$

where $M(t)$ is the moisture content at time t , M_0 is the initial moisture content, K_1 , K_2 are constants, and

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Table 1—Chemical composition of chickpeas and field peas

Grain	Cultivar	Moisture %	Ash %	Fat %	Protein %	Carbohydrate*	Relative size ^b
Chickpea	Tyson	8.60	2.52	3.27	23.1	62.5	377
	Dooen	8.38	2.62	2.98	23.1	62.9	284
	Amethyst	8.34	2.59	3.29	23.3	62.5	391
Intermediate	Semsen	8.03	2.64	3.07	23.8	62.4	231
Kabuli	Kaniva	8.15	2.86	5.05	23.7	60.2	139
	Macareena	8.37	2.91	4.56	23.2	61.0	112
	Garnet	8.39	2.76	4.54	23.4	61.0	137
Field pea	Dun	8.85	2.71	0.98	24.1	63.4	215
	Dundale	8.73	2.63	0.88	23.9	64.0	233
	Progretta	8.52	2.55	0.97	24.0	63.0	186

* Carbohydrate calculated by difference.

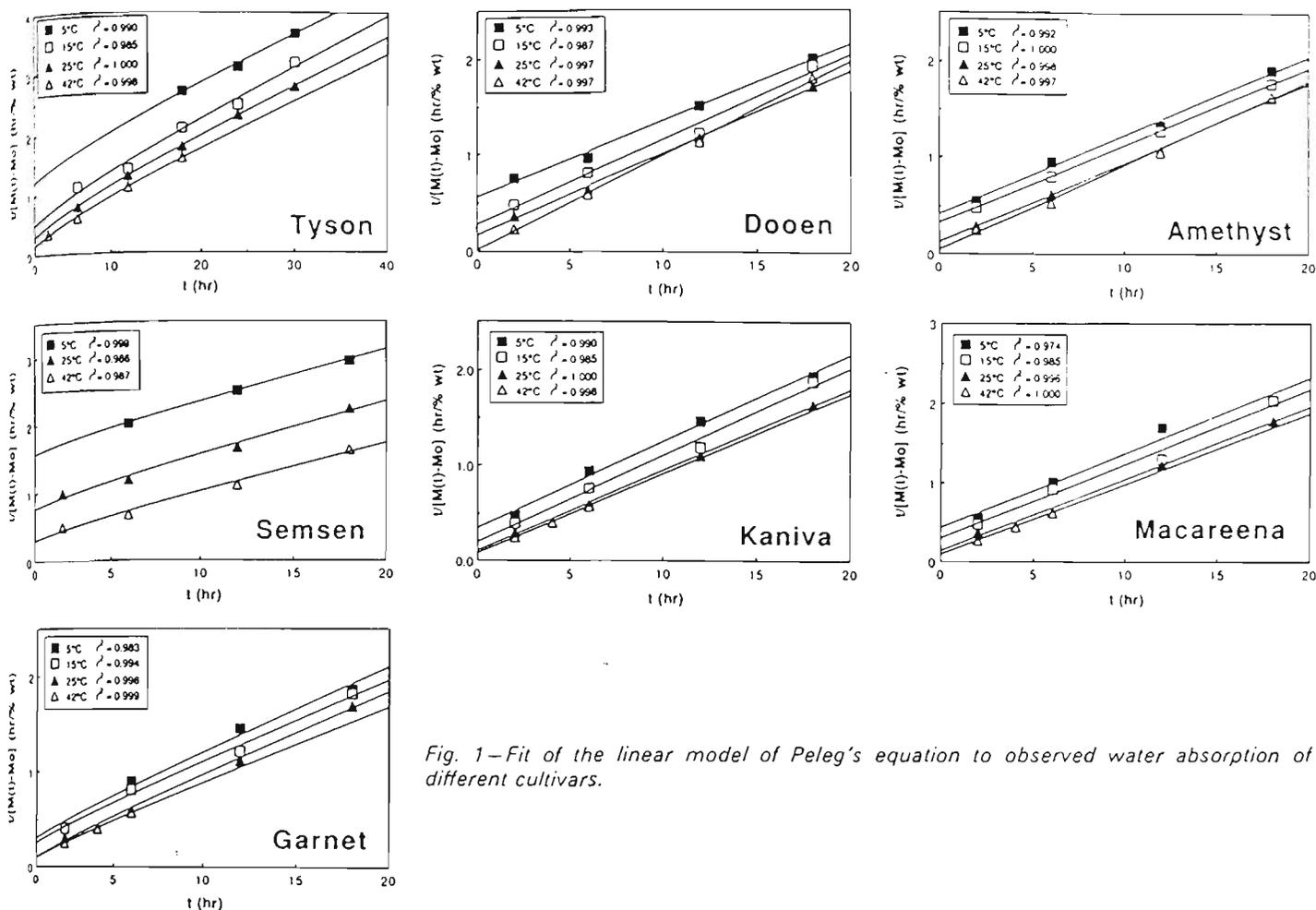


Fig. 1—Fit of the linear model of Peleg's equation to observed water absorption of different cultivars.

$$Meq = Mo + 1/K_2 \quad (2)$$

where Meq is the equilibrium moisture content as $t \rightarrow \infty$. Equation [1] can also be transformed to the linear relationship:

$$t/[M(t) - Mo] = K_1 + K_2 t \quad (3)$$

where $1/K_1$ is the initial rate of absorption. The unit of K_1 is hr/% weight and that of K_2 is the reciprocal of % weight.

RESULTS & DISCUSSION

GROSS chemical compositions of the seven chickpea and three field pea cultivars (Table 1) showed no differences ($P < 0.05$) in moisture, fat, protein, and carbohydrate among the cultivars. The fit of equation [3] to experimental data on water absorption of the seven chickpea cultivars was demonstrated (Fig. 1). Values of constants K_1 and K_2 derived from the linear fit (Table 2 and 3) showed regression coefficient ranges from 0.974 to 1.000 (Table 2) and degree of fit was generally good. The plots of $t/[M(t) - Mo]$ vs. t also showed no apparent curvature. Similar results were found for water absorption of field peas (Table 2 and 3). The capacity of the Peleg model [1] to predict the entire water absorption process, using limited data, is demonstrated in the characteristic moisture absorption curves for chickpea (Macareena and Dooen, Fig. 2) and field pea (Progretta and Dundale, Fig. 2) at three different temperatures. The moisture absorption curves show that water content increased with increased soaking time and temperature. Similar results have been reported for soybean, peanut, pigeon pea, rice and cowpea (Singh and Kulshrestha, 1987; Engels et al., 1987; Hendrickx et al., 1987; Sopade and Obekpa, 1990).

Data for the Tyson cultivar of chickpea at 5°C were not collected at soaking times < 12 hr since weight gains at shorter times were small and Peleg indicated that the model did not apply under such conditions (Fig. 1, Tyson). Generally, for the Desi type, the equation could give a fairly good fit after a

Table 2—Peleg constants (K_1 , K_2) and initial absorption rate ($1/K_1$) of chickpeas and field peas

Grain	Cultivar	°C	K_1 (hr/% wt)	$1/K_1$ (% wt/hr)	K_2 (hr/% wt °C)	
Chickpea	Tyson	42	0.15	6.67		
		25	0.30	3.38		
	Desi	15	0.49	2.06	1.2×10^{-2}	
		5	1.19	0.84		
Chickpea	Dooen	42	0.01	72.3		
		25	0.16	6.12		
	Amethyst	15	0.28	3.62	1.3×10^{-2}	
		5	0.55	1.82		
Chickpea	Amethyst	42	0.04	27.6		
		25	0.12	8.26		
	Intermediate	Semsen	15	0.32	3.13	1.1×10^{-2}
			5	0.41	2.45	
Chickpea	Intermediate	42	0.20	3.39		
		25	0.76	1.31	3.4×10^{-2}	
	Kabuli	Kaniva	5	1.56	0.64	
			42	0.07	14.9	
Chickpea	Kabuli	25	0.10	9.91		
		15	0.19	5.15	0.7×10^{-2}	
	Garnet	Garnet	5	0.34	2.94	
			42	0.09	11.0	
Chickpea	Garnet	25	0.10	10.2		
		15	0.25	4.02	0.6×10^{-2}	
	Macareena	Macareena	5	0.30	3.36	
			42	0.08	12.6	
Chickpea	Macareena	25	0.13	7.66		
		15	0.29	3.42	0.8×10^{-2}	
	Field pea	Dun	5	0.38	2.66	
			42	0.38	2.60	
Field pea	Dun	25	0.66	1.50	4.4×10^{-2}	
		15	1.74	0.57		
	Dundale	Dundale	42	0.37	2.67	
			25	0.68	1.48	3.3×10^{-2}
Field pea	Progretta	15	1.33	0.75		
		42	0.10	9.94		
		25	0.22	4.50	2.4×10^{-2}	
Field pea	Progretta	15	0.44	2.29		

Table 3—Peleg constant K_2 and calculated equilibrium moisture content (Meq) of chickpeas and field peas

Grain	Cultivar	°C	K_2 (% wt ⁻¹)	Mean K_2 (% wt ⁻¹)	Meq (% wt)	Mean Meq (% wt)
Chickpea	Desi	42	7.9×10^{-2}	8.2×10^{-2}	13.7	13.1
		25	8.2×10^{-2}		13.0	
		15	8.6×10^{-2}		12.5	
		5	8.1×10^{-2}		13.2	
	Dooen	42	9.7×10^{-2}	8.7×10^{-2}	11.2	12.9
		25	8.5×10^{-2}		12.7	
		15	8.8×10^{-2}		12.3	
		5	8.0×10^{-2}		13.4	
	Amethyst	42	8.8×10^{-2}	8.3×10^{-2}	12.3	12.9
		25	8.2×10^{-2}		12.3	
		15	8.0×10^{-2}		13.3	
		5	8.2×10^{-2}		13.0	
Intermediate	Semsen	42	7.1×10^{-2}	7.5×10^{-2}	15.0	14.2
		25	6.8×10^{-2}		13.6	
		5	7.6×10^{-2}		14.0	
Kabuli	Kaniva	42	8.4×10^{-2}	8.8×10^{-2}	12.7	12.2
		25	8.5×10^{-2}		12.6	
		15	9.1×10^{-2}		11.8	
		5	9.1×10^{-2}		11.8	
	Macareena	42	9.0×10^{-2}	9.2×10^{-2}	12.0	11.7
		25	9.1×10^{-2}		11.8	
		15	9.4×10^{-2}		11.5	
		5	9.5×10^{-2}		10.6	
	Garnet	42	7.9×10^{-2}	8.6×10^{-2}	13.5	12.5
		25	8.7×10^{-2}		12.3	
		15	8.6×10^{-2}		12.5	
		5	9.0×10^{-2}		11.9	
Field pea	Dun	42	6.9×10^{-2}	7.1×10^{-2}	15.3	15.1
		25	8.5×10^{-2}		12.5	
		15	8.8×10^{-2}		18.1	
	Dundale	42	7.3×10^{-2}	7.6×10^{-2}	14.6	14.0
		25	8.2×10^{-2}		13.1	
		15	7.4×10^{-2}		14.4	
	Progretta	42	7.7×10^{-2}	7.1×10^{-2}	13.9	14.4
		25	7.3×10^{-2}		14.6	
		15	7.3×10^{-2}		14.6	

rather long period of soaking (6–12 hr) and for the Kabuli type during the first 6–7 hr of soaking.

Differences in water absorption rate and water uptake among chickpea cultivars are exemplified by Macareena and Dooen (Fig. 2). In field peas, Progretta had a higher water absorption than the Dundale (Fig. 2). Sopade and Obekpa (1990) suggested that the difference in water absorption between soybean and peanut was due to differences in fat and protein content. However, differences in fat, protein and carbohydrate among our chickpeas and field peas were not significant (Table 1). The initial moisture content of the beans would affect the rate of absorption (Smith and Nash, 1961; Chittenden and Hustrulid, 1966). In our study, the difference in initial moisture was also insignificant. Consequently, there is probably a factor other than gross fat, protein and carbohydrate that contributes to differences in water absorption of different chickpea and field pea cultivars.

Seed size should be considered because water absorption rate is influenced by surface area. The smaller the seed, the larger the relative surface area for given seed weight (Ituen et al., 1985). The reciprocal of constant K_1 derived from equation [3] is used to present the initial absorption rate (Table 2). The results clearly indicated the effect of temperature on initial absorption rate which increased with increasing temperature. The large seed cultivars (Kabuli type) had higher absorption rates than the smaller seed cultivars (Desi type). Average $1/K_1$ values of three Kabuli cultivars at 15 and 25°C were 4.19 and 9.25% wt/hr respectively, while corresponding $1/K_1$ values of three Desi cultivars were 2.93 and 5.91% wt/hr respectively (Table 2). This finding could not be compared with other studies which focused on water absorption between different types of grain legumes rather than different cultivars. However, it suggested that seed size or water absorption surface of seed coat alone was not a major factor affecting water absorption, at least among chickpeas.

King and Ashton (1985) studied water absorption by whole soybeans and soybeans with seed coat partially abraded. They reported that the rate of absorption by the abraded seeds was more than that for unabraded seeds. Similar results have been reported by Singh and Kulshrestha (1987). Sopade and Obekpa (1990) reported no major difference in absorbed water between dehulled and whole peanuts. Chickpea seed coat is much thicker than that of peanut seed which contributes only 5% of seed weight while chickpea seed coat contributes up to 16.4% (Singh et al., 1981; Chavan et al., 1986). In an earlier study on water absorption of soybean, Smith and Nash (1961) reported that the principal factor controlling absorption in whole bean was the seed coat. The ratio of coat thickness to seed size and, to some extent, seed coat structure were the only obvious differences between the Desi and Kabuli chickpea types (Jambunathan and Singh, 1981). Polyacrylamide gel electrophoretic analysis did not distinguish two types of chickpea by protein patterns. Gross chemical composition also showed no significant differences. The seed coat in Desi seed contributed 16% of seed weight while in Kabuli seed the average was 7% (Singh and Jambunathan, 1981). The fact that all Kabuli chickpeas had unexpectedly higher water absorption than Desi chickpeas, suggested that thickness of seed coat and also the seed coat structure had critical effects on water uptake and absorption rate (Singh and Kulshrestha, 1987; Gandhi and Bourne, 1991).

A constant K_3 ($K_1 = K_3T + K_4$) was developed to describe the temperature effect on water absorption rate by plotting K_1 against temperature (Fig. 3). A straight line with K_4 as the ordinate intercept and K_3 as the gradient was obtained. This confirmed that the Peleg constant K_1 varied with temperature as reported by Sopade and Obekpa (1990). Note that at a given temperature, the lower K_1 , the greater the amount of water absorbed.

The temperature effect on water absorption of Desi type chickpea was greater than that of the Kabuli type. All constants

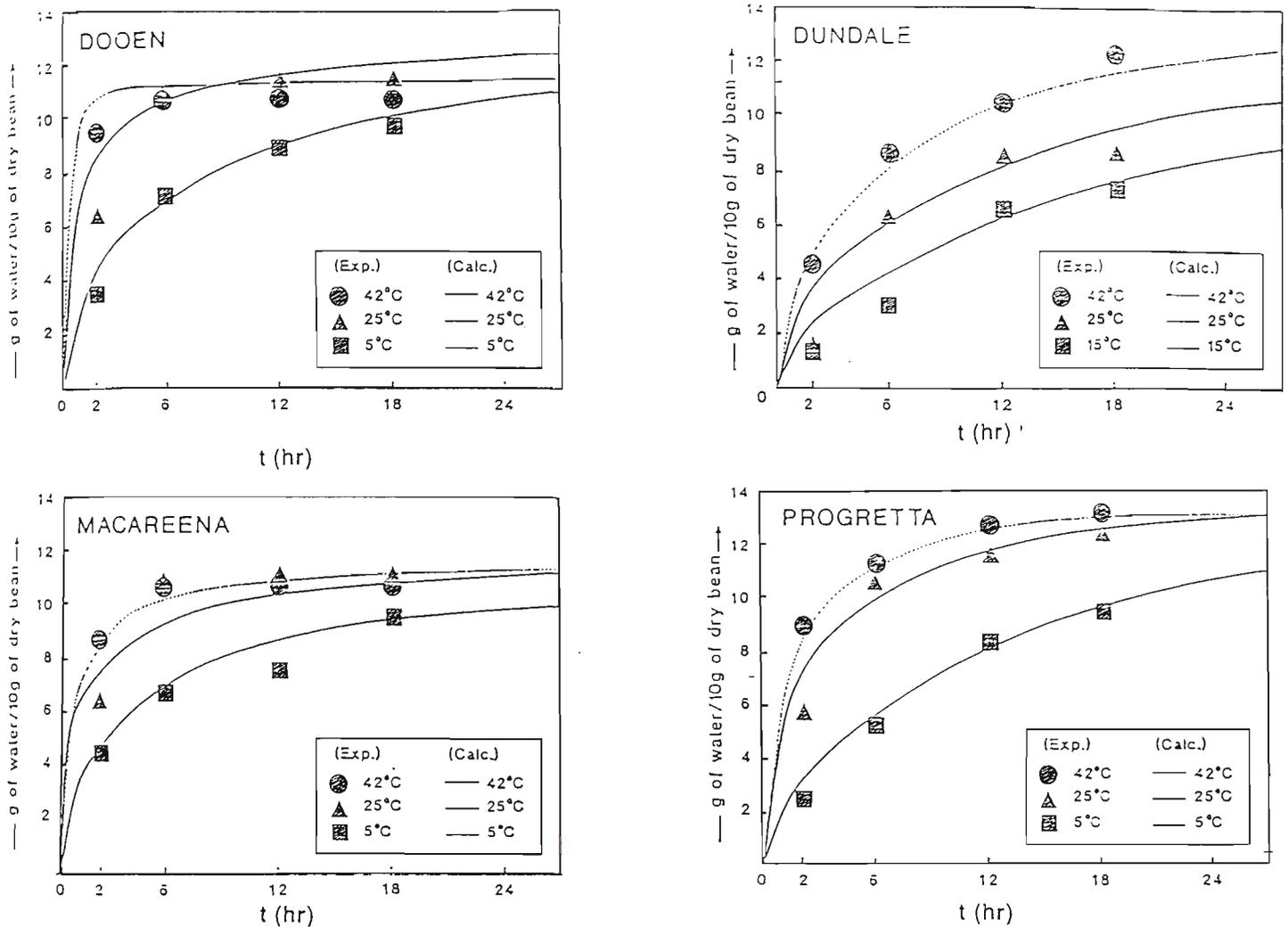


Fig. 2—Experimental and predicted water absorption characteristics of two chickpea cultivars (Dooen and Macareena) and two field pea cultivars (Dundale and Progetta).

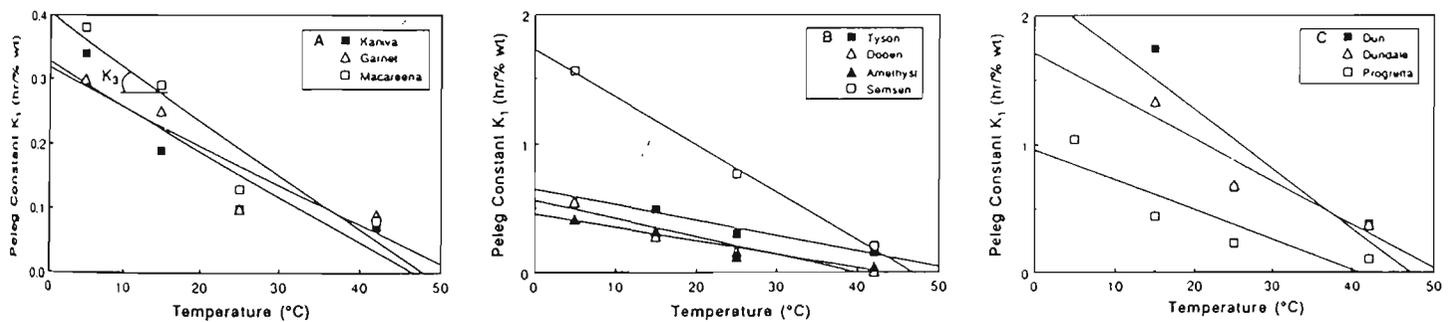


Fig. 3—Effect of temperature on the Peleg constant K_1 of chickpeas and field peas; (A) Kabuli type; (B) Desi type; (C) Fieldpea.

K_3 for the Kabuli type ($\leq 0.8 \times 10^{-2}$) were lower than those of the Desi type ($\geq 1.1 \times 10^{-2}$). Constant K_3 for the field pea ranged from $2.4 - 4.4 \times 10^{-2}$. There was clear correlation between constant K_3 and the seed number of different cultivars (Desi/Kabuli) as well as grain type (chickpea/field pea). Since there was no significant difference in protein pattern or gross chemical composition between Desi and Kabuli chickpea, this constant could serve as a characterizing parameter for grain type based upon water absorption behavior (Fig. 4).

In contrast to K_1 and K_3 constant K_2 was almost unaffected by temperature with mean values ranging from 8.2 to $9.2 \times 10^{-2} \% \text{ wt}^{-1}$ for chickpea and 7.1 to $7.6 \times 10^{-2} \% \text{ wt}^{-1}$ for field pea (Table 3). The independence of K_2 with respect to temperature indicated that the same equilibrium moisture content would be obtained regardless of soaking temperature. The equilibrium moisture content of each cultivar was obtained by inserting the K_2 value into equation [2]. The mean equilibrium

moisture content of chickpea was 12.4% wt and that of field pea was 14.5% wt. Note that the Samsen cultivar with seed size nearer that of field pea than Desi or Kabuli chickpeas also had both K_2 value ($7.5 \times 10^{-2} \% \text{ wt}^{-1}$), and equilibrium moisture content (14.2% wt) closer to those of field peas.

The Peleg model could be used to describe the water absorption of chickpea and field pea cultivars. The initial moisture absorption rate ($1/K_1$) and constant K_3 were influenced by soaking temperature. Constant K_2 was almost unaffected by temperature. Using only short term soaking data the Peleg model could provide a useful approach to calculate equilibrium moisture content of chickpea and field pea at any soaking temperature.

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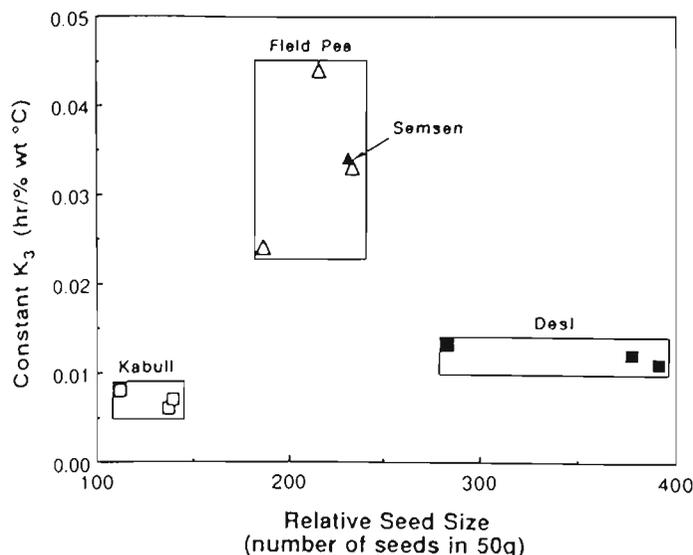


Fig. 4—Correlation between seed size of chickpea or field pea and constant K_3 .

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ment to eliminate interfering microorganisms would not be necessary. Green olive fermentation by *Lb. plantarum* yielded lactic acid which almost exclusively provided the low pH needed for preservation. However, the formation of other products (e.g. flavor compounds) may be desirable for improving product quality. The use of a suitable mixed culture rather than a single lactic strain could be the most appropriate approach for accomplishing that.

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Solubility of Grain Legume Proteins Measured by Infrared Spectroscopy

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ABSTRACT

The application of infrared spectroscopy using a rapid milk analyser (Milkoscan) for the study of grain legume protein solubility was investigated. Solubilities of chickpea, fieldpea and soybean proteins obtained by both the Kjeldahl procedure and infrared spectroscopy were compared. Although the values obtained by the two methods differed at certain pHs (< pH 3 and > pH 9), the Milkoscan still offers a rapid approach for the investigation of grain protein solubility.

INTRODUCTION

Functional properties of vegetable proteins influence their potential for food applications. An important functional property is solubility. To examine their solubility the Kjeldahl procedure is widely used to determine the nitrogen in extracts of the protein over a range of pH levels (Bradstreet, 1965).

Regardless of several modifications including semi-automation, the Kjeldahl procedure is still a time consuming and rather laborious assay (Pearson and Pertz, 1976; Fosdick and Pike, 1982; Hach, Bowden, Kopelowe and Brayton, 1987; Devani, Shishoo, Shah and Suhagia, 1989; Barbano, Clark, Dunham and Fleming, 1990).

With the increasing interest in food applications of vegetable proteins, there is a need for an accurate, rapid and less laborious method to examine their solubility. Infrared spectroscopy may provide a quick, non destructive and easy to perform alternative procedure. Goulden (1964) and other investigators (Biggs, 1979; Sjaunta and Schaar, 1984) successfully estimated proteins in cow milk by infrared absorption. With its accurate results, compared to those obtained from the Kjeldahl method, instrumental infrared estimation of milk proteins has been recognised as an official method for the dairy industry (AOAC, 1978; Van de Voort, 1980). In addition Hung, Kyle and Yu (1988) used a single cell infrared milk analyser - the Milkoscan 104 - to estimate the protein content in soy beverage and aqueous extracts from lupin and peanut seeds. Since the

Milkoscan 104 was found to give as accurate an estimation of protein content in these legume extracts as the Kjeldahl method, this instrument should also serve as a simple means to study the solubility of grain legume proteins.

The solubility of a protein is greatly dependent on the pH of the extraction medium since the pH influences charge and electrostatic balance within and between protein molecules. Proteins of grain legumes are almost insoluble near their isoelectric points (pI), but their solubility increases at both lower and higher pHs, because at these pHs proteins have a net negative or positive charge.

This report evaluates the application of an infrared spectroscopic technique for a study of the solubility of chickpea, field pea and soybean proteins over a wide range of pH by comparing results from both the Kjeldahl procedure and the instrumental infrared spectroscopy method.

MATERIALS AND METHODS

The outlines of the processes to prepare chickpea flour protein extracts (CFPE1, CFPE2, CFPE3 extracted at pH 2, pH 7 and pH 9, respectively) and chickpea protein isolates (CPI1, CPI2, CPI3 extracted at pH 2, pH 7 and pH 9, respectively) are given in Figs 1 and 2.

Chickpea (*C. arictinum* cv Kaniva) grown at Horsham, Victoria, was dehulled, defatted and the resulting flours (100 g) were suspended in deionised water (500 mL), readjusted to pH 2, 7 and 9 with 1M NaOH or HCl and stirred for 1 h at 20°C. The suspension was centrifuged at 4,500 g for 20 minutes and filtered through Whatman paper 541. Portions of the filtered extracts were diluted 2-fold and 4-fold for comparative measurements. The protein contents were measured by both the Kjeldahl procedure and infrared spectroscopy as described below.

The filtered extracts from Fig 1 at pH 2, pH 7 or pH 9 were precipitated at pH 4.2 with 2 M NaOH or 2M HCl and centrifuged at 8000 g for 25 minutes to give an acid precipitated curd with approximately 43% protein. The curds were re-extracted at pH 9 in a 5% suspension for 1 hour at 20°C, centrifuged at 3,500 g for 25 minutes and filtered through Whatman 541 filter paper. The filtered supernatants were precipitated with 2M HCl at pH 4.2 and centrifuged at 8,000 g for 25 minutes. The precipitated curds were

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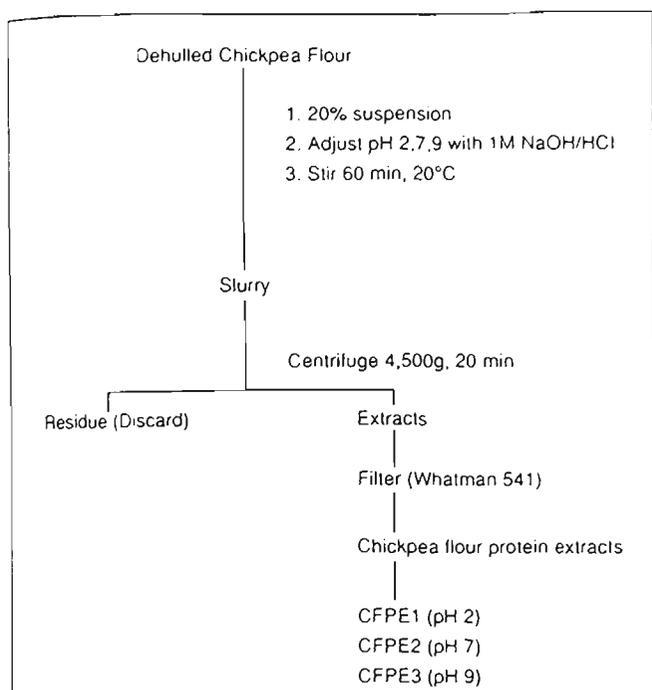


Fig 1. Process for preparation of chickpea flour protein extracts

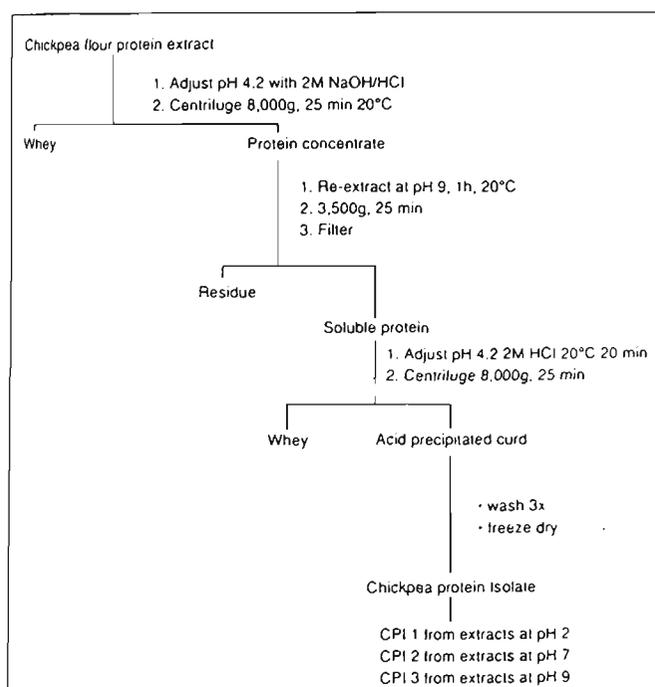


Fig 2. Process for chickpea/field pea protein isolate preparation

washed three times with pH 4.2 water and freeze dried to give protein isolates containing 90-97% protein.

Defatted field pea flour (*P. Sativum* cv Bonzer) from seeds grown at Horsham, Victoria was extracted at pH 9 as described above. The protein content of field pea was 89%.

A commercial Soybean protein isolate (Supro 500E) produced in USA with 87.4% protein content was used.

Kjeldahl procedure

The nitrogen content of protein extracts was measured using a Tecator Kjeltac system (Tecator application Note 750416-RA for cow milk proteins). Each sample (5 g) was added to a digestion tube containing Missouri catalyst ($\text{CuSO}_4/\text{K}_2\text{SO}_4 - 1/300$) (12 g) followed by the addition of concentrated sulphuric acid (20 mL) and 30% hydrogen peroxide (12 mL). The mixture was digested at 420°C until clear. The ammonia formed by adding 40% sodium hydroxide (50 mL) was removed by steam distillation into a 4% boric acid solution and quantitated by titration against sulphuric acid. Recovery was checked using tryptophan (AJAX, 98%). Protein was calculated as % N x 6.25. All samples were assayed in duplicate.

Infrared measurement

The protein extracts (30 mL) were measured in triplicate with the Milkoscan Model 104, (Foss Electric, Hillerod, Denmark). This instrument was calibrated with cow milk samples, having a range of protein contents from 2-4%. The milk samples were assayed by the Kjeldahl procedure, corrected to measure true protein only and protein calculated by $\text{N} \times 6.38$. The Milkoscan 104 calibration for protein measurement was based on the infrared radiation absorbed at 6.5 μm by the nitrogen hydrogen bond within the peptide skeleton of the protein molecules (Vann de Voort, 1980). Consequently the instrument measured only protein nitrogen. Each sample took approximately 1 minute to analyse.

Comparison of Milkoscan and Kjeldahl methods.

Initial comparison of the two methods was done by using chickpea flour protein extracts at three different pHs (CFPE 1, CFPE 2 and CFPE 3) and diluting to three different concentrations, each of which was assayed by both methods. To evaluate the accuracy of the Milkoscan at low level of protein content similar to those in the legume extracts, a commercial skim milk was diluted to several low concentrations and assayed by the two methods.

Assays

Solubility test. 2% suspensions of chickpea isolates (CPI1, CPI2 and CPI3), field pea isolate (FPI) and soy protein isolate (SPI) were prepared over the range of pH 2-10. The filtered extracts were divided into two portions and the soluble protein content assayed by both methods.

Simple amides were used to evaluate the pH effect on the infrared reading of the N-H bond in peptides. A series of 1% N-methyl and N-ethyl acetamide aqueous solutions were adjusted to pH 2-pH 10 by adding 0.1M NaOH or 0.1 M HCl. The concentration of acetamide in the solution was kept constant by adjusting with deionised water to a constant volume.

These solutions ($n = 6$) were measured by the Milkoscan under the same conditions as with the protein solutions.

Electrophoretic analysis. Analytical SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was carried out by a procedure similar to that of Weber and Osborne (1969), using a LKB-2001 vertical electrophoresis apparatus.

Amino acid analysis. Amino acid content was determined as described by Fox, Rayner and Wu (1985) except for tryptophan. Amino acids were analysed by ion exchange chromatography on a Waters HPLC system using post column derivatisation with ninhydrins. Tryptophan was measured following a HPLC method described by Delhave and Landry (1986).

RESULTS AND DISCUSSION

Fig 3 shows the correlation between Kjeldahl and Milkoscan values for protein estimation by linear regression analysis at three different pHs. The slopes for the protein extracts at pH 2, pH 7 and pH 9 were 0.745, 1.011 and 0.982, respectively. The results obtained by the Milkoscan method agreed with those by the Kjeldahl method, for extracts at pH 7 and pH 9, but the results obtained from the Milkoscan were lower than those from the Kjeldahl for extracts at pH 2.

The influence of pH on infrared readings for milk proteins has been mentioned by Goulden (1964) who found that when the pH of milk dropped from 6.7 to 6.5, the infrared reading increased by 0.03%. In naturally soured milk, carboxylic acids were produced

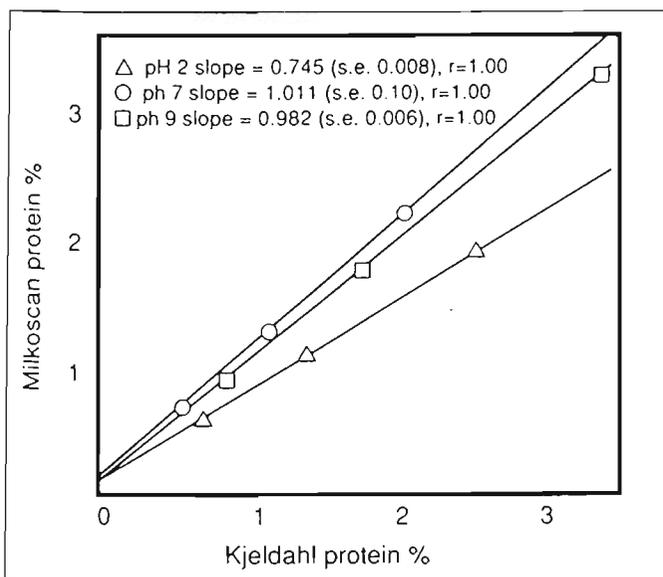


Fig 3. Protein values obtained by the Milkoscan and by the Kjeldahl procedure on the samples of chickpea protein isolates, at pH 2, pH7, pH 9.

and existed predominantly as carboxylate anions with two most intense absorption bands at 1540 cm^{-1} ($6.5\text{ }\mu\text{m}$) and 1410 cm^{-1} ($7.1\text{ }\mu\text{m}$). With the addition of the first absorption band ($6.5\text{ }\mu\text{m}$), due to the carboxylate anions formed in soured milk, the optical density at $6.46\text{ }\mu\text{m}$ for nitrogen - hydrogen bonding of the peptides increased, resulting in an increase in apparent protein content. In contrast, Robertson, Dixon, Nowers and Brink (1981) found that lowering the pH of a milk sample from 5.96 to 5.48 caused a slight decrease in the protein reading (0.063%). These studies showed a different effect of pH on the protein reading. However, the protein changes were small perhaps due to the small pH change investigated, so the influence of pH on the infrared protein reading under strong alkaline and acidic environments warranted further investigation.

To investigate the effect of extraction pHs on the accuracy of the Milkoscan protein reading, the solubility of a purified chickpea protein isolate (CPI3) was measured at a wider range of pH by both methods. The solubility profile obtained by the two methods was different. The Milkoscan gave lower values than the Kjeldahl procedure at very low and very high pHs (pH 2-3 and pH 9-10) but higher values at pHs near the isoelectric point of the proteins (pH 4-6) (Fig 4). A similar observation was obtained from the solubility of other chickpea protein isolates extracted at pH 7 and pH 2 (CPI2 and CPI1). The ratio of the Milkoscan to Kjeldahl protein contents was calculated and tabulated for each pH. This procedure was repeated for field pea protein isolate (extracted at pH 9) and a soy protein isolate (Fig 5).

The result in Table 1 indicated that at the pHs at which the proteins are likely to have a net negative or positive charge, (ie very low, very high pH) the Milkoscan readings were lower than those from the Kjeldahl (ratio less than 1). In a study on aqueous ammonium ion by transmission infrared spectroscopy, Van de Voort, Mills, Paquette and Grunfeld (1986) found the influence of pH over the range of 3.5-8 had little effect on the infrared reading, but a significant effect was observed at pH 8.5. The change was consistent with the shift in the equilibrium from NH_4^+ to NH_3 (aqueous). Since the protein measurement by the Milkoscan was based on the infrared energy absorbed by the nitrogen hydrogen bond within the peptide skeleton of the protein molecules, any factor affecting this bond is likely to cause a change in the Milkoscan reading. To examine this, solutions of two acetamide derivatives - N-methyl and N-ethyl acetamide - were analysed on the Milkoscan at a constant concentration but at different pHs. The Milkoscan readings obtained from solutions of each acetamide derivative, over the pH range 2-10, are given in Table 2. In each case, the Milkoscan reading varied with pH and in each case the highest reading was obtained at the unadjusted pH. The different response to pH change of the two acetamide derivatives appears

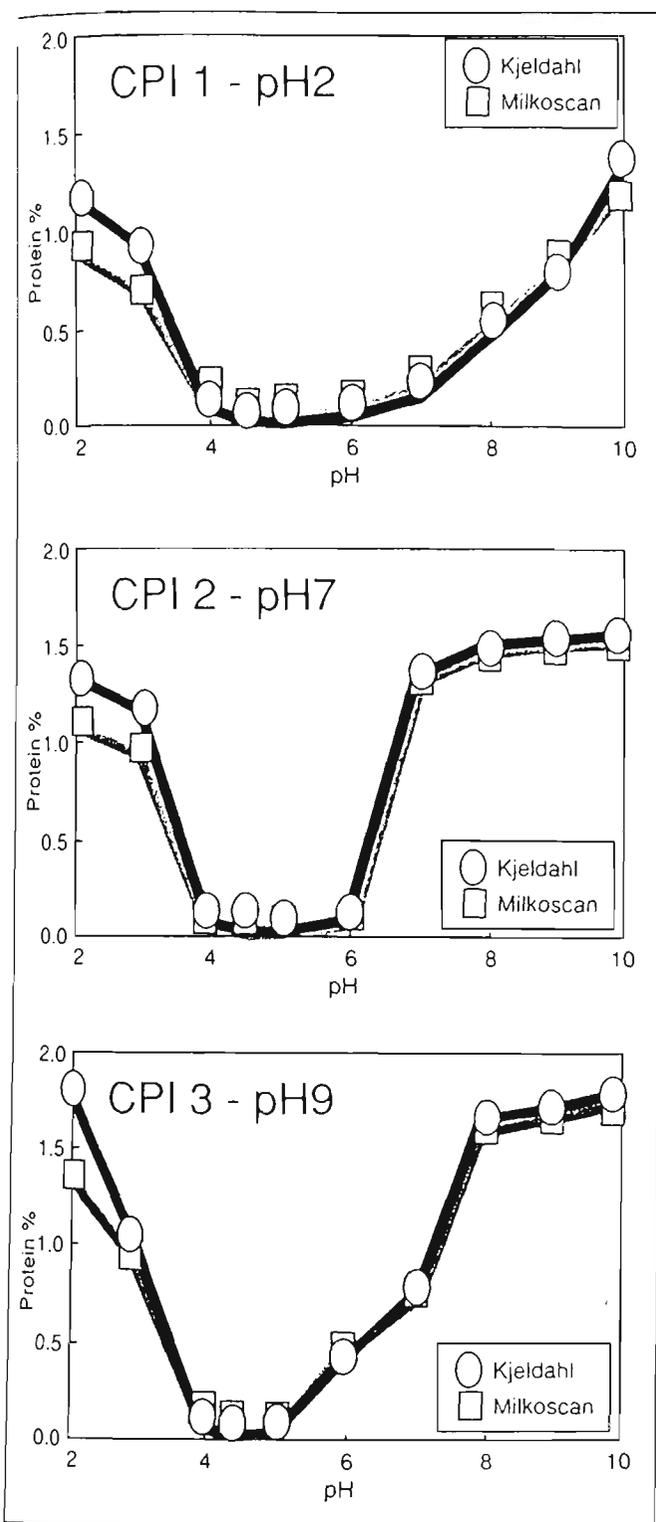


Fig 4. Solubility of chickpea protein isolates prepared at pH 2 (CPI 1), pH 7 (CPI 2) and pH 9 (CPI 3) measured by the Milkoscan and the Kjeldahl procedure

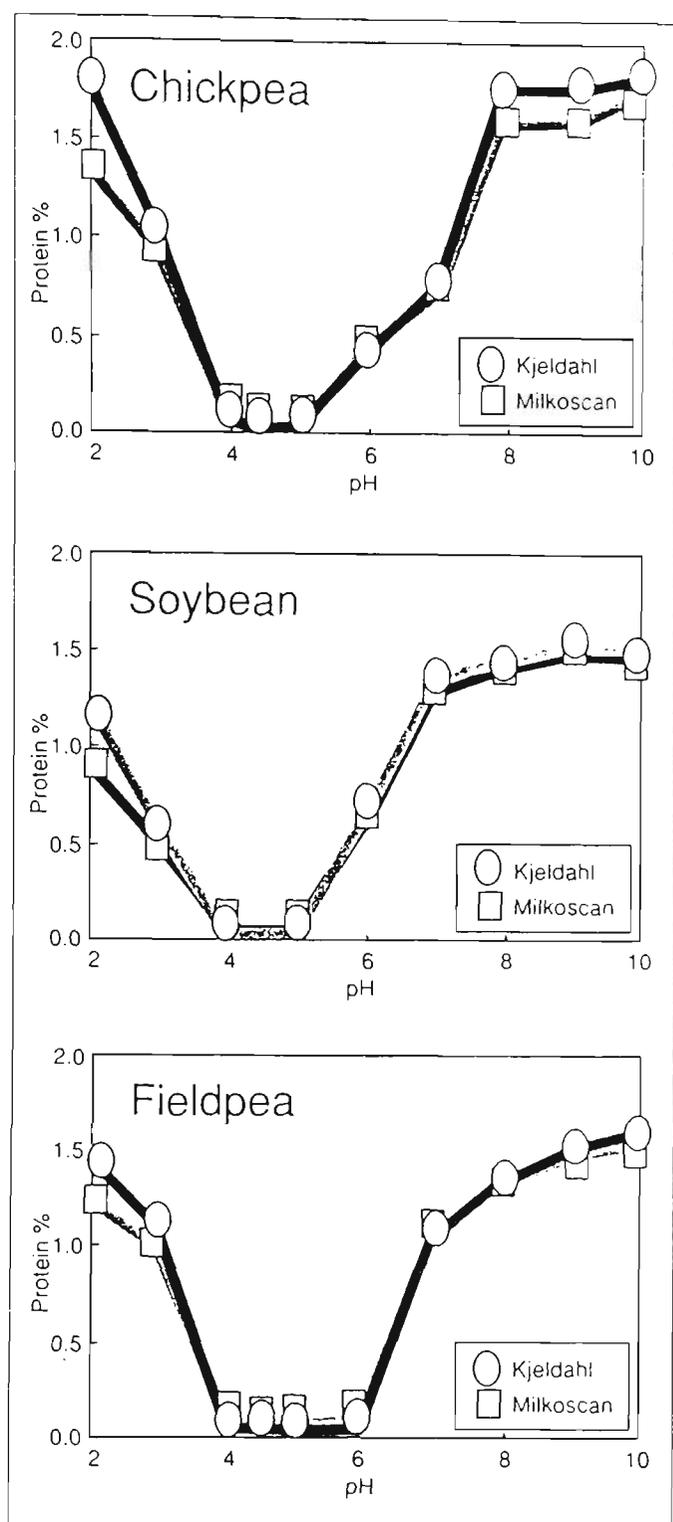


Fig 5. Solubility of chickpea, soybean and field pea protein isolates measured by the Milkoscan and Kjeldahl procedure

to be a consequence of the altered strength of the N-H bond caused by different electron releasing ability of the two alkyl substituents. The behaviour of a protein in aqueous solution is obviously more complicated than either of these simple amide derivatives, but it seems clear from this investigation of the behaviour of the amides that the lower Milkoscan readings obtained from protein solutions under both strongly alkaline and acidic

conditions, are due to the influence of pH on the nature of the peptide bonds.

At pHs close to the pI the protein readings by the Milkoscan were higher than by Kjeldahl. In this study the protein content in the extracts at these pHs (pH 4-6) was low, sometimes lower than 0.02%. At such a low concentration, the instrumental measurement

PP.12 Title: Chickpea Proteins for Human Consumption

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Abstract :

Chickpea, the second most widely consumed grain legume in the world after soybeans, is a staple food crop in many tropical and subtropical climates, providing an economical source of protein for millions of people. Chickpea proteins have a nutritional quality superior to other grain legumes but have not yet been investigated as extensively as soybean proteins.

The aims of this study were to extract and characterise different protein fractions from the seeds and to identify the effects of extraction conditions on the functional properties of these proteins for food applications.

Like other grain legumes, chickpea showed maximum solubility at pH 2 and pH 9-10. The combined effects of five major parameters (extraction pH, temperature, time, solvent, particle size) affecting the yield of extracted proteins have been investigated. Statistical analysis of about 100 extractions indicated particle size and extraction pH had significant effects.

In contrast to many previous studies, the yield of extracted albumin in this study was far higher (100% - 200%). Optimum extraction conditions for glutelins and globulin were identified. *In vitro* digestibility of all fractionated proteins was studied with several enzymes. Their functional properties were also investigated to identify the effects of processing conditions on quality of the isolated proteins.

During this study, a rapid infra-red spectroscopic technique was used to measure the protein content extracts of chickpea. A simple empirical model was shown to be capable of predicting the water absorption of chickpea and to give a positive distinction between two main Kabuli and Desi types whereas composition analysis and electrophoretic analysis failed.

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62. Functional Properties of Native and Modified Chickpea Proteins for Food Applications

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Functional properties of chickpea proteins prepared at a laboratory and pilot scale were investigated. Solubility, emulsifying properties, gel and foam forming properties, water and oil absorption, viscosity and rheological properties of these proteins were studied. The proteins were partially hydrolyzed by papain and trypsin. Acylated chickpea proteins were also prepared with acetic and succinic anhydride under various conditions. Enzymatic and chemical modification of these proteins affected remarkably their functional properties. Dough properties of bread and noodles supplemented with native and modified chickpea protein were investigated to assess the effect of protein supplementation in different food systems.
