Department of Life Sciences

Contribution of process parameters to the particle size distribution and apparent viscosity properties of soy protein isolate- and hydrolysate-based infant formula emulsions

Emma Marie McEvoy
B.Sc. (Hons) Food Technology

Supervisor: Professor Richard J. FitzGerald
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A thesis submitted to the University of Limerick in fulfilment of the requirements for the degree of Masters of Science
DECLARATION

Contribution of process parameters to the particle size distribution and apparent viscosity properties of soy protein isolate- and hydrolysate-based infant formula emulsions

Supervisor: Professor Richard J. FitzGerald

This thesis is presented in fulfilment of the requirements for the degree of Masters of Science.

"It is entirely my own work and has not been submitted to any other university or higher education institution, or for any other academic award in this university. Where use has been made of the work of other people, it has been fully acknowledged and referenced."

Emma Marie McEvoy

Date

13/07/05

Emma Marie McEvoy
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DEDICATION

For Mam and Dad.
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CONTRIBUTION OF PROCESS PARAMETERS TO THE PARTICLE SIZE DISTRIBUTION AND APPARENT VISCOSITY PROPERTIES OF SOY PROTEIN ISOLATE- AND HYDROLYSATE-BASED INFANT FORMULA EMULSIONS

Emma Marie McEvoy

ABSTRACT

Oil-in-water emulsions were manufactured with commercially available soy protein isolate (SPI 1500 and SPI 1651) and soy protein hydrolysate (SPH 1761 and SPH 1762) ingredients. In addition to SPI/SPH, the model emulsions contained corn syrup solids (CSS) and a proprietary vegetable fat blend. The ingredients mixture was pasteurised at 77°C for 30 sec, followed by homogenisation (double pass) using a first stage pressure of 30 bar and a second stage pressure of 170 bar. The objective of the study was to quantify the effect of ingredient composition (SPI/SPH and CSS), and lecithin inclusion on the particle size distribution of the emulsions. The effects of variation in total solids (TS, 12.5 - 26 % w/w), pH (6.4 - 7.5) and homogenisation pressure (110 - 360 bar) on the particle size distribution, and apparent viscosity ($\eta_{\text{app}}$) of the emulsions was also evaluated.

The exclusion of CSS in the emulsions manufactured with SPH 1762 resulted in an increased proportion of smaller particles, compared to the same emulsion containing CSS. There were no real differences in particle size distribution profiles for emulsions manufactured with the fat blend containing varying amounts of lecithin (0 - 2 % (w/w)). However, the absence of added lecithin resulted in model emulsions manufactured with SPI sample 1651 and SPH sample 1761, having particle size distribution profiles with a greater proportion of larger particles. The addition of lecithin to emulsions generated with SPH sample 1762 resulted in a decrease in the average particle size.

No TS related effects were observed on the particle size distributions of model emulsions generated with the SPI samples 1500 and 1651 and SPH sample 1761. However, emulsions made with SPH sample 1762 at low TS (12.5 - 14 %) contained more particles having a lower size distribution than those made at high TS levels (23 - 26 %). Increasing homogenisation pressure generally resulted in a decrease in the mean particle size of SPI emulsions. Emulsions generated with SPH sample 1761 had a bimodal particle size distribution profile at the higher homogenisation pressures. For emulsions generated with SPH sample 1762, an increase in homogenisation pressure resulted in an increase in $\eta_{\text{app}}$. The particle size distribution and $\eta_{\text{app}}$ profiles of SPI sample 1500/1651 and SPH sample 1651 model emulsions were unaffected by pH changes between pH 6.4 and 7.5. However, an increase in pH resulted in a general decrease in average particle size and an increase in $\eta_{\text{app}}$ of the SPH sample 1762 emulsion.

The results demonstrate that ingredient composition and processing conditions have significant effects on the properties of soy protein-based model emulsions.
CHAPTER 1

LITERATURE REVIEW
1.1 SOYBEAN

The soybean, a native of China, is one of the oldest crops of the Far East. For centuries, the Chinese and other Oriental people, including Japanese, Korean and Southeast Asians, have used the bean in various forms as one of the most important sources of dietary protein and oil (Liu, 1997). Soybeans contain high levels of lysine and have a low fat content. The use of soy products in Western countries is increasing with the growing demand for high quality vegetarian food of good nutritional value (Molina et al., 2001). However, the history of soy protein products – flours, concentrates, isolates and their derivatives – is relatively short. In early years, soy protein products were mainly used to meet nutritional needs, but more recently they have been used primarily for their unique functional characteristics. Nowadays, many thousands of tons of concentrated forms of functional soy protein ingredients and products are used by the food industry, feed manufacturers, and other non-food, non-feed industries in a variety of applications (Rhee, 1994).

Despite its nutritional and functional properties, the soybean remained a crop exclusive to the Orient for many centuries. It was first introduced to Europe in about 1912 by the German botanist, Engelbert Kaempfer. Later Carl von Linné, a Swedish botanist, gave a genetic name, *Glycine max*, to soybeans. *Glycine* is the Greek word meaning “sweet”. The word *max* means “large”, referring to the large nodules on the soybean plant. Soybean production in Europe has been mainly limited due to poor climate and soil conditions. Although the early introduction of soybeans into the United States dates back to the mid-eighteenth century, the large-scale official introduction did not occur until the early 1900’s which coincided with breakthroughs in harvesting and processing. Until 1954, China led the world in soybean production and export, but since then the United States has become the world leader (Liu, 1997).
Chapter 1

Soybeans (Figure 1) belong to the pea family *Leguminosae*, subfamily *Papiliconacae* and the genus *Glycine Max*. Its plant is bushy with height ranging from 0.75 to 1.25 m, branching sparsely or densely, depending on cultivars and growing conditions. The soybean is a typical legume differing in colour, size and shape depending on the variety (Liu, 1997).

![Figure 1: Soybeans](taken from: Kansas State University, 2005).

Soybeans represent an economical and valuable agricultural commodity. Among cereal and other legume species, it has the highest protein content (around 40 %); other legumes have protein contents between 20 % and 30 %. The soybean also contains about 20 % oil and 4 to 10 % water. The soybean contains more protein than beef, more calcium than milk and more lecithin than eggs. Other valuable components found in soybeans include phospholipids, vitamins such as thiamine (B1) and riboflavin (B2), as well as minerals such as iron, copper, calcium, potassium, manganese, zinc, cobalt and magnesium. Furthermore, soybeans contain many minor substances, some of which, such as trypsin inhibitors, phytates and oligosaccharides are known to be biologically
active. Others, such as isoflavones, are recently being recognised for their ability to prevent human cancers and other diseases (Liu, 1997).

Figure 2: Structure of a soybean seed (taken from: Liu, 1997).

The soybean seed is essentially devoid of endosperm and consists of a seed coat and a large, well developed embryo (Figure 2). Besides cotyledons, the embryo has three other parts: radicle, hypocotyl and epicotyl. During germination, the radicle becomes the primary root, whereas the hypocotyl lifts the cotyledons above the soil surface. The epicotyl is the main stem and growing point (Liu, 1997).

On average, the oil and protein content together constitute about 60 % of the dry matter in soybeans (Table 1). The remaining dry matter is composed of mainly carbohydrates (about 35 %) and ash (about 5 %). Soybeans, on a wet basis, contain about 35 % protein, 17 % oil, 31 % carbohydrate and 4.4 % ash (Liu, 1997).
Table 1: Proximate compositions of soybeans and their structural components
(taken from: Liu, 1997).

<table>
<thead>
<tr>
<th>Chemical Composition (% dry matter)</th>
<th>% in whole seeds</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hull</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>86</td>
<td>4.3</td>
</tr>
<tr>
<td>Hypocotyl axis</td>
<td>2</td>
<td>41</td>
<td>11</td>
<td>43</td>
<td>4.4</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>90</td>
<td>43</td>
<td>23</td>
<td>29</td>
<td>5.0</td>
</tr>
<tr>
<td>Whole seeds</td>
<td>100</td>
<td>40</td>
<td>20</td>
<td>35</td>
<td>5.0</td>
</tr>
</tbody>
</table>

### 1.2 Soy Protein

As already outlined, the component present in the greatest amount in soybeans is protein, averaging about 40% of total dry matter (Table 1). In general, cultivated soybeans comprise approximately 8% hull, 90% cotyledons and 2% hypocotyl axis (Table 1). Cotyledons contain the highest percentage of both protein and oil, whereas the hull has the lowest content of these components. The hypocotyl axis has a protein content similar to cotyledons but its lipid content is about half that in cotyledons. Since the cotyledon is the major component in the whole seed, its composition is very close to that of the whole seed, despite differences in composition among structural parts (Liu, 1997).

Soy proteins are used in foods as functional and nutritional ingredients and as a substitute for animal proteins (Qi et al., 1997). The most popular plant protein source serving as an ingredient in food formulation is soy protein. It is not a homogenous protein but is made up of several different types of proteins (Liu, 1997). The functional
properties are directly related to the physiochemical properties of these proteins, thus a detailed knowledge of the characteristics of soy proteins is essential for understanding and manipulating their properties in foods (Kinsella, 1979). Different soy protein ingredients are manufactured on the basis of having appropriate functional properties for food applications and consumer acceptability. The functional characteristics such as water sorption, solubility, gelation, surfactancy, ligand-binding and film formation, affect the behaviour of proteins in food systems during processing, manufacturing, storage and preparation (Kinsella, 1979).

1.2.1 AMINO ACID COMPOSITION OF SOY PROTEIN

Legumes have been recognised as an excellent source of protein for humans and as an excellent source of protein for balancing the amino acid composition of cereal and vegetable products, which often comprise the bulk of the human diet (Kolar et al., 1985). Soy protein, like proteins of most other leguminous plants, is low in sulphur containing amino acids such as methionine, cystine and are also low in threonine (Table 2). However, soy protein contains sufficient lysine, which is deficient in most cereal proteins (Liu, 1997). The amount of essential amino acids required for various age groups have been estimated by the Food and Nutrition Board (FNB). The essential amino acid composition of SPI meets or exceeds this pattern (Table 2). Soy protein is reported to be a highly digestible source of all the essential amino acids (Kolar et al., 1985).
Table 2: Essential amino acid composition (mg amino acid per gram protein) of different soy protein products (taken from: Kolar et al., 1985).

<table>
<thead>
<tr>
<th>Essential Amino Acid</th>
<th>Soy Flour</th>
<th>Soy Concentrate</th>
<th>Soy Isolate</th>
<th>FNB* pattern for infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>26</td>
<td>26</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>51</td>
<td>48</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>Leucine</td>
<td>77</td>
<td>79</td>
<td>82</td>
<td>70</td>
</tr>
<tr>
<td>Lysine</td>
<td>69</td>
<td>64</td>
<td>64</td>
<td>51</td>
</tr>
<tr>
<td>Cysteine + Methionine</td>
<td>32</td>
<td>28</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Phenyalanine + Tyrosine</td>
<td>89</td>
<td>89</td>
<td>92</td>
<td>73</td>
</tr>
<tr>
<td>Threonine</td>
<td>43</td>
<td>45</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>13</td>
<td>16</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Valine</td>
<td>15</td>
<td>50</td>
<td>50</td>
<td>48</td>
</tr>
</tbody>
</table>

* Food and Nutrition Board

1.2.2 INDIVIDUAL SOY PROTEIN COMPONENTS

The classification and fractionation of soy proteins is quite complex. Soy proteins were initially classified according to their sedimentation velocity into 2S, 7S, 11S and 15S fractions (Table 3). The 2S fraction contains trypsin inhibitors and cytochrome and constitutes about 8% of the total protein in soybeans. The 7S fraction contains globulins and enzymes (lipoxygenase and amylase) and constitutes about 35% of the protein. The 11S fraction is considered to be a single protein and constitutes about 52% of the total protein. The 15S fraction is a polymer form of the 11S fraction and constitutes about 5% of the protein. The major soy globulins were classified into
glycinin and α-, β- and γ-conglycinin based on their different immunological responses. The 11S fraction is believed to be mainly composed of glycinin with the globulin portion of the 7S fraction being conglycinin (Rhee, 1994).

Table 3: Approximate distribution of the different components of soy proteins (taken from: Kinsella, 1979).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Content (%)</th>
<th>Principal components</th>
</tr>
</thead>
<tbody>
<tr>
<td>2S</td>
<td>8</td>
<td>Trypsin inhibitor, Cytochrome</td>
</tr>
<tr>
<td>7S</td>
<td>35</td>
<td>Globulins (conglycinin), Lipoxygenase, Amylase</td>
</tr>
<tr>
<td>11S</td>
<td>52</td>
<td>Globulins (glycinin)</td>
</tr>
<tr>
<td>15S</td>
<td>5</td>
<td>Polymers</td>
</tr>
</tbody>
</table>

1.2.3 MOLECULAR STRUCTURES OF 7S AND 11S GLOBULINS

The storage proteins, 7S (conglycinin) and 11S (glycinin) are the principal components of soy protein. The relative quantities of these proteins vary according to the literature, however, approximate values are given in Table 2.

The 7S globulins of soybean are classified into three major fractions with different physiochemical properties, i.e., β-conglycinin, γ-conglycinin and basic 7S globulin. β-Conglycinin is the most prevalent of these and accounts for 30-50 % of the total seed proteins. β-Conglycinin has a molecular mass of 150-200 kilo Daltons (kDa). Four subunits have been identified: three major subunits i.e., α' (72 kDa), α (68 kDa) and β (52 kDa) and one minor subunit γ, which is similar in size to that of the β subunit (Utsumi et al., 1997). All three major subunits are rich in asparagine, glutamine, leucine and arginine. Two subunits, α' and α are very similar in amino acid composition. Both
lack cysteine and have low levels of methionine. The $\beta$ subunit contains no methionine (Liu, 1997).

$\gamma$-Conglycinin is a glycoprotein containing about 5% carbohydrate. It is a trimer composed of three identical subunits and has a total molecular mass of 170 kDa. The N-terminal amino acid sequence of $\gamma$-conglycinin differs from that in the other globulins (Utsumi et al., 1997).

Basic 7S globulin (Bg) is a glycoprotein having a higher isoelectric point (pH 9.05-9.26) than the other globulin species. Bg has a molecular mass of 168 kDa and is composed of four subunits consisting of a high molecular weight polypeptide and a low molecular weight polypeptide, which are linked by disulfide bridges (Utsumi et al., 1997).

Most of the structural studies on soy proteins have been conducted on the 11S (or glycinin) fraction because it is a protein that can be easily prepared in a relatively pure form. It is the largest single fraction of total seed protein (25 – 35%, Table 3) and accounts for over 40% of the total seed globulins (Liu, 1997). There are three types of acidic ($A_1$, $A_2$ and $A_3$) and three types of basic ($B_1$, $B_2$ and $B_3$) subunits, with different molecular weights, in the 11S protein molecule. Monomeric glycinin consists of six subunits: three acidic and three basic subunits. The acidic and basic subunits are held together by hydrophobic interactions and disulfide bonds. Dimeric glycinin has been considered to consist of two identical monomer units of glycinin held together by hydrophilic interactions, i.e., electrostatic and/or hydrogen bonding, and has a molecular weight of approximately 360 kDa (Rhee, 1994).
1.3 TYPES OF SOY PROTEIN PRODUCTS

A range of soy protein ingredients are available commercially, these include soy protein isolates, soy flours and grits, soy concentrates, soy protein hydrolysates and textured soy proteins (Rhee, 1994). The composition of the different products are summarised in Table 4. The manufacturing procedures for these different ingredients are briefly reviewed below.

1.3.1 SOY FLOURS AND GRITS

Soy flours and grits are produced by grinding and screening soybean flakes either before or after the oil is removed. These products contain between 40 and 54 % protein (Table 4). They are the least refined forms of soy protein ingredients used for human consumption and may vary in particle size, fat content and degree of protein denaturation. Depending on the type and level of oil present, soy flours and grits are commonly divided into 1) full-fat flours, 2) high-enzyme flours, 3) defatted flours, 4) defatted grits and 5) lecithinated/re-fatted flours.

1) Full-fat soy flours are becoming increasingly important in food. They are the least refined soy protein ingredient containing about 40 % protein. They are prepared by grinding dehulled cotyledons to a specific size. These full-fat soy flours are produced primarily in Europe and Asia and are mainly used in the baking industry and in the production of soymilks.

2) High-enzyme flours are prepared by grinding defatted flakes with minimum heat denaturation to maintain a high nitrogen solubility index (NSI) and usually contain 52-54 % protein. These flours are used in baking applications to increase mixing tolerance.
and bleaching in bread. High-enzyme flours are also used as starting raw materials for preparation of functional concentrates and isolates.

3) Defatted flours are prepared by finely grinding defatted flakes to pass through No. 100 U.S. Standard Screen. They generally contain 52-54 % protein. Controlled moist heat treatment is used to produce white (85-90 nitrogen solubility index (NSI)), cooked (20-60 NSI) and toasted (< 20 NSI) grits. Defatted flours are used in a variety of food applications where a wide range of protein solubilities are required.

4) Defatted grits are similar to defatted flours, except a screen size between No. 10 and No. 80 is used during fractionation. They are generally used in ground meat and bakery products.

5) In the production of lecithinated/refatted flours about 0.5-30 % lecithin or vegetable oil is blended with defatted flours. These products are usually used to improve water dispersibility and emulsifying capacity in baking applications (Rhee, 1994).

Table 4: Typical composition of different soy protein preparations (taken from: Kinsella, 1979).

<table>
<thead>
<tr>
<th>Component</th>
<th>Soy flours (%)</th>
<th>Concentrates (%)</th>
<th>Isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>56.0</td>
<td>72.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Fibre</td>
<td>3.5</td>
<td>4.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Ash</td>
<td>6.0</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>33.5</td>
<td>17.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>
1.3.2 **SOY PROTEIN CONCENTRATES (SPC)**

SPC’s are prepared from dehulled and defatted soybean flakes by removing most of the water-soluble, non-protein constituents. These products usually contain at least 70% protein on a moisture-free basis (Table 4). There are three basic processes for preparation of soy protein concentrates: acid leaching at pH 4.5, extraction with 70-90% aqueous ethanol and extraction with water after heat denaturation of the protein with moist heat. “Functional concentrates” are prepared by subjecting a low water-soluble soy protein concentrate to heat treatment by steam injection, in order to increase solubility and functionality. Homogenisation can also be used to further improve functionality of SPC. The functional concentrates act as emulsifiers and emulsion stabilisers in a range of food applications. They bind water and fat and they offer adhesive properties similar to those of SPI’s (Rhee, 1994).

1.3.3 **SOY PROTEIN ISOLATES (SPI)**

Soy protein isolate is the most refined soy protein product, possessing many useful functional properties (Tsumura *et al.*, 2005). The origin of isolated soy protein can be traced to the development of products for the paper coating industry in the 1930’s. Unmodified food soy proteins were on the market in 1957. The production of meat analogs based on the spinning process first patented by Boyer received much publicity in the 1950’s and 1960’s. However, the large expectations for this product were never fully realised. Isolated soy protein products texturised by thermoplastic extrusion and/or heat fibering are the major structured soy isolates available to the food industry (Kolar *et al.*, 1985).
Isolated soy proteins, containing over 90% protein (Table 4), are the most highly purified of the commercial soy protein ingredients. This high degree of purification provides maximum latitude in the formulation of food products, minimizes flavour and colour contribution due to the soy protein ingredients, has the most favourable image with consumers, and allows for the widest variety of functional properties of the different soy ingredients (Kolar et al., 1985).

Soy isolates are prepared from minimally heat-treated soy flour (see Section 1.3.1) or defatted soybean flakes by dissolving the protein in dilute alkali (pH ~ 8.0), removing the insoluble materials by centrifugation (or filtration) and precipitation of the protein at pH 4.5. This isoelectric curd may be dried or usually it is neutralized with sodium hydroxide (potassium or calcium hydroxide may also be used) and spray dried (Kinsella, 1979). Neutralisation to form Na-, K- or Ca-proteinates make the isoelectric isolates more soluble and functional. These neutralised isolates are used where emulsification, emulsion stabilisation, water and fat absorption, adhesive and fiber forming properties are desired. They are therefore used in a wide variety of meat and dairy product analogs (Rhee, 1994).

Isolated soy proteins have found widespread application in formulated food products of many types and are also used directly as nutritional supplements. SPI as part of a modified diet has been found to be effective in lowering blood cholesterol levels and as a high-quality protein source. They may be processed into several formats including spray-dried freeflowing powders, dry granules and fibrous forms. Isolated soy protein is generally recognized as safe (GRAS) by the Food and Drug Administration (Kolar et al., 1985).
1.3.4 **SOY PROTEIN HYDROLYSATES (SPH)**

Enzymatic hydrolysis had been used for many centuries to modify the functional and nutritional properties of food proteins in the production of traditional foods such as cheeses and fermented plant foods. Enzymatic proteolysis has been shown to modify functional properties such as foaming, gelation and emulsification, increase solubility and to liberate biologically active peptides from certain proteins (Spellman *et al.*, 2003).

A useful way to increase protein solubility of both native and modified soybean isolates is through hydrolysis with proteinases (Adler-Nissen, 1976). Partially hydrolyzed soy protein ingredients are produced by controlled hydrolysis of soy proteins with proteolytic activities (e.g., pepsin, papain, bromelain) to reduce the molecular size of proteins to large peptides (3000 – 5000 Daltons). This enzyme treatment substantially improves acid solubility and whipping properties. Fully hydrolyzed proteins (i.e.,
hydrolyzed soy protein (HSP), soy sauce, etc.) are prepared from soy grits usually by acid hydrolysis to produce flavouring agents (Rhee, 1994). Many studies have demonstrated that limited enzymatic hydrolysis of soy protein isolates improved functional properties including solubility, emulsifying and foaming properties (Qi et al., 1997, Walsh et al., 2003).

**1.3.5 Textured Soy Proteins**

Textured soy proteins (TSP) are prepared to impart a structure (i.e., fibre or chunk) for use as food ingredients. They are frequently made to resemble meat, seafood or poultry in structure and appearance when hydrated. Textured soy flours and soy protein concentrates are prepared by thermoplastic extrusion or steam texturisation of soy flours, alcohol or heat-denatured concentrates. The compositions of these products are similar to the corresponding raw materials. Fibrous foods, ground meat products, poultry and seafood products are examples of food systems which use these products successfully. Structured soy protein concentrates are extruded into different shapes and sizes and are also used in poultry, meats and seafoods. Structured soy protein isolates are prepared by extruding soy protein isolate or by extruding a solution of soy protein isolate into an acid-salt bath to coagulate the protein into fibres. The fibres are then put together with binders to form fibre tows or fibre bundles. These products can be used in poultry and seafoods and as food analogs (Rhee, 1994).
Chapter 1

1.4 APPLICATIONS OF SOY PROTEIN INGREDIENTS

By converting soy into various forms of foods, soybeans have been the major source of dietary protein for millions of people in the Orient for centuries. The extent of conversion of soy protein into foods in the rest of the world is still very small as compared to usage of soybean oil. However, due to an ever-increasing population, demand for food protein has increased significantly. Although animal protein has been a major source for meeting such demands, there is now a strong incentive to use low cost plant protein in the world economy (Liu, 1997).

Functional properties are affected by composition, structure and conformation of ingredient proteins as well as by the manner in which they are used. Therefore, knowledge of the physiochemical properties of component proteins as well as the surrounding conditions is critical for understanding the mechanisms of particular functional traits (Rhee, 1994).

A number of dairylike products such as imitation milks and cheeses, non-dairy frozen desserts, yoghurts and coffee whiteners have been developed from soybean and soy protein ingredients. This helps to lower cost, improve nutritional value, reduce allergic responses, alleviate lactose intolerance and improve functional characteristics (Rhee, 1994).

Soy protein ingredients are used as partial replacements of meat, binders, flavour enhancers, emulsifiers, brine ingredients and as meat analogues. They contribute to nutrition, flavour and critical functional properties. The quality of whole meat products can also be improved by injecting soy protein brine, this helps to tenderise and reduce cooking losses (Rhee, 1994). Soy proteins incorporated in sausage batters can increase emulsifying properties, viscosity, gel forming and water holding of comminuted meat.
products (CMP), (Zayas, 1997). The poultry industry has been developed using SPI’s, e.g., poultry luncheon meats (Kolar et al., 1985).

Soy protein ingredients are used in a variety of bakery products for various functional and nutritional reasons. Soy flours are commonly used in bakery products, mainly for economic reasons, as replacers for more expensive milk and nonfat dry milk. Some of the benefits of using soy flours in bread include improved water absorption, better dough handling, tenderising effects, firmer body, resiliency, improved crust colour and better keeping quality (Rhee, 1994).

Specially processed soy protein ingredients can also be used as aerating and whipping agents. Partially hydrolysed soy proteins possess good foaming and foam-stabilising properties, which make them suitable for use as aerating agents. These soy protein ingredients can be used alone or in combination with egg albumen or whole egg to improve whipping properties, i.e., whipping rate and whip stability. These modified soy protein ingredients are also used in coffee whiteners and whipped desserts (Rhee, 1994).

Most commercial infant formulae are manufactured using bovine milk as a basic ingredient. However, these formulae can also be soy protein or protein hydrolysate based. In all the formulae the protein content is adapted to adjust the essential amino acid composition to that of human milk (Algeria et al., 1999). Infant formulae can be described as heat sterilised and dehydrated emulsions. The protein, fat, carbohydrate and other minor ingredients are blended together with water, this solution is then homogenised and subjected to heat treatments before final sterilisation or spray drying (McSweeny et al., 2004).

Soy based formulae are often used when an infant displays an intolerance to lactose or milk proteins. Soy formulae contain purified soy proteins, carbohydrates composed of maltodextrin, corn starch and, in some cases, sucrose, and fat, which is a mixture of
vegetable oils. It is necessary for these formulae to be supplemented with methionine, carnitine, copper, zinc, iron and calcium in order to provide a nutritionally adequate intake (Maldonado et al., 1998).

Hydrolysed protein formulae were developed in order to reduce or eliminate protein allergenicity and to prevent and/or treat allergic disorders. There are two types of preparation (a) formulae with a high degree of hydrolysis, commonly known as semi-elemental; and (b) partially hydrolysed formulae with a low degree of hydrolysis (Maldonado et al., 1998).

1.5 FUNCTIONAL PROPERTIES OF SOY PROTEINS

Functional properties of proteins are those physical and chemical properties which affect the behaviour of proteins in food systems, during processing, storage, preparation and consumption. These characteristics influence the quality and organoleptic attributes of foods (Damodaran, 1996 & 1997 (a)). Functional properties can be classified into three main groups: (a) hydration properties (dependant on protein-water interactions), (b) properties related to protein-protein interactions and (c) surface properties. Proteins have many functional roles on food systems, these are summarised in Table 5.

Soy protein ingredients must possess appropriate functional properties, e.g., water sorption, solubility, gelation, surfactancy, ligand-binding and film formation, for food applications and consumer acceptability. These functional properties reflect the composition and conformation of the proteins, their interactions with other food components and they are affected by processing treatments and the environment. Because functional properties are influenced by the composition, structure and conformation of ingredient proteins, knowledge of the physical properties of component
protein is essential for understanding the mechanism of particular functional traits (Kinsella, 1979).

**Table 5: Functional properties of proteins required in various foods** (adapted from Kinsella, 1979).

<table>
<thead>
<tr>
<th>Food</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages</td>
<td>Solubility at different pH, heat stability, viscosity</td>
</tr>
<tr>
<td>Soups, sauces</td>
<td>Viscosity, emulsification, water retention</td>
</tr>
<tr>
<td>Dough formation, baked products (e.g., bread, cakes)</td>
<td>Heat denaturation, gelation, emulsification, foaming, browning</td>
</tr>
<tr>
<td>Dairy products (e.g., processed cheese, ice cream, desserts)</td>
<td>Emulsification, fat retention, viscosity, foaming, gelation, coagulation</td>
</tr>
<tr>
<td>Egg substitutes</td>
<td>Foaming, gelation</td>
</tr>
<tr>
<td>Meat products (e.g., sausage)</td>
<td>Emulsification, gelation, cohesion, water and fat absorption and retention</td>
</tr>
<tr>
<td>Meat extenders (e.g., texturised vegetable protein)</td>
<td>Insolubility, hardness, chewiness, cohesion, heat denaturation</td>
</tr>
<tr>
<td>Food coatings</td>
<td>Cohesion, adhesion</td>
</tr>
<tr>
<td>Confectionary products (e.g., milk chocolate)</td>
<td>Dispersibility, emulsification</td>
</tr>
</tbody>
</table>

The primary factors affecting the behaviour of the protein are the amino acid composition, the sequence of the residues and the molecular weight. There are also other properties such as the tertiary and quaternary structures, hydrophobicity, net charge and charge distribution, as well as the resulting isoelectric point and the
flexibility of the molecule, which also affect the behaviour of the protein, but these are all determined by the primary factors. However, the behaviour of the protein also depends on the properties of the environment. The extrinsic factors, i.e., character of the solvent, temperature, pH, ionic strength, divalent cations, denaturants, other macromolecules, lipids and activity of enzymes are also important in the selection of a particular protein ingredient for a specific food application (Sikorski, 2001).

1.5.1 HYDRATION PROPERTIES

The interactions of soy proteins with water are important in relation to dispersibility, water absorption and binding, swelling, gelation, viscosity and surfactant properties. These properties influence the functionality of soy proteins in meat, bakery and beverage systems (Kinsella, 1979).

Wettability is important in food formulations. It is affected by surface polarity, texture and area, and by the size and microstructure of the protein particles. The major factors affecting dispersibility are temperature, ionic composition, pH and degree of agitation of the solvent (Kinsella, 1979).

Water-sorption and water-binding by soy proteins are very important in a variety of meats, bakery products and cheeses. When substituting for conventional proteins, soy proteins must have suitable water-binding capacities, in addition to other needed properties. In order to facilitate adjustments in food formulations when interchanging protein sources, the water-binding capacity of different proteins must be determined (Kinsella, 1979).
In addition to water-sorption, soy preparations possess water-holding capacities, i.e., the ability to physically hold water against gravity. This is related to viscosity of food systems and is influenced by pH, ionic strength and temperature. As soy proteins absorb water, they swell. Swelling is an important functional property in foods like processed meat, doughs and custards where the proteins should imbibe and hold water without dissolving and concurrently impart body, thickening and viscosity (Kinsella, 1979).

The water absorbing capacity (WAC) of SPI was affected by protein solubility and the presence of salt. The WAC was high in soy protein samples with a nitrogen solubility index (NSI) in the range of 36.3 - 68.1 %. A decrease in WAC was caused by the presence of 0.2 M NaCl, as a result of increased aggregation in all samples (Wagner & Añón, 1990).

### 1.5.2 Solubility

Kinsella (1979) described solubility as the amount of protein in a sample that goes into solution or exists as a colloidal dispersion under specified conditions of temperature and pH and is not sedimented by low centrifugal force. Generally, good aqueous solubility of soy proteins is required since this influences many other functional properties. A highly soluble protein is necessary to obtain optimum functionality in uses where gelation, solubility, emulsifying activity and foaming are required (Kinsella, 1979). Protein-containing additives with high solubility are also easier to incorporate into foods than proteins of low solubility (Zayas, 1997). Dickinson (1991) also reported that poor solubility is generally associated with poor functionality. Therefore, solubility is an important factor in product quality.
Protein insolubility increases with an increase of hydrophobicity and with a decrease in zeta potential (Hayakawa & Nakai, 1985). Protein-protein interactions in an aqueous medium are accelerated by hydrophobic interactions between the nonpolar groups on the protein. The content of sulphhydryl groups is also related to the insolubilisation of soybean proteins. The tendency for soy isolate solubility is to decrease with an increase in the number of free SH groups (Wagner & Añón, 1990).

The methods of preparation affect soy protein ingredient solubility, with heat treatment, especially moist heat (steam), being the most significant. Heat which is applied to remove solvent, destroy antinutritive factors, inactivate enzymes, dry and volatilise bound off-flavours, all reduce the solubility of soy protein. The extent of which depends on the intensity and duration of the heat treatments (Kinsella, 1979). Soy flours with various solubilities are produced by food manufacturers and utilized depending on their protein dispersibility index (PDI). PDI is the ratio between the water dispersable protein and the total amount of protein. Solubility is the most important factor in product quality for some foods such as high-protein drinks, soft drinks and soy milk (Zayas, 1997).

Generally the solubility of soy protein increases with increasing degree of hydrolysis (DH), however, it generally does not reach 100% solubility even at very high DH (Adler-Nissen, 1986). Walsh et al., (2003) found that a slight decrease in solubility of SPI occurred at a DH of 1%, this was probably due to the heat treatment given after hydrolysis in order to inactivate the enzymes and to terminate the reaction.

At pH values above and below the isoelectric pH (pI), proteins carry a net positive or a net negative charge, respectively. Electrostatic repulsion and hydration of charged residues promote solubilisation of the protein. When solubility is plotted against pH, most food proteins exhibit a U-shaped curve, with minimum solubility occurring at the pI of proteins (Damodaran, 1996). Achouri et al., (1998), obtained the typical pH-
solubility profile of SPI displaying a bell-shaped curve with minimum solubility at pI (pH 4-5). As the pH diverges from this value (pI) or at constant pH, SPI solubility in dilute aqueous solutions increases, as the salt concentration is increased (van Megen, 1974). Native soy protein exhibited low solubility at acidic pH (2.0 to 5.0), but after hydrolysis, solubility was significantly increased between pH 2.0 and 9.0 (Chiang et al., 1999). Furthermore, the combined use of transglutaminase cross-linking and hydrolysis has the potential to generate improved solubility of soy protein ingredients for use in low pH food and beverage products (Walsh et al., 2003).

1.5.3 EMULSIFICATION

An emulsion is a colloidal dispersion of liquid (or partially crystalline) droplets in a liquid continuous medium (Figure 4), (Dickinson, 1991) Emulsions are important elements in the formulation of foods (especially oil-in-water emulsions), and therefore have to be prepared in such a way as to be stable, often over long periods of time. Reactions such as aggregation of flocculation, which lead to creaming and possibly coalescence, must be avoided (Dalgleish, 1997).

![Figure 4: Oil-in-water emulsion](taken from: University of British Columbia, 2005).
The properties of protein-stabilised emulsions are affected by several factors. These include intrinsic factors such as pH, ionic strength, temperature, presence of low-molecular-weight surfactants, sugars, oil-phase volume, type of protein and melting point of oil used; and extrinsic factors such as type of equipment (i.e., homogeniser, blender), rate of energy input and shear rate (Damodaran, 1996).

The multiphasic state of foods is mainly due to thermodynamic incompatibility among its major components. Fats, because of their energetically unfavourable interaction with water tend to separate and exist as a separate phase. Although proteins and polysaccharides are soluble in water, because of their limited thermodynamic compatibility they tend to form protein-rich and polysaccharide-rich regions within the aqueous phase. Their stability against phase separation during storage is critically dependent on the type of surfactants present at the interfaces of the various dispersed phases. Two types of emulsifiers are being used on foods: low molecular mass surfactants, such as lecithin, mono- and diglycerides, sorbitan monostearate etc., and high molecular mass surfactants, such as proteins and certain gums. These emulsifiers have a hydrophilic end which is attracted to the water phase and a hydrophobic end which interacts with the oil phase (Figure 5). At similar bulk concentration (w/v), low molecular mass surfactants decrease the surface or interfacial tension to a greater extent than the high molecular mass surfactants. This difference is mainly due to differences in orientation and configuration of these surfactants at an interface, (Damodaran, 1997 (b)).
Proteins are generally surface-active and have good emulsifying properties which are therefore beneficial for manufacturing various emulsified foods (Elizalde et al., 1996). Since the exact secondary and tertiary conformation of a protein at the interface is difficult to decipher, its configuration is usually depicted in terms of “trains”, “loops” and “tails”. The trains are the hydrophobic segments that lie flat on the interface, making contact with both the aqueous and oil phases (only the nonpolar side chains of amino acids are orientated towards the oil phase). The loops are the polypeptide segments between the trains that are suspended into the aqueous phase and the tails are the N- and C- terminal segments that are invariably suspended in the aqueous phase (Figure 6), (Damodaran, 1997 (b)). Soy proteins aid in the formation of emulsions, primarily by decreasing interfacial tension between the water and oil, and also by helping to stabilise the emulsion by forming a physical barrier at the interface (Molina et al., 2001).
Figure 6: Diagrammatic representation of the orientation of a protein emulsifier at the oil/water interface (taken from: Damodaran, 1997 (b)).

The most important functionality of soy proteins in processed meats and other foods is their emulsifying property, together with fat and water retention. The emulsion capacity (EC), emulsion stability (ES) and emulsifying activity (EA) of soy proteins are useful functional characteristics which play an important role in the development of soy protein-containing products for use as a food. EC may be expressed as the amount of oil (ml) that is emulsified under specific conditions per g of protein. ES refers to the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing and flocculation. EA refers to the maximal interfacial area (cm$^2$) stabilised per g of protein for an emulsion (Zayas, 1997). EC, ES and EA all depend on the properties of proteins and conditions of emulsification, and they vary with the source of protein, its concentration, pH, ionic strength (salt type and concentration) and viscosity of the system (Damodaran, 1997 (b)). Webb et al., (2002) found the ES of SPI to be significantly greater that that of whey protein isolate (WPI) and sodium caseinate (SC). Utsumi et al., (1997) reported that soy proteins had a higher EA when compared to the dairy proteins as a result of the high emulsifying ability of β-conglycinin. The use of soy proteins in emulsified food systems can promote fat binding to reduce cooking loss, improve EC and maintain stability of the emulsion system (Zayas, 1997).
Commercial preparation of soy isolates can cause physical and chemical changes to the protein, these changes can in turn affect the functional properties of the protein. Therefore, different commercial isolates vary widely in their emulsifying properties depending on differences in their composition, conformation, net charge and structure (Elizalde et al., 1996).

Soy proteins play two roles in emulsification, i.e., forming and stabilising oil-in-water and water-in-oil emulsions. The stabilising effect of soy proteins in emulsions is related to high electrical charge and the hydrophilic-lipophilic structure which enables soy proteins to orient at the oil/water interface with the hydrophilic segments oriented towards the water phase and the lipophilic segments towards the oil phase. This interaction lowers the interfacial tension between two immiscible phases, i.e., oil and water, and thereby facilitates emulsification (Elizade et al., 1991). Protein-lipid and protein-water interactions are the major factors of emulsion formation and affect the appearance, colour, texture and yield of the finished products (Zayas, 1997).

The emulsifying properties of soy proteins may be improved by heat denaturation due to increased exposure of hydrophobic groups and therefore increased surface hydrophobicity leading to decreased surface tension at oil-in-water interfaces (Zayas, 1997).

McWatters & Holmes, (1979) measured the emulsifying properties of soy flour over the pH range of 2-10, in three dispersion media, i.e., distilled water, 0.1 M NaCl (low salt) and 1.0 M NaCl (high salt). The EC of the water and low-salt suspensions were similar and quite low at pH 4 but improved significantly at pH levels below or above pH 4. In high-salt suspensions, EC generally increased from pH 2 to pH 10. While no emulsions were formed by the water and low-salt suspensions at pH 4, thick mayonnaise-like emulsions were formed by the high salt treatment at this pH. Multiple regression analyses showed that pH was the primary determinant of the emulsifying properties of
the soy flour. Molina et al. (2001) found that pressure treatment at neutral pH can improve the EA of soy proteins but, in most cases it does not improve the ES.

Enzymatic and chemical modifications have been used to improve protein functionalities. Enzymatic modification has an advantage over chemical methods because it causes minimal undesirable side reactions or products (Qi et al., 1997). In general, a limited degree of hydrolysis usually improves the emulsifying properties, whereas excessive hydrolysis often causes loss of some of these functionalities (Kuehler & Stine, 1974). Qi et al. (1997) found that pancreatin hydrolysis could alter molecular weight, increase surface hydrophobicity and improve the functional properties (solubility and EA) of soy protein isolate. Soy protein peptides prepared by papain modification and ultrafiltration had high emulsifying properties (Wu et al., 1998). Kim et al., (1990) found that the EC of SPI’s enzymatically modified with trypsin and alcalase increased, but no significant improvement in EC resulted from rennet treatment.

**Emulsion Instability**

There are four principle mechanism for the destabilisation of emulsions. Creaming: a separation caused by the upward motion of emulsion droplets that have lower density than the surrounding medium. Flocculation: an aggregation of droplets when the kinetic energy released during collisions bring the droplets over the repulsive force barrier and into a region where attractive forces operate and cause the droplets to attach to each other. Coalescence: when two droplets colloid, they lose their identity and form a single larger one. Oswald ripening: caused by the diffusional transport from small droplets into larger ones, when the chemical potential of the liquid in the droplet decreases as the droplet radius increases (Walstra, 1996).
Chapter 1

The concentration of emulsion droplets and the droplet size are the key parameters in determining emulsion instability. Coalescence is dominant at high concentrations of emulsion droplets (> 10 to 50 %), flocculations occurs at low concentrations and small droplets (< 5 % and 1μm in size) and creaming occurs at low concentrations and large droplets (< 10 to 50 % and > 2 to 5 μm in size), (Walstra, 1996).

Methods of analysis of particles

Emulsion stability is a measure of the rate at which an emulsion creams, flocculates or coalesces. The rate of these changes can be measured by determining the size and distribution of the oil droplets in the emulsion. Stoke's law states that the velocity at which a droplet moves is proportional to the square of the radius (Huang et al., 2001).

There are many methods used for the analysis of particles including sieving, light microscopy, electron microscopy and sedimentation analysis. The method used for the analysis of particle size in this study was laser diffraction. The technique of laser diffraction relies on the fact that particles passing through a laser beam scatter light at an angle that is inversely proportional to their size (small particles scatter light at high angles whereas large particles scatter light at low angles), (Figure 7). It is therefore possible to calculate particle size distributions if the intensity of light scattered from a sample is measured as a function of angle (Malvern Instruments Ltd., 2005).
1.5.4 VISCOSITY

Rheology is the study of the change in form and the flow of matter, embracing elasticity, viscosity and plasticity. Viscosity can be further defined as the internal friction of a fluid caused by molecular attraction, which makes it resist a tendency to flow. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the greater the amount of force required to cause this movement, which is called shear. Newtonian fluids have constant viscosity dependent on temperature but independent of the applied shear rate. These fluids show a straight line relationship between shear stress ($S$) and shear rate ($F'$). A non-Newtonian fluid is broadly defined as one for which the relationship $F'/S$ is not constant. When the shear rate is varied, the shear stress does not vary in the same proportion. The viscosity of such fluids will therefore change as the shear rate is varied. Thus the experimental parameters of viscometer model, spindle and speed all have an effect on the measured viscosity of non-Newtonian fluid. This measured viscosity is called the “apparent viscosity” of the fluid (Dairy Processing Handbook, 1995).

Product formulation, texture control and mouthfeel properties all require knowledge of the viscosity and flow properties of protein dispersions. Viscosity can be used to
evaluate the thickening power of soy proteins which are of practical interest in fluid foods (soups, beverages, batters) and in comminuted meats. The viscosity of protein dispersions is mostly influenced by the hydrodynamic properties of the component protein i.e., molecular weight, size, hydration and shape of the molecule. These are in turn influenced by temperature, pH, ionic strength and also processing treatments as they affect molecular conformation, structure, aggregation state, hydration and swelling (Kinsella, 1979).

Tsumura et al., (2005) studied two soy protein hydrolysates which were produced from an SPI in which either the glycinin or β-conglycinin fractions had been selectively enzymatically hydrolysed. The apparent viscosity of the two hydrolysates was lower than that of the control SPI, with the reduced-glycinin hydrolysate being particularly low among the three.

1.5.5 GELATION

Protein gels are composed of three-dimensional matrices or networks of intertwined, partially associated polypeptides, in which water is entrapped. Gels are characterised by a relatively high viscosity, plasticity and elasticity (Kinsella, 1979). In the last few years approximately 1 % of the soybean protein produced has been used for human food, mainly to improve texture, the rest is used for animal feed. Therefore the most important property of soy proteins is their ability to form a gel with good water holding capacity upon heating (van Vliet et al., 2002). Conditions during gel formation in food products vary greatly due to variations in pH, salt content, the combination of ingredients, etc. These all affect the properties of the gel formed (Renkema et al., 2000). The first use of soy protein as a food, in the Orient, was in the form of a gel known as tofu. Soy gels have the capacity to act as a matrix and to hold moisture, lipids,
polysaccarides, flavours and other ingredients. The characteristic property of soy protein gels is a considerably higher water holding capacity (WHC) than in milk and other protein gels. Therefore, soy proteins can be used in gel systems in which syneresis is undesirable, e.g., yoghurt (Zayas, 1997).

The major components of soybean protein isolate, 11S (glycinin) and 7S (β-conglycinin) globulins are affected in a distinct manner according to treatments or modifications. Sorgentini et al., (1995) found that heat treatment induced dissociation, denaturation and aggregation of 7S and 11S subunits. At an adequate protein concentration this treatment leads to gelation phenomenon (Puppo & Añón, 1998, 1999). Gelling properties of soybean proteins could be improved by interaction with other gelling agents such as gums or polysaccharides (Molina Ortiz & Wagner, 2004).

Nakamura et al., (1984) examined the gelation and gel properties of glycinins using five soybean cultivars having different subunit compositions. It was found that the gelling characteristics of glycinin differed significantly among cultivars, arising from the differences in the nature of protein itself as well as the protein concentration.

Boatright & Hettiarachchy, (1995) found that improvement in protein solubility contributed by added antioxidants corresponded to increased gel fracturability, hardness and adhesiveness of heat set SPI gels. The gel strength of soy protein isolate was markedly improved through aqueous alcohol washing of the soy flake (Hua et al., 2005). Hermansson (1985) found that at pH 7.0, β-conglycinin-rich gels (purity 70 %) had a less regular and more cross-linked network than gels made by glycinin. The β-conglycinin strands show a complex aggregation pattern, possibly in the form of double spirals. Increasing ionic strength at neutral pH values led to the formation of coarser gels.
1.5.6 **FOAMING**

A foam is a coarse dispersion of gas bubbles in a liquid or solid continuous phase (usually water), (Dickinson, 1991). The capacity of proteins to form stable foams with gas by forming impervious protein films, is an important property in confectionary products such as cakes, soufflés, whipped toppings, fudges, etc. Protein foams consist of gas droplets encapsulated by a liquid film containing soluble surfactant protein. This in turn lowers the interfacial tension between the gas and water, which facilitates deformation of the liquid and expansion against its surface tension. Proteins for foaming should be soluble in the aqueous phase. They should concentrate at the interface, unfold to form cohesive layers of protein around air bubbles as they are formed. The proteins should possess sufficient viscosity and mechanical strength to prevent rupture and coalescence (Kinsella, 1979).

Significant variability in the foaming capacity of soy proteins has been reported on the literature. This may be related to the different level of protein denaturation during preparation of different soy protein ingredients. The foam stability of a SPI dispersion was far less than a WPI dispersion (Webb *et al.*, 2002). There is little information available on the foaming properties of isolated 7S and 11S globulins. It has been reported that the foaming characteristics of the 11S protein, glycinin, may be limited by its relatively stable oligomeric structure (Zayas, 1997). Hua *et al.*, (2005) discovered that washing soy flakes with aqueous alcohol before extraction to prepare SPI, resulted in improved foaming stability of the SPI. This may be due to the fact that the residual lipids in defatted soy flakes were substantially removed by alcohol washing.

Unmodified vegetable proteins, including soy proteins, often exhibit best foaming properties at a pH below 4, which limit their application (van Vliet *et al.*, 2002). Molina Ortiz & Wagner, (2002) studied the effect of enzymatic hydrolysis on structure
characteristics of soy protein isolates. They discovered that at pH 4.5, isolates exhibited low capacity to form and stabilise foams, this however improved after enzymatic hydrolysis of the isolates.

It is clear therefore that various commercially available soy protein products are available. These products find applications in a whole range of foods where functional attributes such as solubility, emulsification, foaming, gelation, etc. are central characteristics in the final food product.

1.6 OBJECTIVE OF STUDY

OVERALL OBJECTIVE
- To study the emulsifying properties of commercially available SPI and SPH ingredients in model infant formulations.

SPECIFIC OBJECTIVES
- To assess contribution of ingredient composition and lecithin concentration to the particle size distribution of SPI and SPH stabilised model emulsions.

- To determine the contribution of total solids (TS), homogenisation pressure and pH to the particle size distribution and apparent viscosity of SPI and SPH stabilised model emulsions.
Chapter 2

Contribution of Ingredient Composition and Lecithin Concentration to the Particle Size Distribution of Soy Protein Emulsions
2.1 ABSTRACT

The main objective of this work was to quantify the effects of adjusting ingredient composition and lecithin concentration on the particle size distribution of two soy protein isolate (SPI sample 1500 and SPI sample 1651) and two soy protein hydrolysate (SPH sample 1761 and SPH sample 1762) stabilised model emulsions. Model emulsions were manufactured using SPI/SPH, corn syrup solids (CSS) and a commercially obtained fat blend. These ingredients were passed through a laboratory pasteuriser (77°C for 30 sec), followed by homogenisation (double pass) at a first stage pressure of 170 bar and a second stage pressure of 30 bar. For the ingredient composition study, three emulsions were generated, i.e., a model emulsion containing protein, CSS and fat blend, a model emulsion without protein and a model emulsion without CSS. For the lecithin effect study, a model emulsion was made using the fat blend containing 5 different lecithin concentrations (0 – 2 % (w/w)). No major effects on particle size distribution were evident when CSS was removed from emulsions made with SPI sample 1500, SPI sample 1651 and SPH sample 1761. Surprisingly, the absence of CSS in the emulsion manufactured with SPH sample 1762 resulted in an increase in the proportion of smaller particles, after the 2nd homogenisation pass and on storage at 5°C for 22 h. Although there were no real differences in particle size distribution profiles for emulsions manufactured with fat containing varying amounts of lecithin, there was an obvious effect when lecithin was removed, particularly from those emulsions made with SPI sample 1651 and SPH sample 1761. With these soy protein ingredients, the particle size distribution profiles tended to be bimodal with an increased proportion of larger sized particles. The presence of lecithin greatly improved the particle size distribution of the emulsion generated with SPH sample 1762, particularly after the 1st homogenisation pass. The proportion of smaller particles increased with a corresponding decrease in larger particles.
2.2 INTRODUCTION

An infant's first months of life are characterised by fast growth and a high rate of physiological development. Therefore, the quantity and quality of the supply of energy and essential nutrients, including amino acids must be sufficient to support the infants needs (Algeria et al., 1999). Human milk is the best food for infants, for its nutritional and metabolic advantages, psychological effects and the role played in preventing diseases. When breastfeeding is impossible, it must be substituted by an infant formula that satisfies the nutritional needs of the infant (Maldonado et al., 1998).

Special formulae can be used for infants with certain nutritional needs. Infants presenting intolerance to lactose may be fed soy formulae or others based on cow milk from which the lactose has been removed, to be replaced by another carbohydrate, usually maltodextrin or corn syrup solids (Maldonado et al., 1998). Soy protein based formulae are also preferentially fed to infants who have developed, or who are susceptible to the development of allergenic reaction to cows milk protein. Soy proteins exhibit high emulsifying properties compared to other plant proteins (Zayas, 1997). Soy proteins play a central role in the functional characteristics and behaviour of formulated infant products. Recently, formulae with higher protein, fat, carbohydrate and calcium levels than regular formulae have been developed for the specific nutritional requirements of premature infants. It is important to form a stable emulsion and to select the ideal processing parameters to optimize stability. It is therefore necessary to understand how the ingredients in a formula influence emulsification and thermal properties (McSweeney et al., 2004). Lecithins are important ingredients in the commercial manufacture of emulsions. Commercial lecithins are mixtures of several phospholipids and fats (Agboola et al., 1998).
The objective of the present study was to investigate the contribution of variation in soy protein ingredient type and lecithin concentration to the particle size distribution profiles of two SPI and two SPH ingredients in model infant formula emulsions.
2.3 MATERIALS AND METHODS

2.3.1 MATERIALS

Soy protein isolates (SPI 1500, SPI 1651) and soy protein hydrolysates (SPH 1761, SPH 1762) were obtained from DuPont Protein Technologies (137 Rue De L’Université, 75007 Paris, France). Corn syrup solids (Roquette Frères 62080 Lestrem, France/Cerestar France, 7 Rue du Maréchal Joffre, 59842 Haubourdin Cedex BP 109, France) and fat blend A, a proprietary blend of vegetable fat/oil used in infant formulations, was obtained from a commercial supplier.

Sodium dihydrogen orthophosphate dihydrate, di-sodium hydrogen orthophosphate dehydrate, L-leucine and Kjeldahl catalyst tablets were obtained from BDH Laboratory Supplies (Poole, BH15 1TD, UK). Boric acid, NaOH, HCl and sodium dodecyl sulphate (SDS) were supplied by Sigma Chemical Co. (Poole, Dorset, UK).

Low nitrogen H₂SO₄ was purchased from Lennox Laboratory Supplies (Naas Road, Dublin 12) and anti-bumping granules were supplied by Scientific & Chemical Supplies Ltd. (West Midlands, WV14 OQZ, UK). Trinitrobenzenesulphinic acid (TNBS) was obtained from Pierce (Rockford, IL 61105, U.S.A.).
CHARACTERISATION OF SPI AND SPH

COMPOSITIONAL ANALYSIS

2.3.2 MOISTURE CONTENT

This was performed using the IDF procedure (1991). Samples (1.5 g) of SPI and SPH were dried to a constant weight at 70°C under reduced pressure of 1,000 mbar in a vacuum heating oven (Sanyo Gallenkamp PLC (Model no: OVA031.XX1.5), Riverside Way, Uxbridge, Middlesex UB8 2YF, UK) for 24 h. The analysis was carried out in duplicate and values expressed as the mean ± SD.

2.3.3 NITROGEN CONTENT

Nitrogen content was determined using the macro-Kjeldahl procedure (IDF Standard 20B: 1993) with some modifications as follows:

Low nitrogen sulphuric acid (25 ml) was added to SPI and SPH samples (0.3 g) in Kjeldahl tubes. Two Kjeldahl tablets and some anti-bumping granules were then added. A blank sample consisting of distilled water (0.3 g) was prepared with each batch of samples tested.

The Kjeldahl tubes were then placed in a heating block (Buchi Digestion Unit K-435, Buchi Labortechnik, Mason Technology, Dublin 8) for the digestion process. The heating block was initially run at setting 4 for 30 min, this was increased to setting 9 for 2 h in order to obtain complete digestion.

On complete digestion the Kjeldahl tubes were allowed to cool at room temperature prior to distillation using a Buchi Distillation Unit (Model B-323, Buchi Labortechnik,
Mason Technology, Dublin 8). Distilled water (85 ml) and 40 % (w/v) NaOH (140 ml) was added to the Kjeldahl tubes which were subsequently distilled for 8 min. The ammonia distilled off was collected in conical flasks containing 50 ml of 4 % (w/v) boric acid.

The borate formed during distillation was then titrated with standard 0.1 N HCl to a pH of 4.6. The end point was determined using a pH meter (Model 3310, Analytica Laboratory Suppliers, Sandyford Industrial Estate, Foxrock, Dublin 18).

**Calculation of nitrogen content:**

\[
\% \text{ N} = 1.4007 \left( V_s - V_b \right) \frac{M}{W}
\]

where:

- \( V_s \) is the volume (ml) of standard 0.1 N HCl used in titration of the sample
- \( V_b \) is the volume (ml) of standard 0.1 N HCl used in titration of the blank
- \( M \) is the molarity of the standard HCl (0.1 M)
- \( W \) is the mass (g) of the sample being tested

The molecular weight of nitrogen is 14.0067

The percentage protein was calculated by multiplying the percentage nitrogen by the protein conversion factor. The Kjeldahl conversion factor used for soy protein was 6.25 (Kolar *et al.*, 1985).
2.3.4 **DETERMINATION OF THE DEGREE OF HYDROLYSIS (DH)**

The TNBS method used was that described by Adler-Nissen (1979). This procedure is based on the reaction of TNBS with primary amino groups in proteins and peptides. Degree of hydrolysis (DH) was defined as the percentage of the total number of peptide bonds in the protein which has been cleaved during hydrolysis. In an intact protein, no peptide bonds are cleaved and therefore DH = 0.

\[
DH \% = \frac{\text{Number of peptide bonds cleaved}}{\text{Total number of peptide bonds}} \times 100
\]

**Preparation of reagents for TNBS assay:**

Phosphate buffer (0.2125 M, pH 8.2) was prepared from two solutions; Solution A (NaH$_2$PO$_4$) and Solution B (Na$_2$HPO$_4$). Solution A was prepared by dissolving 3.23 g of sodium dihydrogen orthophosphate dihydrate (NaH$_2$PO$_4$·2H$_2$O) in distilled water and bringing the final volume to 100 ml. Solution B was prepared by dissolving 37.38 g of di-sodium hydrogen orthophosphate dihydrate (Na$_2$HPO$_4$·2H$_2$O) in distilled water and bringing final volume to 1000 ml. Solution A was then added to Solution B until a pH of 8.2 was reached at room temperature. The approximate ratio was 43 ml of Solution A : 1000 ml Solution B.

A 0.1 % (w/v) TNBS solution was prepared by dissolving 1 ml of TNBS reagent (5 % w/v) in distilled water and making up to 50 ml in a volumetric flask. This solution was covered with aluminium foil in order to exclude all light. (This solution was prepared daily and stored at 4°C until required for use).
A 1 % (w/v) solution of SDS was prepared by dissolving 1 g of SDS in distilled water and making to 100 ml with distilled water.

**Preparation of test samples:**

Samples of SPI and SPH (1 % (w/v) protein) were dissolved in distilled water and allowed to stir for 30 min at room temperature. Dilutions (1:10) were then made in 1 % SDS.

Dilutions of a L-leucine stock solution giving between 2 and 28 mg/ml of amino nitrogen were prepared in distilled water. These were then used to generate a standard curve to demonstrate the linearity of the absorbance following reaction with TNBS (abs @ 340 nm). The standard curve was performed on one occasion and for subsequent determinations a single standard value of 28 mg/ml L-leucine was tested in order to confirm reproducibility of the assay solutions.

**Assay:**

Duplicate aliquots (0.25 ml) of test sample solutions (1 % (w/v) protein or protein equivalent) were added to test tubes containing 2 ml of 0.2125 M phosphate buffer pH 8.2. (Duplicate blank samples were also prepared using 0.25 ml aliquots of 1 % SDS). TNBS (2 ml) was then added to each tube. The contents were mixed using a vortex and covered with aluminium foil. The tubes were placed in a covered water bath (to exclude all light) at 50°C for 60 min. After incubation, the reaction was stopped by adding 4 ml of 0.1 N HCl to each tube. The tubes were then allowed to cool for 30 min at room temperature again making sure to exclude light at all times.
The absorbance at 340 nm of the sample/standard solutions were then read against the blanks using an Ultraspec 2000 UV/visible spectrophotometer (Pharmacia Biotech, Cambridge, England).

**Calculations:**

\[ DH = \frac{AN_2 - AN_1}{Npb} \]

where:

- \( AN_1 \) is the amino nitrogen content of the protein substrate before hydrolysis (mg/g protein) - \( AN_1 \) was obtained from SPI.
- \( AN_2 \) is the amino nitrogen content of the protein substrate after hydrolysis (mg/g protein).
- \( Npb \) is the nitrogen content of the peptide bonds in the protein substrate (mg/g protein).

A value of 108.6 is used for \( Npb \) of soy protein (Kolar *et al.*, 1985).

### 2.3.5 Preparation of Model Emulsions

Distilled water was heated to 70°C and fat blend was heated to approximately 50 °C, on hot stirring plates. CSS were then added to the water, followed by the soy protein isolate/hydrolysate test sample and finally the fat blend to give a final TS of 24 % (w/w). The quantities of fat, protein and carbohydrate in the emulsion were 36, 18 and 69 g/L, respectively. The ingredient mixture was then left to stir for an hour at 70 °C, before
passing through a laboratory pasteuriser (Armfield FT 43, Armfield Ltd., Ringwood, Hampshire, England), (77°C for 30 sec). After this high temperature short time (HTST) step the solution was allowed to cool to 66°C and then homogenised (double pass) using a two-stage laboratory-scale homogeniser (APV 1000, APV Products, Rohlomsvej 8, DK-2620 Albertslund, Denmark) at a first stage pressure of 170 bar and a second stage pressure of 30 bar.

Table 1: Ingredient composition for model emulsion at 24 % total solids (w/w).

<table>
<thead>
<tr>
<th>Soy Protein Sample</th>
<th>Fat (g)</th>
<th>SPI/SPH (g)</th>
<th>CSS (g)</th>
<th>H₂O* (g)</th>
<th>Total batch size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI 1500</td>
<td>70.24</td>
<td>42.00</td>
<td>145.12</td>
<td>769.90</td>
<td>1025.00</td>
</tr>
<tr>
<td>SPI 1651</td>
<td>70.40</td>
<td>45.30</td>
<td>145.12</td>
<td>770.16</td>
<td>1025.00</td>
</tr>
<tr>
<td>SPH 1761</td>
<td>70.34</td>
<td>44.78</td>
<td>145.12</td>
<td>770.24</td>
<td>1025.00</td>
</tr>
<tr>
<td>SPH 1762</td>
<td>69.92</td>
<td>47.26</td>
<td>145.12</td>
<td>769.48</td>
<td>1025.00</td>
</tr>
</tbody>
</table>

*Moisture content of the soy protein isolate (SPI) and soy protein hydrolysate (SPH) (varied for the different samples, see Table 2) and corn syrup solids (CSS, 4.94 %) were taken into account.

(a) For the ingredient composition study, three emulsions were made. Firstly a model emulsion containing protein, CSS and fat blend, secondly a model emulsion without protein and thirdly a model emulsion without CSS.

(b) For the lecithin effect study, a model emulsion was made using fat blend containing 5 different lecithin concentrations (0 – 2 % (w/w)).
2.3.6 **MEASUREMENT OF THE PARTICLE SIZE DISTRIBUTION OF EMULSIONS**

The particle size distribution of each emulsion was determined by laser light scattering using a Malvern Mastersizer 2000 with a Hydro 2000S sample dispersion system, interfaced with Mastersizer 2000, version 5.1 software (Malvern Instruments Ltd., Malvern, UK).

The optical parameters selected were a particle refractive index of 1.449, a particle absorbance of 0.005 and a dispersant refractive index of 1.330. Emulsion droplets were sized using distilled water as the dispersant. Test samples were added to the dispersion unit, which was set at a speed of 1820 rpm, until a laser obscuration of approximately 20% had been achieved.

### 2.4 RESULTS AND DISCUSSION

**COMPOSITIONAL ANALYSIS**

Table 2: Properties of soy protein isolate and soy protein hydrolysate ingredients.

<table>
<thead>
<tr>
<th>Ingredient Code</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>DH (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>4.59 ± 0.11</td>
<td>85.71 ± 0.53</td>
<td>0</td>
<td>6.99</td>
</tr>
<tr>
<td>1651</td>
<td>3.74 ± 0.28</td>
<td>80.91 ± 0.32</td>
<td>0</td>
<td>6.78</td>
</tr>
<tr>
<td>1761</td>
<td>3.55 ± 0.23</td>
<td>80.39 ± 0.45</td>
<td>1.91 ± 0.12</td>
<td>6.89</td>
</tr>
<tr>
<td>1762</td>
<td>4.96 ± 0.04</td>
<td>76.16 ± 0.33</td>
<td>6.14 ± 0.25</td>
<td>6.63</td>
</tr>
</tbody>
</table>

*Mean values quoted are for independent triplicate determinations (with the exception of moisture which was using duplicate determinations).*
The results for the characterisation of the SPI and SPH ingredients are summarised in Table 2. SPH sample 1762 had the highest moisture content at 4.96 %, while SPH sample 1761 had the lowest moisture content at 3.55 %. SPI samples 1500 and 1651 and SPH sample 1761 all had a protein content of between 80 – 86 %, while SPH sample 1762 had a protein content of 76.16 %. As SPI samples 1500 and 1651 are unhydrolysed they both had a DH of 0 %. SPH sample 1761 had a DH of 1.91 %, while SPH sample 1762 had a DH of 6.14 %. SPI sample 1500 had the highest pH of 6.99 and SPH sample 1762 had the lowest pH of 6.63.

**PARTICLE SIZE DISTRIBUTION**

All of the particle size distribution profiles are means of independent duplicate analyses. Error bars are not included for clarity and presentation purposes, but there was little difference in the results. The particle size distribution profile for the emulsions generated without soy protein, following storage at 5°C over a period of 22 h are represented in Figure 1. There was no major change in the profiles over 6 h, they had an average particle size of ~ 0.7 µm. In all cases the 2nd homogenisation pass improved the profiles, by generally increasing the proportion of smaller particles. This corresponds with the findings of Dalgleish (1997) who stated that the introduction of a second homogenisation pass reduces particle size and therefore increases emulsion stability. After storage for 22 h, the profile developed a third population of particles between ~ 10 and 100 µm (Figure 1 (c)). This is indicative of destabilisation during storage. There appears to be no similar reports in the literature with respect to the removal of the protein source on particle size distribution of soy protein stabilised model infant formula emulsions.
The particle size distribution profiles for emulsions manufactured using SPI sample 1500 are shown in Figure 2. The average particle size for the profiles after the 1\textsuperscript{st} homogenisation pass was \( \sim 0.39 \) \( \mu m \), with all profiles displaying a shoulder at \( \sim 1.5 \) \( \mu m \). Again in all cases the 2\textsuperscript{nd} homogenisation pass resulted in the profile shifting to the left, to an area of smaller particles and also a loss of the shoulder at \( \sim 1.5 \) \( \mu m \). These emulsions appeared to be relatively stable on 22 h storage at 5\textdegree C.

Emulsions generated using SPI sample 1651 had particle size distribution profiles that can be seen in Figure 3. The average particle size for all populations after the 1\textsuperscript{st} homogenisation pass was \( \sim 0.4 \) \( \mu m \). The 2\textsuperscript{nd} homogenisation pass resulted in the profile shifting slightly to the left. All emulsions had a shoulder at \( \sim 1.02 \) \( \mu m \) after both 1\textsuperscript{st} and 2\textsuperscript{nd} homogenisation pass. There was no obvious change in particle size during storage at 5\textdegree C (Figure 3 (a), (b) and (c)).

The particle size distribution profiles for emulsions containing SPH sample 1761 are represented in Figure 4. After the 1\textsuperscript{st} homogenisation pass the emulsion had two major peaks, the first at \( \sim 0.3 \) \( \mu m \) and the second at \( \sim 1.0 \) \( \mu m \). The latter of these peaks was significantly reduced on the 2\textsuperscript{nd} homogenisation pass. These emulsions appeared to be relatively stable on 22 h storage at 5\textdegree C.

Emulsions manufactured with SPH sample 1762 resulted in distinctly bimodal particle size distribution profiles (Figure 5). The first of these populations had an average particle size of \( \sim 0.22 \) \( \mu m \) and the second an average of \( \sim 10.74 \) \( \mu m \). This may be due to the instability of emulsions manufactured with this sample, causing the development of larger oil droplets. In the case of this SPH sample, the 2\textsuperscript{nd} homogenisation pass had an adverse effect in that it shifted the larger of the populations to the right, to an area of larger particle size. After 10 min at 5\textdegree C, the 2\textsuperscript{nd} homogenisation pass caused the development of a small group of particles between \( \sim 56 \) \( \mu m \) and 112 \( \mu m \) (Figure 5 (b)), but this disappears after 22 h storage at 5\textdegree C (Figure 5 (c)). Although there were no
reports of this phenomenon in the literature, Roesch & Corredig, (2003) found that emulsions containing > 2 g of soy protein concentrate (SPC) per 100 g of emulsion resulted in a bimodal particle size distribution profile. One population of oil droplets had an average particle size of about 0.5 μm and the second population of particles were > 10 μm.

The removal of CSS from the emulsion generated with SPI sample 1500 had no obvious effect on the particle size distribution profile (Figure 6). There was a similarity with the particle size distribution profiles for emulsions manufactured with SPI sample 1500 and CSS (Figure 2), in that the 2nd homogenisation pass removed the shoulder on the right of the profile. The average particle size of profiles over all storage times was ~ 0.4 μm. The particle size distribution profiles for emulsions manufactured with SPI sample 1651 without CSS can be seen in Figure 7. Similar to the emulsions generated with CSS, the 2nd homogenisation pass increased the amount of smaller particles. The average particle size for all profiles was ~ 0.33 μm, this was similar to the average particle size of emulsions manufactured using SPI sample 1651 containing CSS (Figure 3). The particle size distribution profiles of emulsions containing SPH sample 1761 in the absence of CSS are shown in Figure 8. Again there was no real obvious effect, with the population of particles having a shoulder on the right after the 1st homogenisation pass and the 2nd homogenisation pass resulting in a shift to an area of smaller particles. This emulsion had less larger particles than the emulsion generated with SPH sample 1761 without CSS (Figure 4). There is a very obvious difference in the particle size distribution profiles of emulsions manufactured with SPH sample 1762 not containing CSS (Figure 9). Firstly after the 1st homogenisation pass and over 10 min storage time, a bimodal profile was evident with peaks at ~ 0.34 μm and 3.56 μm. Over time the proportion of particles in the larger peak began to decrease (Figure 9 (a), (b) and (c)). Over 22 h storage time at 5°C and after the 2nd homogenisation pass, the profiles changed resulting in a larger proportion of smaller particles (between ~ 0.09 μm and 1.18 μm) and a reduced proportion of larger particles (between ~ 1.36 μm and 16.26 μm), (Figure 9 (a)
and (c)). The result of this decrease in particle size means the likelihood of creaming occurring is reduced, as according to Stoke’s Law, the creaming rate decreases as the droplet size decreases (Iwabuchi et al., 1991). From these results it can be concluded that in order to generate stable model infant formula emulsions with SPH sample 1762, it is necessary to have CSS in the emulsion and also the 2\textsuperscript{nd} homogenisation step plays an important role in generating particles having smaller size distributions.

Mitidieri & Wagner, (2002) reported that, in general, denatured protein (denatured soy protein isolate (DSI) and denatured whey soy protein (DWSP)) produced slightly higher interfacial area than native proteins (native soy isolate (NSI) and native whey soy protein (NWSP)). It was a coincidence that emulsions manufactured with denatured proteins had droplets with lower median diameter than emulsions manufactured with native proteins. Although the SPI and SPH used in the present study may not have undergone thermal denaturation, it may be possible to make comparisons with the hydrolysed soy proteins. SPH sample 1762, having a DH of 6.14 %, gave a bimodal particle size distribution. However, SPH sample 1761, having a much lower DH of 1.91 %, had a monomodal distribution similar to that of the emulsions manufactured with the SPI samples.
Figure 1: Particle size distribution profiles for emulsions manufactured @ 24% TS without protein (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), 1st pass, 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 2: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass — 1st pass, —— 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 3: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), --- 1st pass, ----- 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 4: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), –– 1st pass, –– 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 5: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), —— 1st pass, ——— 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 6: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 @ 24% total solids, without corn syrup solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), first pass, second pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 7: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 @ 24% total solids, without corn syrup solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), --- 1st pass, ---- 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 8: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 @ 24% total solids, without corn syrup solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), 1st pass, 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
Figure 9: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 @ 24% total solids, without corn syrup solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), 1st pass, 2nd pass); (a) after 10 min; (b) after 6 h; (c) after 22 h. (Profiles are means of independent duplicate analyses).
The particle size distribution profiles for SPI and SPH emulsions manufactured with the fat blend containing varying amounts of lecithin (0 - 2.0 %) are represented in Figures 10 - 13. In Figure 10, it can be seen that lecithin had no major effect on the particle size distribution of emulsions manufactured with SPI sample 1500. After the 1st homogenisation pass the population of particles was bimodal, particularly for the 0 % lecithin sample (Figure 10 (a)). After the 1st homogenisation pass, there was the evidence of the formation of a second peak, between ~ 10 and 100 µm. The 2nd homogenisation pass resulted in a shift to the left, to smaller particle sizes (Figure 10 (b)). Dagleish (1997) stated that although coalescence may occur after homogenisation, the likelihood of this happening is reduced by a second homogenisation step.

The absence of lecithin has an obvious effect on the particle size distribution profiles of emulsions manufactured with SPI sample 1651 (Figure 11). In both profiles, after 1st (Figure 11 (a)) and 2nd (Figure 11 (b)) homogenisation passes, the particle size distribution profile for the 0 % lecithin samples was bimodal when compared to the profiles for emulsions containing lecithin.

Again there was a notable effect of the removal of lecithin from emulsions generated with SPH sample 1761 (Figure 12). After the 1st homogenisation pass (Figure 12 (a)) the sample containing 0 % lecithin had a bimodal particle size distribution profile with the fist peak at ~ 0.29 µm and the second peak at ~ 1.18 µm, while after the 2nd homogenisation pass (Figure 12 (b)) the profile had developed a third group of droplets between 3.09 µm and 56.39 µm. The reason for this occurrence is not known.

Distinct bimodal particle size distribution profiles for emulsions manufactured with SPH sample 1762 are represented in Figure 13. After the 2nd homogenisation pass (Figure 13 (b)), there was no real lecithin effect on the emulsions. However, after the 1st homogenisation pass (Figure 13 (a)) the profile for the emulsion without lecithin was quite different to those of the emulsions that contained lecithin. The height of the
smaller of the peaks increased (from \(~ 1.77 \%\) in emulsions without lecithin to \(~ 4 \%\) in those that did contain lecithin), almost doubling in size, indicating the formation of a larger amount of smaller particles. The larger of the peaks for the emulsion generated without lecithin decreased, with a corresponding reduction in the mean diameter of the larger particles (Figure 13 (a)). After the 2\textsuperscript{nd} homogenisation pass the absence of lecithin had no effect on the particle size distribution profile, with the profiles being similar to those of emulsions that contained lecithin. However, there was still a bimodal distribution with peaks at \(~ 0.22 \mu\text{m}\) and \(~ 8.15 \mu\text{m}\).

Emulsions containing lecithin would be expected to be more stable than those that do not. Lecithins are structurally like fats but contain phosphoric acid. Most importantly, they have an electrically charged end and a noncharged end. The electrically charged or polar end of this is water-loving or hydrophilic and easily dissolved in water. The uncharged or nonpolar end is fat-loving or hydrophobic and easily dissolved in fat or oil. The result in an oil-in-water mixture is that the emulsifier dissolves part of itself in water and part in oil (Potter & Hotchkiss, 1995).

Although there are no direct comparisons in the literature for soy protein, Agboola \textit{et al.}, (1998 (b)) studied oil-in-water emulsions containing 4 \%\% whey protein hydrolysate (WPH), prepared in a two-stage homogeniser. They looked at the effect of adding two levels (0.1 and 0.25 \%\%) of either unmodified commercial soy lecithin or hydroxylated lecithin and also the effect of retorting at 121 °C for 16 min. The retorting resulted in immediate destabilisation of the lecithin-free emulsions. Addition of unmodified lecithin slightly improved the stability of the retorted emulsions, but did not prevent creaming and coalescence. However, addition of hydroxylated lecithin markedly improved the creaming stability after retorting and prevented coalescence.

Soy lecithin has a strong affinity for soy proteins and has been utilised to produce a soy lecithin-soy isolate (SI) complex to improve its emulsifying properties. The EC of the
lecithin-SI complex increased as the ratio of soy lecithin to soy isolate increased (Hirotsuka et al., 1984). In the present study, lecithin improves the particle size distribution of most of the emulsions manufactured with the SPI and SPH samples, by increasing the proportion of smaller particles. However, for the emulsion manufactured with SPH sample 1762, the particle size distribution profiles showed that without lecithin, there was a large amount of small particles and a small amount of large particles. When lecithin was added to this emulsion the opposite was true. This may in some way be due to the fact that this particular soy protein ingredient was very insoluble and hard to keep in solution.

From the findings in the present study, it can be seen that the presence of CSS in model infant formulae emulsions manufactured with SPI samples 1500 and 1651 and SPH sample 1761, showed no real effect on the particle size distribution profiles of the emulsions over 22 h storage at 5°C. However, emulsions generated with SPH sample 1762 without CSS appeared to be more stable, with a larger proportion of small particles.
Figure 10: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 @ 24% total solids with ---0%, ---0.4%, ---0.8%, ---1.0%, ---1.5% and ---2.0% lecithin, (a) 1st pass and (b) 2nd pass homogenisation.
Figure 11: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 @ 24% total solids with ---0%, ---0.4%, ---0.8%, ---1.0%, ---1.5% and ---2.0% lecithin, (a) 1st pass and (b) 2nd pass homogenisation.
Figure 12: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 @ 24% total solids with ---0%, ---0.4%, ---0.8%, ---1.0%, ---1.5% and ---2.0% lecithin, (a) 1st pass and (b) 2nd pass homogenisation.
Figure 13: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 @ 24% total solids with ---0%, 0.4%, 0.8%, 1.0%, 1.5% and 2.0% lecithin, (a) 1st pass and (b) 2nd pass homogenisation.
CHAPTER 3

CONTRIBUTION OF TOTAL SOLIDS (TS), HOMOGENISATION PRESSURE AND pH TO THE PARTICLE SIZE DISTRIBUTION AND APPARENT VISCOSITY OF SOY PROTEIN EMULSIONS
3.1 **ABSTRACT**

The main objective of this work was to quantify the effects of adjusting the total solids (TS), pH and homogenisation pressure on some emulsifying properties of two soy protein isolate (SPI) and two soy protein hydrolysate (SPH) ingredients in model infant formula emulsion systems. Model emulsions were manufactured using SPI/SPH, corn syrup solids (CSS) and a commercial fat blend. These ingredients were passed through a laboratory pasteuriser (77°C for 30 sec), followed by homogenisation (double pass), at a first stage pressure of 170 bar and a second stage pressure of 30 bar. For the total solids (TS) effect study, ingredients were blended to different TS levels (12.5 – 26 % (w/w)) prior to emulsification. For the homogenisation pressure effect study, homogenisation (single pass) pressures were 100, 210 and 310 bar (first stage) and 10, 30 and 50 bar (second stage). For the pH effect study, the pH of the SPI and SPH emulsions were adjusted to pH’s in the range of 6.4 to 7.5, before homogenisation. No TS effects were observed on particle size distributions of model emulsions generated with the SPI samples 1500 and 1651 and SPH 1761. However, emulsions made with SPH sample 1762 at low TS were more stable than those made at high TS levels. As expected, increasing homogenisation pressure resulted in emulsions generated with SPI samples 1500 and 1651 having lower volume average particle diameters ($d_{43}$) values. Surprisingly, increasing homogenisation pressure resulted in an increase in apparent viscosity ($\eta_{app}$) of emulsions manufactured with SPH sample 1762. For emulsions generated with SPH sample 1762, an increase in pH resulted in a general decrease in $d_{43}$, which corresponded to an increase in $\eta_{app}$ value.
3.2 INTRODUCTION

Soy protein-based foods are now one of the fastest-growing categories in the food industry, resulting in a demand for manufacturing soy protein ingredients with a range of functional properties (Tsumura et al., 2005). One important application of soy proteins is in infant formulations. Infant formulae can be milk, soy protein or protein hydrolysate based. In all of these formulae the protein content is adjusted to give the same essential amino acid content to that of human milk (Algería et al., 1999). Infant formulae have been designed to provide infants with the required nutrients for optimal growth and development (Alles et al., 2004).

Some of the most important properties of emulsion-based foods are determined by the sizes of the oil droplets they contain. Therefore it is important to control and measure the size of the droplets in emulsions. Droplet size distributions are largely determined by the quantity and the quality of the emulsifier, the energy input of the homogeniser used, the temperature used during homogenisation and the viscosity of the continuous phase (Egelandsdal et al., 2001). Under some circumstances, droplets can combine to form larger droplets (Robins et al., 2002). Coalescence is likely to occur during or immediately after homogenisation, this coalescence may however be reduced by introducing a second homogenisation step (Dalgleish, 1997). High-pressure technology has been applied in many areas of food science research, and it has been shown to affect not only fat globule size, but also macromolecules or colloids, and it may also modify the structure and functional properties of biopolymers. To date, very little information is available on the effect of dynamic high pressure on the emulsifying properties of proteins, especially on commercial soy protein ingredients (Roesch & Corredig, 2003).

Particles in an emulsion tend to aggregate because of attractive inter-atomic forces, but aggregation is opposed by repulsive charge interactions (or enhanced by attractive
charge interactions), which arise from material absorbed at the interface. The net charge of a protein is highly dependent on pH; therefore if the pH of an emulsion is close to that of the isoelectric point of the proteins, i.e., where their net charge approaches zero, this situation may favour aggregation of emulsion droplets (Dalgleish, 1997).

In the industrial manufacture of ready to feed soy protein formulae, the ingredient mixture is usually compounded and subsequently processed at high TS. However, there appears to be no information available on the contribution, if any, of the TS level at homogenisation on the resulting emulsion. The pH range to be used in the manufacture of infant formulae is dictated by the EU Directive (1991).

The objective of the present study was to investigate the contribution of variation in totals solids (TS), homogenisation pressure and pH to the particle size distribution and apparent viscosity of two SPI and two SPH model emulsions.
3.3 MATERIALS AND METHODS

3.3.1 MATERIALS

Materials used were as listed in Chapter 2.

3.3.2 PREPARATION OF MODEL EMULSIONS

Model emulsions with the SPI and SPH samples were blended as described in Chapter 2, with the following modifications:

(a) For the TS effect study, ingredients were blended to different TS levels (12.5 – 26 % (w/w)) prior to heat treatment @ 77°C for 30 sec followed by a double pass through a two stage homogeniser (first stage 170 bar; second stage 30 bar).

(b) For the homogenisation pressure effect study, ingredients were blended at 24 % TS, heat treated @ 77°C for 30 sec and the homogenisation (single pass) pressures used were 100, 210 and 310 bar (first stage) and 10, 30 and 50 bar (second stage).

(c) For the pH effect study, ingredients were blended at 24 % TS and the pH was adjusted to 6.4-7.5. The samples were then heat treated @ 77°C for 30 sec and homogenised. The homogenisation (double pass) pressures used were (first stage 170 bar; second stage 30 bar).
3.3.3 MEASUREMENT OF THE PARTICLE SIZE DISTRIBUTION OF EMULSIONS

The particle size distribution of each emulsion was determined, as described in Chapter 2, by laser light scattering using a Malvern Mastersizer 2000 with a Hydro 2000S sample dispersion system, interfaced with Mastersizer 2000, version 5.1 software (Malvern Instruments Ltd., Malvern, UK).

3.3.4 DETERMINATION OF APPARENT VISCOSITY

A Brookfield Programmable DV-II+ Viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) was used to measure fluid viscosity at different shear rates. The ultra low (UL) adapter was connected to a Brookfield refrigerated circulating bath model TC-500 by a ULA-40Y water jacket. The control keys on the DV-II+ viscometer were used to set the required speed and to select spindle. Before any readings were taken, the viscometer was auto zeroed as per manufacturers instructions.

Calibration of the UL Adapter:

When the UL adapter was used to examine the apparent viscosity of a given test sample, the water bath was stabilised at the required temperature. A 16 ml aliquot of viscosity standard fluid (4.6 mPa.s) (Brookfield Engineering Laboratories, Middleboro, MA, USA) was dispersed into the UL tube. The spindle was then attached to the DV-II+. The sample tube was attached to the mounting channel. The viscosity was then
measured and recorded when the viscosity standard, sample chamber and spindle reached test temperature (25°C).

**Measurement of apparent viscosity:**

Aliquots (16 ml) of the SPI and SPH emulsions were placed in the UL adapter (LVDV-II+), attached to the Brookfield Programmable DV-II+ viscometer. The test sample, sample chamber and spindle (LVDV-II+) were then equilibrated to 25°C. Finally, the apparent viscosity was measured and recorded. The $\eta_{\text{app}}$ values were obtained at a shear rate of 40 rpm. The temperature was controlled at 25°C with a Brookfield TC-500 circulating water bath connected to the water jacket of the UL adapter. The $\eta_{\text{app}}$ values reported were averages of five determinations.
3.4 RESULTS AND DISCUSSION

PARTICLE SIZE DISTRIBUTION

All of the particle size distribution profiles are means of independent duplicate analyses. Error bars are not included for clarity and presentation purposes, but there was little difference in the results. Figures 1 – 4 show the particle size distribution profiles for model emulsions manufactured with the SPI and SPH samples at different TS levels. Emulsions containing SPI sample 1500 had a monomodal distribution (Figure 1). After the 1st homogenisation pass, emulsions manufactured containing SPI sample 1500 (17 % TS) had particles ranging in size from 0.4 µm to 7.1 µm with greatest the proportion of particles being ~ 0.3 µm. After the 2nd homogenisation pass the particles ranged from 0.4 µm to 1.6 µm with a peak at around 0.2 µm (Figure 1 (d)). At all TS concentrations the 2nd homogenisation pass resulted in a decrease in the average particle size. The particle size distribution profiles for emulsions generated with SPI sample 1651 at different TS are represented in Figure 2. Profiles at all TS levels were monomodal with a peak at ~ 0.34 µm. As with SPI sample 1500 the 2nd homogenisation pass shifted the profile slightly to the left, corresponding with a reduction in the average size of the droplets. Emulsions manufactured with SPH sample 1761 resulted in particle size distribution profiles which can be seen in Figure 3. At 26 % TS (Figure 3 (a)), the 2nd homogenisation pass greatly improved the profile by decreasing the amount of particles between ~ 0.77 µm and 10 µm. The profiles at the remaining TS levels also had monomodal populations of particles with mean particle sizes of ~ 0.3 µm. The shoulder on the profiles after 1st pass homogenisation was removed after the 2nd homogenisation step. SPH sample 1762 had a distinctly bimodal distribution at 26 and 23 % TS (Figure 4 (a) and (b)). The smaller peak with droplets between 0.1 µm and 0.7 µm in size and the larger peak had particles between 2.4 µm and 32.4 µm. Surprisingly, the 2nd homogenisation pass seemed to have an adverse effect on emulsions manufactured with
SPH sample 1762 as it increased the mean size of the largest particles from 32.4 μm to 74.3 μm. This is unusual as it would be expected the 2nd homogenisation pass would further break up the droplets, as is seen in the case of emulsions manufactured with the other soy protein samples. The formation of these large droplets may be related to the inability of the short peptides in the SPH to adequately stabilise the greater surface area produced by homogenising at high pressures.

Several studies have reported the effects of hydrolysis on the emulsifying properties of soy protein, because these properties greatly affect the use of soy protein ingredients in many food applications (Jung et al., 2005). In the majority of the studies reporting on the emulsifying properties of soy protein modified by protease, emulsification properties were improved (Qi et al., 1997; Wu et al., 1998). However, some hydrolysis conditions led to small or no changes in EC (Don et al., 1991). Jung et al., (2005) found that emulsification capacities of SPI and SPC's were improved by hydrolysing to 2% DH. Emulsions manufactured with SPI sample 1761 which had a DH of 1.91 % had particle size distribution profiles indicating that these emulsions may be inherently more stable that emulsions manufactured with SPH sample 1762 which had a DH of 6.14 %. Utsumi et al., (1997) reported that soy proteins had higher EA when compared to the dairy proteins as a result of the high emulsifying ability of β-conglycinin.

Although no direct comparisons with this study can be made with the literature, McCrae et al., (1999) studied the emulsification properties of whey protein in reconstituted skim milk, containing anhydrous milk fat at 4 and 18 % by modifying the protein concentration. At a similar protein-to-fat ratio, the fat content was not associated with changes in initial particle size, but was related to stability during storage. In the more concentrated solutions, creaming was inhibited and no longer affected time-dependent aggregation of fat globules, except under the most extreme conditions (i.e., at high initial particle size and at a high storage temperature).
Although variation in TS levels did not have a major effect for emulsions manufactured with SPI samples 1500 and 1651, and SPH sample 1761, it can be seen that generating an emulsion with SPH sample 1762 at low TS levels (Figure 4 (f)) results in a more stable emulsion with a lower mean particle size. This may have consequences for infant formulae manufacturers in how they process different soy protein ingredients.
Figure 1: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 (two stage homogenisation (1\textsuperscript{st} stage 170 bar, 2\textsuperscript{nd} stage 30 bar), double pass —— 1\textsuperscript{st} pass, —— 2\textsuperscript{nd} pass) at different total solids levels: (a) 26.0\%, (b) 23.0\%, (c) 20.0\%, (d) 17.0\%, (e) 14.0\%, (f) 12.5\%. (Profiles are means of independent duplicate analyses).
Figure 2: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass —— 1st pass, —— 2nd pass) at different total solids levels: (a) 26.0%, (b) 23.0%, (c) 20.0%, (d) 17.0%, (e) 14.0%, (f) 12.5%. (Profiles are means of independent duplicate analyses).
Figure 3: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass --- 1st pass, ---- 2nd pass) at different total solids levels: (a) 26.0%, (b) 23.0%, (c) 20.0%, (d) 17.0%, (e) 14.0%, (f) 12.5%. (Profiles are means of independent duplicate analyses).
Figure 4: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass — 1st pass, — 2nd pass) at different total solids levels: (a) 26.0%, (b) 23.0%, (c) 20.0%, (d) 17.0%, (e) 14.0%, (f) 12.5%. (Profiles are means of independent duplicate analyses).
Figures 5 – 8 show the particle size distribution profiles for model emulsions manufactured with SPI and SPH samples at different homogenisation pressures. As expected, the average particle size for the SPI samples decreased with increasing homogenisation pressures, e.g. SPI sample 1500 had an average particle size of ~ 0.6 μm at an homogenisation pressure of 100/10 bar (1st stage/2nd stage), which decreased to ~ 0.2 μm on homogenisation at 310/50 bar (Figure 5). The particle size distribution profiles for emulsions generated with SPI sample 1651 using different homogenisation pressures can be seen in Figure 6. At the lowest homogenisation pressure used (100/10 bar) the average particle size was ~ 0.39 μm, while at the highest homogenisation pressure used (310/50 bar) the average particle size was ~ 0.22 μm. SPH sample 1761 became distinctly bimodal at higher homogenisation pressures with the larger peak being between 0.1 μm and 1 μm (Figure 7). SPH sample 1762 had a bimodal distribution at all pressures (Figure 8), but at higher pressures there was an increase in particles of smaller size. After homogenisation at 100/10 bar the smaller particles ranged from 0.1 μm to 0.8 μm while after the homogenisation pressure of 310/50 bar they were between 0.02 μm and 0.8 μm in size. However, there was also an increase in larger particles with an increase in homogenisation pressure. The average particle size of the larger population of droplets was ~ 7.1 μm after homogenisation at 100/10 bar. After homogenisation at 310/50 bar the average particle size of the group of droplets increased to ~ 21.4 μm. There are sufficient surface active peptides to cover the smaller droplets. However, at higher homogenisation pressures the surface area is larger and there are not enough peptides to go around, resulting in the formation of larger droplets.

There have been similar reports made for emulsions formed using highly hydrolysed whey proteins (Agboola et al., 1998 (a)), where higher proportions of small particles and hence lower average sizes were formed with increasing homogenisation pressure. However, formation of large emulsion droplets (> 10 μm) can also be seen for SPH sample 1762 (Figure 8). This may be related to the inability of the short peptides in the
SPH sample to adequately stabilize the greater surface area produced by homogenising at higher pressures (Agboola et al., 1998 (a)).

Molina et al., (2001) studied the effect of static high pressure (200-600 MPa) on the emulsifying properties of soy protein isolates and demonstrated that, while high pressure dissociated 7S subunits, the dissociation of 11S led to aggregation by changing its surface hydrophobicity and as a consequence, its solubility. The higher dynamic pressures used in the present study may have had a similar effect.

Roesch & Corredig, (2003) found that high pressure homogenisation (80 MPa) caused the disruption of soy protein concentrates (SPC’s) and protein aggregation. They also found that after homogenisation and/or heat treatment, the suspensions were still characterized by a greater distribution of large particles, but the mean particle size was smaller than for untreated SPC. Homogenisation appeared to have had the same effect on the SPI’s used in the present study.

Modifications of emulsifying properties of SPI’s by static high-pressure processing were studied by Puppo et al., (2005). SPI solutions (10 g/l) in two pH conditions: alkaline (pH 8) and acidic (pH 3) were treated by high-pressure at various pressure levels (200, 400 and 600 MPa for 10 min at 10°C). Oil-in-water emulsions (30/70) were subsequently prepared with untreated and high-pressure treated SPI’s. The results indicated that high-pressure treatment improved the EA of SPI at pH 8. Emulsions prepared with high-pressure treated SPI at pH 8 showed a smaller average droplet size and an increase of the percentage of proteins absorbed at the interface. In the present study dynamic pressure was used. Static and dynamic pressures may both lead to protein denaturation leading eventually to better surface coverage and more stable emulsions. For SPI at pH 3, acidification contributed to a modification of emulsifying properties: i.e., to an increase in droplet size and in depleted flocculation. In general, the high-pressure treatment resulted in an increase of adsorbed proteins and a decrease of
flocculation. Again this reinforces the fact that high homogenisation pressure has an improving and stabilising effect on soy protein emulsions.
Figure 5: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 @ 24% total solids and different first and second stage homogenisation pressures, single pass. (a) 100/10, (b) 100/30, (c) 100/50, (d) 210/10, (e) 210/30, (f) 210/50, (g) 310/10, (h) 310/30, (i) 310/50; (1st stage/2nd stage (bar)). (Profiles are means of independent duplicate analyses).
Figure 6: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 @ 24% total solids and different first and second stage homogenisation pressures, single pass. (a) 100/10, (b) 100/30, (c) 100/50, (d) 210/10, (e) 210/30, (f) 210/50, (g) 310/10, (h) 310/30, (i) 310/50; (1st stage/2nd stage (bar)). (Profiles are means of independent duplicate analyses).
Figure 7: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 @ 24% total solids and different first and second stage homogenisation pressures, single pass. (a) 100/10, (b) 100/30, (c) 100/50, (d) 210/10, (e) 210/30, (f) 210/50, (g) 310/10, (h) 310/30, (i) 310/50; (1st stage/2nd stage (bar)). (Profiles are means of independent duplicate analyses).
Figure 8: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 @ 24% total solids and different first and second stage homogenisation pressures, single pass. (a) 100/10, (b) 100/30, (c) 100/50, (d) 210/10, (e) 210/30, (f) 210/50, (g) 310/10, (h) 310/30, (i) 310/50; (1st stage/2nd stage (bar)). (Profiles are means of independent duplicate analyses).
The particle size distribution profiles for model emulsions manufactured with the SPI and SPH samples at different pH values are summarised in Figures 9 – 12. Altering the pH of model infant formula emulsions generated with SPI sample 1500 had no real effect on the particle size distribution profiles (Figure 9). The average particle size was ~ 0.4 μm. At pH 6.4, SPI sample 1651 (Figure 10) had a group of droplets with an average size of ~ 0.3 μm. At pH 7.5, the same emulsion had particles with an average size of ~ 0.28 μm. Therefore, there was no major change in the particle size distributions with a change in pH. The particle size distribution profiles for emulsions manufactured with SPH 1761 at different pH’s are represented in Figure 11. After the 1st homogenisation pass the average particle size was ~ 0.33 μm, while after the 2nd homogenisation pass this was reduced to 0.29 μm. In general, the 2nd homogenisation pass increased the proportion of smaller particles, improving all profiles. SPH sample 1762 was distinctly bimodal up to pH 7.0 (Figure 12 (a), (b) and (c)), with a maxima at ~ 0.25 μm and at ~ 6.2 μm. At pH 7.3 (Figure 12 (d)) the profile was monomodal, with an average particle size of ~ 0.3 μm. At pH 7.5 (Figure 12 (e)), and after the 1st homogenisation step the particle size distribution profile for emulsions manufactured with SPH sample 1762 were almost monomodal with an average droplet size of ~ 0.29 μm. While after the 2nd homogenisation pass the profile had a population peaking at ~ 0.26 μm and a smaller population of particles at ~ 3 μm.

There appears to be no similar work on the effect of pH on the particle size distribution of different soy protein stabilised emulsions. Hunt & Dalgleish, (1994) conducted similar experiments on emulsions made with whey protein isolate (WPI). Emulsions made with various concentrations (0.5 – 2.5 wt %) of WPI were most stable at pH 7.0 and least stable at pH 5.5. They found that at pH 7.0 the particle size distribution of the emulsion was monomodal, but that at pH 5.5 the distribution appeared to be bimodal. This pH is close to the pI of β-lactoglobulin (pI = 5.2) and α-lactalbumin (pI = 4.1 – 4.8) and therefore, solubility is likely to be reduced at this pH. They also concluded that the behaviour of the whey proteins depended on the variations of the tertiary and quaternary
structures with pH. These facts may also be true for the SPI and SPH samples used in this study. Alteration on pH had the greatest effect on emulsions generated using SPH sample 1762.

Although pH has no great effect on SPI samples 1500 and 1651, and SPH sample 1761 and in their use in model infant formula emulsions, it is an important factor to consider manufacturing an emulsion using SPH sample 1762. From the particle size distribution profiles, it can be seen that the optimum pH for manufacturing model infant formula emulsions using this SPH would appear to be pH 7.3 (Figure 12 (d)). The reason for looking at pH's in the range of 6.4 and 7.5, is to keep within the guidelines stated in the EU Directive with regard to manufacture of infant formulae.
Figure 9: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1500 (two stage homogenisation (1\textsuperscript{st} stage 170 bar, 2\textsuperscript{nd} stage 30 bar), double pass —— 1\textsuperscript{st} pass, —— 2\textsuperscript{nd} pass) at pH values: (a) 6.4, (b) 6.7, (c) 7.0, (d) 7.3, (e) 7.5. (Profiles are means of independent duplicate analyses).
Figure 10: Particle size distribution profiles for model emulsions manufactured with soy protein isolate (SPI) sample 1651 (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass —— 1st pass, —— 2nd pass) at pH values: (a) 6.4, (b) 6.7, (c) 7.0, (d) 7.3, (e) 7.5. (Profiles are means of independent duplicate analyses).
Figure 11: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1761 (two stage homogenisation (1\textsuperscript{st} stage 170 bar, 2\textsuperscript{nd} stage 30 bar), double pass \textemdash\textemdash 1\textsuperscript{st} pass, \textemdash\textemdash 2\textsuperscript{nd} pass) at pH values: (a) 6.4, (b) 6.7, (c) 7.0, (d) 7.3, (e) 7.5. (Profiles are means of independent duplicate analyses).
Figure 12: Particle size distribution profiles for model emulsions manufactured with soy protein hydrolysate (SPH) sample 1762 (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass — 1st pass, — 2nd pass) at pH values: (a) 6.4, (b) 6.7, (c) 7.0, (d) 7.3, (e) 7.5. (Profiles are means of independent duplicate analyses).
Apparent Viscosity (\(\eta_{\text{app}}\))

Apparent viscosity (\(\eta_{\text{app}}\)) profiles for model emulsions manufactured with the SPI and SPH samples at different homogenisation pressures are shown in Figures 13 and 14. For the SPI samples no major change in \(\eta_{\text{app}}\) at a shear rate of 40 rpm was evident with increasing homogenisation pressures. SPI sample 1500 had an average \(\eta_{\text{app}}\) of approximately 8 mPa.s, (Figure 13 (a)), while SPI sample 1651 had an average of approximately 6 mPa.s, (Figure 13 (b)). It was not possible to measure the \(\eta_{\text{app}}\) of SPH sample 1761 at 40 rpm (Figure 14 (a)), except at the lowest homogenisation pressure of 1\(^{st}\) stage 100/2\(^{nd}\) stage 10 bar. This was because it was not possible to achieve the necessary torque of between 10-100 \% at 40 rpm. SPH sample 1762 shows a slight increase in \(\eta_{\text{app}}\) with increasing homogenisation pressure with some differences after 1\(^{st}\) and 2\(^{nd}\) stage pressures (Figure 14 (b)). After homogenisation at 100/10 bar, the emulsion containing SPH sample 1762 had a \(\eta_{\text{app}}\) value of 6.36 mPa.s. Following homogenisation at 310/50 bar this emulsion had a \(\eta_{\text{app}}\) value of 13.84 mPa.s.

Mitidieri & Wagner, (2002) reported that the rheological behaviour of emulsions manufactured with native and denatured soy proteins was similar. They were all Newtonian emulsions, therefore collision frequency between droplets during homogenisation and stirring should be practically equivalent for all emulsions, assuming that differences in droplet size distributions were not that important. However, the emulsion samples used in this experiment were non-Newtonian fluids, since the viscosity of these fluids change as the shear rate is varied. Therefore it is not possible to make direct comparisons with the work of Mitidieri & Wagner, (2002)

Although there are no direct comparisons with this study in the literature, Xie & Hettiarachchy, (1997) reported that an increase in viscosity of SPI based emulsions due to the addition of xanthan gum contributed greatly to increased emulsifying activity and
emulsion stability of the dispersions. It is expected that high-pressure homogenisation causes the formation of small fat globules and therefore decreases the viscosity. There is seen to be little or no change in the $\eta_{app}$ of emulsions manufactured with SPI samples 1500 and 1651 (Figure 13 (a) and (b)), and SPH sample 1761 (Figure 14 (a)) at different homogenisation pressures. However there was an increase in $\eta_{app}$ of emulsions manufactured with SPH sample 1762 (Figure 14 (b)), with an increase in homogenisation pressure. This may be related to the poor solubility of this particular SPH.
Figure 13: Apparent viscosity (40 rpm) profiles for model emulsions manufactured with soy protein isolate (SPI) samples (a) 1500 and (b) 1651 @ 24% total solids and different first stage (100, 210 & 310 bar) and second stage (10, 30 & 50 bar) homogenisation pressures, single pass. (Profiles are means of independent duplicate analyses).
Figure 14: Apparent viscosity (40 rpm) profiles for model emulsions manufactured with soy protein hydrolysate (SPH) samples (a) 1761 and (b) 1762 at 24% total solids and different first stage (100, 210 & 310 bar) and second stage (10, 30 & 50 bar) homogenisation pressures, single pass. (Profiles are means of independent duplicate analyses).
The $\eta_{\text{app}}$ profiles for model emulsions manufactured with SPI and SPH samples at different pH values are shown in Figures 15 and 16. Emulsions generated with SPI sample 1500 showed a general trend of an increase in $\eta_{\text{app}}$ with an increase in pH (from 8.51 mPa.s at pH 6.4 to 9.95 mPa.s at pH 7.5), (Figure 15 (a)), with a slight decrease on the 2nd homogenisation pass (from 8.04 mPa.s at pH 6.4 to 9.85 mPa.s at pH 7.5). The $\eta_{\text{app}}$ of emulsions manufactured with SPI sample 1651 at pH 6.4 was ~ 7.40 mPa.s. This decreased with an increase in pH with a minimum $\eta_{\text{app}}$ value of ~ 6.06 mPa.s at pH 7.0 (Figure 15 (b)). Emulsions manufactured with SPH sample 1761 over all pH ranges, had an average $\eta_{\text{app}}$ of ~ 3.4 – 3.8 mPa.s (Figure 16 (a)). SPH sample 1762 had a trend of a decrease in $\eta_{\text{app}}$ with an increase in pH (from 10.42 mPa.s at pH 6.4 to 4.99 mPa.s at pH 7.5) (Figure 16 (b)). After the 2nd homogenisation pass the $\eta_{\text{app}}$ also decreased (from 12.72 mPa.s at pH 6.4 to 6.56 mPa.s at pH 7.5) with a minimum $\eta_{\text{app}}$ at pH 7.3 (4.32 mPa.s after 1st pass homogenisation and 5.19 mPa.s after 2nd pass homogenisation).

Although there are no direct comparisons in the literature, Waniska & Kinsella, (1988) demonstrated that maximum surface viscosity of $\beta$-lactoglobulin solutions occurred near the isoelectric point. At this pH, electrostatic repulsion is minimised allowing hydrophobic residues to stabilise a more compact tertiary structure.

One of the most important limitations of soy protein as a food ingredient is its poor solubility at pH ~ pI. The pI of the SPI and SPH samples used in the present study is not known but it may obviously have some effect on the resultant emulsions. Malhotra & Coupland, (2004) studied the interactions of SPI with an anionic and a non-ionic surfactant as a function of pH. The viscosity of the non-ionic and surfactant-free samples were low (~ 3 mPa.s) and largely pH independent, while the anionic surfactant containing samples were much more viscous, particularly at high pH (greater that the isoelectric point).
Hutton & Campbell, (1977) studied the viscosity properties of a soy concentrate and a soy isolate. SPC dispersions increased in viscosity as pH increased from pH 5.0 to 7.0. This increase may be related to changes in the solubility of the concentrate under these conditions. This is similar to the results obtained for emulsions manufactured with SPI sample 1500, where an increase in pH corresponded to an increase in $\eta_{app}$ (Figure 15 (a)). Hutton & Campbell, (1977) however also found that as the pH of SPI dispersions increased, the dispersions decreased in $\eta_{app}$. The effect of pH occurred primarily between pH 6.0 and 7.0. This is similar to the results for emulsions manufactured with SPI sample 1651, which showed a general decrease in $\eta_{app}$ with an increase in pH (Figure 15 (b)).

The $\eta_{app}$ of the SPI and SPH samples is important for their use in infant formulae. The $\eta_{app}$ needs to be as low as possible after the evaporation stage and prior to spray-drying in order to get efficient spray-drying taking place. From the results of the present study it can be seen that emulsions manufactured with SPH sample 1761 (Figure 16 (a)), over the pH range studied, had the lowest $\eta_{app}$. While emulsions generated using SPH sample 1762 showed the lowest $\eta_{app}$ to occur at pH 7.3 (Figure 16 (b)). From the particle size distribution profiles, it can be seen that the optimum pH for manufacturing model infant formula emulsions using this SPH would also appear to be pH 7.3 (Figure 12 (d)).
Figure 15: Apparent viscosity (40 rpm) profiles for model emulsions manufactured with soy protein isolate (SPI) samples (a) 1500 and (b) 1651 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass 1st pass, 2nd pass) and at different pH values (6.4 - 7.0). (Profiles are means of independent duplicate analyses).
Figure 16: Apparent viscosity (40 rpm) profiles for model emulsions manufactured with soy protein hydrolysate (SPH) samples (a) 1761 and (b) 1762 @ 24% total solids (two stage homogenisation (1st stage 170 bar, 2nd stage 30 bar), double pass ▼ 1st pass, ▲ 2nd pass) and at different pH values (6.4 – 7.0). (Profiles are means of independent duplicate analyses).
SUMMARY

No adverse effects on particles were determined, since problem within GSP was resolved.

Porcine embryo medium with 20% sample C602, SPS samples L921 and SPS sample L761.

No weight loss was observed in these samples. Allowing SPS samples C602 and L761 to be assessed at a 6-month period.

Although these were no significant differences in particle size distribution, product formulation was improved with a selected combination of a suitable solvent and an effective formulation.

The selection of solvent in emulsion systems with SPS sample L761 resulted in a significant decrease in the particle size, particularly after the 6-month storage period.

No adverse effects were observed on particles. The distribution of a selected medium was generated with the SPS samples L921 and L761 and SPS sample L761. However, emulsion samples C602 and SPS sample L761 at 6-month storage period showed no significant change in particle size.

Increasing homogeneity was observed in SPS samples with lower than the determined values. Increasing homogeneity was observed in the 6-month period of storage, particularly with SPS sample C602.
The main outcomes of this study include:

- No major effects on particle size distribution were evident when CSS was removed from emulsions made with SPI sample 1500, SPI sample 1651 and SPH sample 1761. Surprisingly, the absence of CSS in the emulsion manufactured with SPH sample 1762 resulted in an increase in smaller particles, after the 2\textsuperscript{nd} homogenisation pass and on storage at 5°C for 22 h.

- Although there were no real differences in particle size distribution profiles for emulsions manufactured with fat containing varying amounts of lecithin, there was an obvious effect when lecithin was removed, particularly from those made with SPI sample 1651 and SPH sample 1761. Exclusion of lecithin for these ingredients resulted in an increase of the proportion of larger particles. Therefore, the addition of lecithin is important in the manufacture of infant formulae emulsions when using these soy protein ingredients.

- The addition of lecithin to emulsions generated with SPH sample 1762 resulted in a decrease in the average particle size, particularly after the 1\textsuperscript{st} homogenisation pass.

- No TS effects were observed on particle size distributions of model emulsions generated with the SPI samples 1500 and 1651 and SPH 1761. However, emulsions made with SPH sample 1762 at low TS (12.5 – 14 %) contained more particles having a lower size distribution than those made at high TS levels (23 – 26 %).

- Increasing homogenisation pressure resulted in SPI emulsions with lower mean particle diameter ($d_{43}$) values. Increasing homogenisation pressure resulted in an increase in apparent viscosity ($\eta_{app}$) of emulsions manufactured with SPH sample
1762, therefore emulsions manufactured using this SPH might be expected to be more stable when homogenised at low pressures.

- For emulsions generated with SPH sample 1762, an increase in pH resulted in a general decrease in $d_{43}$, which corresponded to an increase in $\eta_{app}$ value.

**Future Work:**

- Perform stability studies on the emulsions over long storage periods e.g., up to 6 months, to determine the susceptibility of the emulsions manufactured with the different ingredients under different processing conditions to creaming, flocculation and coalescence.

- To determine at a molecular level the events taking place at the oil/water interface during the inclusion of different soy protein ingredients and during the application of different processing conditions.
REFERENCES


