Characterisation and Processing Attributes of Isolated Wheat Protein Ingredients in Dairy-type Emulsions

A thesis submitted for the degree of Master of Applied Sciences (Food Technology)

by

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Statement of Authenticity

I certify that this thesis entitled “Characterisation and Processing Attributes of Isolated Wheat Proteins in Dairy-type Emulsions” submitted for the degree “Master of Applied Science” is the result of my own research, except otherwise acknowledged and has not been submitted for a higher degree to any other university or institution. Copies of original data are held by the Department of Food Science, RMIT and Food Science Australia (CSIRO), Melbourne.

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Signed:

Date:
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMF</td>
<td>Anhydrous Milk Fat</td>
</tr>
<tr>
<td>AUD</td>
<td>Australian dollar</td>
</tr>
<tr>
<td>CC</td>
<td>Coffee creamer</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton (measure of molecular weight)</td>
</tr>
<tr>
<td>DEW</td>
<td>Dried egg white</td>
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<tr>
<td>IC</td>
<td>Imitation Creamer</td>
</tr>
<tr>
<td>ISC</td>
<td>Imitation Sweetened Creamer</td>
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<tr>
<td>IWP</td>
<td>Isolated Wheat Protein</td>
</tr>
<tr>
<td>MT</td>
<td>Metric tonne</td>
</tr>
<tr>
<td>NaCAS</td>
<td>Sodium caseinate</td>
</tr>
<tr>
<td>NFDM</td>
<td>Non-Fat Dry Milk</td>
</tr>
<tr>
<td>NZMP</td>
<td>New Zealand Milk Powder</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometre</td>
</tr>
<tr>
<td>Pa.s</td>
<td>Pascal second, unit of (absolute) viscosity</td>
</tr>
<tr>
<td>RCP</td>
<td>Recombined Creamer Powder</td>
</tr>
<tr>
<td>RFCMP</td>
<td>Recombined Full Cream Milk Powder</td>
</tr>
<tr>
<td>RHFP</td>
<td>Recombined High Fat Powder</td>
</tr>
<tr>
<td>RSCM</td>
<td>Recombined Sweetened Condensed Milk</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SCM</td>
<td>Sweetened Condensed Milk</td>
</tr>
<tr>
<td>SMP</td>
<td>Skim Milk Powder</td>
</tr>
<tr>
<td>SPI</td>
<td>Soy Protein Isolate</td>
</tr>
<tr>
<td>SWPI</td>
<td>Solubilised Wheat Protein Isolate</td>
</tr>
<tr>
<td>γ</td>
<td>Shear rate (/sec)</td>
</tr>
<tr>
<td>%w/v</td>
<td>% weight per volume</td>
</tr>
<tr>
<td>η</td>
<td>Viscosity (Pa.s)</td>
</tr>
<tr>
<td>τ</td>
<td>Shear stress (Pa)</td>
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GLOSSARY OF TERMS

**Apparent viscosity:** This is the viscosity of a Non-Newtonian fluid at a given shear rate

**Coalescence:** Coalescence is the fusion of two or more droplets to form a single large droplet

**Consistency index:** It is the constant in the power law model, indicator of the consistency of the food system

**CC:** Coffee Creamer is the dairy powder formed using sodium caseinate

**Flocculation:** The flocculation leaves the two droplets intact but aggregated to each other

**Hysteresis loop area:** It is an indication of the extent of the breakdown of the structure of finished product on application of shear

**IC:** Imitation Creamer is the coffee creamer equivalent formed using IWP

**RCP:** Recombined Creamer Powder is the Recombined Full Cream Milk Powder/Recombined High Fat Powder equivalent made using IWP

**RHFP:** Recombined High Fat Powder is the dairy powder containing more than 70% fat and is formed using Skim Milk Powder

**RFCMP:** Recombined Full Cream Milk Powder is the dairy powder containing formed using casein

**Permanent viscosity:** It is the rate of change of viscosity over the domain of shear rate measured

**Shear rate:** Shear rate is the rate of change of velocity at which one layer of fluid passes over an adjacent layer

**Shear stress:** This is the stress component applied tangential to the plane on which the force acts
**Water activity:** Water activity ($a_w$) refers to unbound water in the food system

**Yield stress:** It is the minimum shear stress corresponding to the first evidence of flow

**25% RSCM:** 25% direct replacement of casein with IWP with overall protein content 7%

**50% (6% protein):** 50% replacement of casein with total protein content decreased from 7% to 6% followed by corresponding increase in sugar content

**50% (5% protein):** 50% replacement of casein with total protein content decreased from 7% to 5% followed by corresponding increase in sugar content
ABSTRACT

Recombined dairy products are commonly produced using various dry milk ingredients, fat, sugar and water. This research work examined the possibility of replacing milk protein, specifically casein/caseinates, with Isolated Wheat Protein (IWP) in various recombined dairy products. These include Recombined Sweetened Condensed Milk (RSCM), Recombined Full Cream Milk Powder (RFCMP), Recombined High Fat Powder (RHFP) and Coffee Creamer (CC).

These dairy products were first manufactured at laboratory-scale with certain modifications in the standard manufacturing process. This was done to achieve finished product with the desirable properties of commercial product. Next, the partial or full replacement of casein/caseinate using IWP in the selected dairy-type products was investigated. And last, the emulsifying properties of both casein and IWP were compared in terms of a range of functional parameters critical to particular dairy products.

IWP performed better than casein in RSCM in terms of emulsifying properties since only partial replacement (25% and 50% with 1% reduced overall protein) was sufficient to compete with 100% casein in terms of viscosity, the most critical parameter for resulting product “Imitation Sweetened Creamer (ISC)”. The free fat content and emulsion stability, the most critical parameters for spray dried emulsions, particularly, RFCMP, RHFP and CC, performed functionally the same as Recombined Creamer Powder (RCP) made using IWP. Consequently IWP can be used to totally replace casein in a RFCMP, RHFP and CC-type emulsion products. Similar products made using dairy and non-dairy fats showed that casein and IWP can effectively stabilise emulsions whatever the type of fat/oil used.

This study showed that Isolated Wheat Protein performed better than casein/caseinates in the selected liquid emulsion system (RSCM), but was only comparable in the spray-dried emulsions (RFCMP, RHP and CC).
CHAPTER 1: INTRODUCTION

This work was conducted during 2004-06 as part of a major research project co-funded by (NFIS) / Food Innovation Grant (FIG) and Manildra Group in conjunction with Food Science Australia and RMIT University. It addressed an R&D objective with potential for direct economic benefits to the Australian Food Industry.

The consumption of recombined milk and milk products has been steadily growing in many parts of the world since the technology was developed in the middle of 20th century (Davidson 1999). As a result, various recombination plants have been established to supply milk products to those areas where the dairy industry was non-existent or there was insufficient supply of milk to fulfil market demands. These areas were largely South East Asia, the Middle East, South America and Africa. Worldwide, gross sales of recombined products industry in 2002 were estimated around US $5-6 billion (Sanderson 2004).

Recombined milk and milk products can be divided into three main sectors:

- Traditional recombined milk products – including sweetened condensed milk, evaporated milk, pasteurised and UHT milk, cheeses
- “Imitation” products – fat-filled milks, sweetened creamers and whey-based blends.

  The aim of these is to provide low-cost nutrition to the lower socio-economic sector
- “Added-value and speciality products” – cultured milk, dairy desserts and fortified milks.

In the world dairy trade, Australian dairy exports were ranked third following New Zealand and European Union (Dairy Australia 2005). Recent statistics for milk powders exported by Australia and the various countries importing these dairy products are shown in Figure 1.1 and Figure 1.2 respectively.
Figure 1.1: Australian dairy industry product exports for 2003-04 (Dairy Australia 2005)

Skim milk powder (SMP) and whole milk powder (WMP) were two of the most exported Australian dairy products after cheese (as shown in Figure 1.1). SMP (costing AUD 2250 per metric tonne (MT) on protein basis) contains about one third protein content, so assuming the cost is entirely due to protein content, the price of milk proteins is equivalent to AUD 6750/MT. As casein is approximately 80% of the total protein content, it is valued at around AUD 8000/MT (Pearce 2006). So SMP being an expensive source of protein and highly...
traded (Figure 1.3), there is a continuous quest among manufacturers to find cost-effective non-milk protein alternatives. Various attempts have been made to study the non-milk protein functionality in a range of products in order to achieve similar functionality as casein/caseinate. Ahmedra, Prinayawiwatkul & Rao (1999) studied the functional properties of non-milk protein, that is, solubilised wheat protein isolate (SWPI) compared with sodium caseinate (NaCAS), dried egg white (DEW), non-fat dry milk (NFDM), and soy protein isolate (SPI). The functionality factors studied were solubility, pH, ability to undergo controlled interactions in order to provide viscosity and surfactant properties to stabilise emulsions and foams etc. SWPI had high solubility at pH range 6.5-8.5 and superior foaming capacity and stability. Also, the water holding capacity, fat absorption capacity and emulsifying properties of SWPI were comparable to other commercial proteins. So when SWPI was incorporated at 5%, 10%, 15% or 20% into ice cream, chocolate chip cookies, banana nut muffins, and hamburger patties respectively, it maintained the desired functional properties. However, few published studies have been carried out to incorporate non-milk proteins in dairy-type products. Therefore this study was undertaken to investigate the feasibility of partial or total replacement of casein by Isolated Wheat Protein (IWP) in a range of dairy-type products for both technical and economic advantages. IWP is a novel protein ingredient developed and marketed by Manildra Group. It is a chemically modified form of gluten and sells for substantial less than casein/caseinate.
The objectives of this research work were:

- To select dairy-type products from existing popular consumer ranges for investigation
- To compare the emulsifying properties of caseins and IWP
- To evaluate a range of formulations for the selected dairy-type products in terms of functionality using partial or total replacement of casein/caseinate by IWP.

**Figure 1.3:** Trends followed by dairy product exports in ‘000 tonnes from year 2000-05 (Dairy Australia 2005)
CHAPTER 2: BACKGROUND STUDY AND LITERATURE REVIEW

This section reviews the functional properties performed by proteins in any real food system. This encompasses the structure of both milk proteins, caseins and whey proteins and also wheat proteins as their significant similarities and differences will determine their interchangeability in foods, particularly dairy-type emulsions. The denaturation of caseins and whey protein structure and the interactions between the two during food processing will also be addressed.

2.1 PROTEIN INGREDIENTS

Proteins, as food components, perform two functions - those related to the physicochemical properties essential for maintaining product characteristics e.g. viscosity, emulsion formation and stability, hardness, gel strength, appearance and those related to nutritional requirements (Parris & Barford 1989). Akiva (1978) classified these functional properties in relation to specific physicochemical properties. Firstly, properties related to the attraction of protein molecules with water and its solutes were termed “Hydrophilic” such as foaming, whipping, water binding, wetting and stickiness. Secondly, properties related to the amphiphilic nature of proteins termed “Hydrophobic-hydrophobic interactions” such as emulsification, fat absorption and fat holding capacity. Thirdly, properties related to protein-protein interactions termed “Intermolecular interactions” and lastly, impact of heat treatment on these interactions such as viscosity, gelation, elasticity etc. In this study on dairy-type emulsions, the emphasis will be towards hydrophobic-hydrophilic interactions and impact on these interactions during heat treatment as it is a fundamental step in any food processing.

In real food systems, these interactions get complex because of the presence of various protein and non-protein components, posing difficulties in understanding the actual contribution of protein functionality to food product. Published literature (Larsson 1990) showed various indirect approaches followed by researchers to understand protein functionality such as
formulation of simple model food systems which contain two or at most three components where interactions can be quantified to a degree (Halling 1981, Dickinson 1982, Graham et al. 1976, Parker 1987, Stainsby 1986 & Mitchell 1986); a study of protein molecular structure in the food mixture, before and after single process steps (Tornberg et al. 1977, Walstra 1983 & Tornberg 1980) and a study of specific properties in model systems of protein and non-protein components of different but similar known proteins (Hermasson 1986).

Milk is a traditional source of high quality protein in the human diet. Its general composition varies depending upon fat content and protein composition according to Rasic & Kurman (1978), and is recognised as an issue affecting the processing of food products. Among various proteins, the structural and functional properties of milk proteins have been extensively studied compared with any other food proteins (Dalgelish 1997) and helped in better understanding their behaviour in real food systems.

2.1.1 Structural and functional properties of milk proteins

Milk proteins contain two main classes namely caseins and whey proteins. Both of these milk protein classes are quite heterogenous as the protein groups within each class have different molecular, physical and chemical properties (Fox and Kelly 2003). These variations within the same protein group can also be attributed to genetic factors i.e. differences in the sequences of amino acids determined by the DNA of the genes (Fox and Kelly 2003).

Caseins occur as casein micelles in milk, which are complexes of colloidal calcium phosphate and caseins (Slattery and Evard 1973, Schmidt 1982 & Walstra 1990). These micelles are spherical aggregates with diameters ranging from 40 to 300 nm and show considerable variation in composition, structure and size distribution (Swaisgood 1996). Due to absence of the higher levels of secondary and tertiary structures, caseins are flexible and unstable structures whereas whey proteins are compact, globular proteins and contain
disulphide bonding to stabilise their structure with major proteins including \(\alpha\)-lactalbumin, \(\beta\)-lactoglobulin, bovine serum albumin and immunoglobulins (Fox 2001). Various models described the casein micelle structure known to contribute significantly to functionality, however, none of which is universally agreed. Only a brief summary of three of the models is presented in this section. In the Slattery and Evard (1973), Schmidt (1982) & Walstra (1990) sub-micellar model, casein molecules are thought to be present as sub-micelles linked via colloidal calcium phosphate and held together by hydrophobic interactions. In the Holt (1992) model, casein was considered to aggregate around the calcium phosphate to develop a micro-gel structure. A more recent model proposed a dual binding of the casein micelles, where bonding is via hydrophobic interactions and also through colloidal calcium phosphate (Horne 1998).

Despite the different views regarding casein micelle structure, it was agreed that \(\kappa\)-casein is mostly found on the micelle surface, where it acts to stabilize the micelle in milk due to its amphiphilic structure and its sensitivity to calcium (Swaisgood 1996, Dalgelish 1997, Horne 1998 & Fox and Kelly 2003).

Boye et al. 1997 proposed the effect of food processing on structural and conformational changes in the properties of proteins. This resulted in the loss of certain characteristics of the native proteins. Jonas et al. (1976) reported that the casein micelles are highly stable to heating contributing to their high surface activity and exceptional water binding capacity, fat emulsification properties and whipping ability. Also they are viscous and soluble in neutral and alkaline conditions. However, unlike caseins, whey proteins become denatured on heating (Fox 2001). And the presence of both types proteins during heating results in interaction of whey proteins with casein micelles and the formation of intermolecular disulphide bond (Jang and Swaisgood 1990 & Corredig and Dalgelish 1999). This may explain the heat stability of concentrated milk products made from heated milk.
Individual proteins can be modified from the milk by lowering pH to 4.6 either by the addition of dilute mineral acid or by converting some of the lactose into lactic acid through the action of added culture termed as caseinates which differ in their heat sensitivity as compared to native proteins (Srinivasan, Singh & Munro 2002). It was reported that unlike caseins, caseinates do not aggregate in the form of micelles and can be manufactured by precipitating caseins from milk. Caseinates are more functional than caseins in various food applications (Srinivasan, Singh & Munro 2003) in terms of most important functional properties such as viscosity and solubility (Muller & Hayes 1963, Hayes et al. 1968, Fox & Mullvihill 1982, Hooker et al. 1982).

Differences in the structures as discussed above have impacted on emulsification properties of caseins and caseinates in finished product (Fox 2001). The caseinates formed by the acid precipitation are regarded as non-aggregated caseins, and milk powder manufactured by spray drying without prior acidification of casein are considered aggregated by Euston & Hirst (1999). The explanation given was that casein molecules in milk powder can retain some of their original, inflexible aggregated structure whereas non-aggregated caseinates have open and flexible structure resulting in the emulsification properties of caseinates are better than milk powders. Also, caseinates impart more solubility and higher viscosity to the finished product.

Although highly-functional milk proteins are very important and are widely used industrially, the focus of R&D on just a few proteins limits both the variety of proteins available and the development of fundamental knowledge of the structure-function relationship. Realistically, it was important to study the functional properties of other potential food proteins in various real food systems such as wheat proteins (Welsh 1979).

**2.1.2 Structural and functional properties of wheat proteins**

Vital wheat gluten, as commercially manufactured, is a complex mixture containing approximately 75% proteins, 8% moisture and varying amounts of starch, lipid and fibre
(Batey & Gras 1981). The two main protein types present in wheat gluten are gliadins and glutenins. They are similar in many aspects especially their dispersibility in aqueous ethanol resulting in their classification as prolamines, having high contents of amino acids namely proline and glutamine. However, they are different in aspects of their functionality; gliadin gives gluten elasticity while glutenin gives it strength (Guthrie 1912). However, gluten utilisation as an ingredient in other food types is limited by its insolubility in water at neutral pH, hence it has poor emulsifying properties and water-holding properties (Mimouni, Raymond, Merle-Desnoyers & Ducastaing 1994). These characteristics limit its use as a protein in food applications.

Researchers focussed on modifying gluten in order to enhance its water binding and solubility properties. A number of methods have been developed to facilitate the use of gluten as a food ingredient (Wu et al. 1976, Sarkki 1979, Mimouni et al. 1994). Deamidation is one of the processes used to improve the functionality of the gluten (Batey & Grass 1981, Wu, Nakai & Powrie 1976). It converts some of the abundant hydrophobic glutamine residues in the gluten protein to glutamic acid residues and hence increases the hydrophilic properties. The deamidation can be achieved by acidic or alkaline hydrolysis (Batey & Gras 1981). Acidic deamidation results in converting glutamine residues to glutamic acid and possibly some peptide hydrolysis leading to the formation of lower-molecular-weight polypeptides, which in turn enhances its solubility properties (Batey & Grass 1981). However, it also exposes the natural lipid to oxidative rancidity, especially at the higher temperatures used in the early developments. Acidic deamidation results in binding an increased amount of water, up to 200 times their weight of water (Maningat, Bassi & Hesser 1994). Deamidation by alkaline treatment results in breaking disulphide bonds of cystine and creates cross linking due to the formation of lysinoalanine but also causes saponification of the lipid, resulting in an unpleasant soapy flavour (Batey & Gras 1981). Ahmedra (1999) investigated the commercial deamidated gluten product termed ”solubilised wheat protein isolate (SWPI)” for its
functionality relative to NaCAS, non-fat dry milk (NFDM), soy protein isolate (SPI) and dried egg white (DEW) in some real food systems. His research work reported exceptional functional properties of solubilised wheat protein isolate, showing its suitability to partially replace casein in various food systems. Literature regarding protein structure left some aspects undisputed. These arise of acid deamidated wheat protein compared with casein and whey protein structure; and in relation to functional properties of wheat proteins in the presence of milk proteins and other ingredients were lacking. This background information would have been valuable for fully understanding the wheat protein behaviour.

2.2 FOOD EMULSION SYSTEMS

An emulsion is considered to be a “heterogenous system”, consisting of one immiscible liquid intimately dispersed in another in the form of droplets (Bourne 1990). Such systems possess a minimal stability, which may be attenuated by the presence of surface active agents. For this purpose, proteins being potentially surface active molecules may unfold their three dimensional structure at the water-oil interface, adsorbing at the interface and helping in stabilising the emulsion. This behaviour is attributed to the interactions of hydrophobic parts of proteins with the oil phase and hydrophilic parts with aqueous phase.

Basically, two types of food emulsions can be found in most foods as shown in Figure 2.1 (A), however, there are emulsions reported containing more than two phases so called “Multiple emulsions”. Multiple emulsions could be a water-in-oil emulsion emulsified in another water phase as shown in Fig 2.1 (B).

- **Oil-in-water emulsions**: If the continuous phase is water, then it is an oil-in-water emulsion. e.g. evaporated milk, condensed milk etc
- **Water-in-oil emulsions**: If the continuous phase is oil, then it is a water-in-oil emulsion e.g. butter
Emulsifiers enhance formation and stabilisation of emulsion because they can adsorb to the oil droplets and protect them from coalescence. Proteins can act as emulsifiers because of the mixture of hydrophilic and hydrophobic functional groups on their component amino acids (McClements 2004).

Two stages are important in protein-stabilised emulsions: adsorption of protein molecules to the interface and the consequent stabilisation of emulsion droplets.

### 2.2.1 Adsorption of protein molecules

The adsorption of protein molecules at the oil-water interface depends on its functionality in the aqueous and oil phase. There are both intermolecular and intramolecular interactions. Intramolecular interactions include hydrophobic bonds, electrostatic interactions and the
cross-linking disulphide bonds whereas intermolecular interactions include hydration forces between the outer surfaces of the protein molecules (Eisenberg and McLachlan 1986). The emulsion formation results in the reduction of interfacial surface tension by proteins becoming adsorbed at the oil-water interface. The interfacial protein adsorption can be divided into three stages as shown in Figure 2.2.

Firstly, interfacial tension decreases rapidly as the protein adsorption is diffusion controlled. The conformation changes with protein denaturation and the hydrophobic groups start to interact more closely with oil phase. Hence a layer of protein molecules is formed at the interface.

Secondly, there occur conformational changes at the interface and also an energy barrier to the new approaching molecules in order to penetrate the molecules already at the interface.

Thirdly, there is development of protein-protein interactions along with slow rearrangements of adsorbed molecules.
Figure 2.2: Schematic diagram of globular protein adsorption at the oil water interface in Regime I-III (Beverung et al. 1999).
Brash and Horbett (1987) characterised various molecular properties of proteins that can influence the surface activity such as size, charge, structure and solubility properties. Jackson and Pallansch (1961) found the interfacial activity of the milk proteins to be in the decreasing order as: \( \beta \)-casein > casein micelles > serum albumins > \( \alpha \)-lactoglobulin > \( \alpha_s \), \( \kappa \)-casein > \( \beta \)-lactoglobulin.

### 2.2.2 Emulsion stabilisation

The mechanism of colloidal stability at an interface was first explained by Derjaguin, Landau, Verway, Overbeek by DLVO theory in 1940s. The attractive Van der Waal forces and the electrostatic repulsion between electrical double layers resulted in the stability of colloidal particles. However, this theory is not valid for some food emulsions with negligible electrostatic interactions. In those cases, stability is predicted due to mutual polymeric repulsion forces of hydrophilic portions of adsorbed protein molecules extended into the aqueous phase. So the protein stabilised emulsions are supposedly stabilised by a combination of electrostatic and polymeric interactions.

An emulsion is considered to be unstable with the coming together of particles, namely, creaming or flocculation (Dickinson 1994a). Creaming occurs because of the density differences of the two phases of the emulsion. This makes droplets either settle (termed “Sedimentation”) or float on the surface (termed “Creaming”). Another phenomenon of emulsion instability is flocculation which occurs due to the formation of aggregates of droplets. Coalescence is the fusion of two or more droplets to form a single large droplet as shown in Figure 2.3. The likely explanation is that a protein film that forms a “protective skin” around droplets and protects droplets from coalescence (Larsson & Friberg 1990).
The flocculation leaves the two droplets intact but aggregated to each other. In coalescence the thin liquid film between them bursts and one large droplet is formed. The stability of an emulsion can possibly be achieved by two main mechanisms i.e. either the droplets can be prevented from reaching each other through an energy barrier or their movement can be slowed due to increased viscosity of the continuous phase.

So the above discussion illustrates that the structure and viscosity contributed by a specific protein is critical in a real food system as it determines the overall functional properties.

2.3 FOOD APPLICATIONS

The food processing industry is giving increased emphasis to the production of alternative protein products as functional ingredients in an expanding number of formulated food products (Welsch 1979). These food emulsions are considered important because they improve palatability, mouth feel, flavour, texture and general appearance. Different types of food applications for the novel ingredients include:

- Replacement of traditional ingredients that are in foods at low levels for their functional rather than nutritional contribution
• Fortification of foods to increase protein levels

• Partial or complete replacement of traditional proteins that make a nutritional contribution to foods.

2.3.1 Advantages of using wheat proteins

Wheat proteins provide additional flexibility in formulating foods due to their lower cost, availability and functionality. Several dairy food systems e.g. fluid milk, infant formula, coffee whitener, cheese, sour cream, offer opportunities for utilising wheat proteins in place of milk proteins.

2.3.2 Disadvantages of using wheat proteins

• Numerous minor compounds may be present in wheat proteins that adversely affect the flavour and colour of the finished products

• Severe treatments to wheat protein ingredients may adversely alter solubility and functionality of the resulting product.

This research studied the functional properties (not nutritional aspects) of the group of novel wheat protein ingredients developed by Manildra Group called “Isolated Wheat Protein”.
CHAPTER 3: PROJECT HYPOTHESIS AND PLAN

3.1 THESIS HYPOTHESIS

IWP will perform as effectively as caseins or caseinates, both in liquid and spray dried dairy-type emulsions.

3.2 THESIS OBJECTIVES

The aim was to determine the feasibility of using IWP in the wide range of dairy-type products were:

- To select target dairy-type products from existing popular consumer ranges
- To compare the emulsifying properties of casein/caseinates and IWP in dairy-type products
- To evaluate a range of formulations of the selected dairy-type products in terms of their functionality using a combination of both casein/caseinates and wheat proteins

3.3 RATIONALE OF THE STUDY

The benefits of doing this research will be:

- An increased scientific understanding of the physical and functional properties of wheat proteins in both liquid and spray dried dairy-type products
- Determining emulsifying properties, which will provide valuable insight for the dairy industry for standardising the particular protein functionality in target dairy-type products
- Beneficial to the community in terms of increasing the variety of products in the market and their affordability to all incomes.
- Potential to capture a huge share of the international market especially due to significantly lower protein ingredient cost, which would in turn benefit the Australian economy.
CHAPTER 4: FUNCTIONAL PROPERTIES OF CASEINS AND ISOLATED WHEAT PROTEIN

This section summarises the investigation undertaken to determine the functional properties of IWP, in order to develop various dairy-type products using IWP. It was carried out by comparing functional properties of IWP with both NaCAS and SMP since these were the two main sources of caseins in most dairy products. The main functional properties investigated were solubility, water holding / fat absorption capacity and emulsifying capacity / stability.

4.1 INTRODUCTION

Functionality refers to any property of a food ingredient that affects its utilisation except for its nutritional utilisation (Kinsella 1976). Functional properties of proteins not only affect their role in the physical behaviour of food during preparation, processing and storage but also the sensory characteristics of food. Various sources of proteins can be used in order to achieve desirable functional characteristics e.g. increased viscosity, gelation etc. as well as sensory characteristics like texture, flavour and appearance in a finished product.

Worldwide the main sources of proteins include milk, meat, fish, soybean, eggs and wheat. Wheat is sought after as a protein source made popular among food processors in order to use it in both existing and new product development products (Welsch 1979). Its abundant production has been reported to reach 600 million tons in year 2004 (FAO 2005). This great quantity of production is mainly used for wheat flour, however, industry uses smaller quantities of wheat to produce starch, pasta, gluten, dextrose, alcohol and other products. A minor, but industrially very important and growing use of wheat is as a source of gluten and starch. The major limitation associated with wheat protein utilisation is its insolubility (Mimouni et al. 1994). The chemical and enzymatic modification of gluten as stated in section 2.1.2 are reported important for its commercial use in a range of food products.
4.2 LITERATURE REVIEW

There are various methods reported from different laboratories to characterise emulsifying properties as follows. Furthermore, different apparatus used in the emulsion formation results in different emulsifying properties. In addition to different apparatus used, emulsifying conditions influenced the emulsifying properties like amount and type of protein, type of oil used etc. The emulsion capacity, emulsion stability and droplet size distribution are the most common methods of quantifying emulsifying properties. However, study of the solubility of proteins is the base for all the above mentioned emulsifying properties as they are affected substantially by solubility (Kinsella 1976; Hettiarachchy et al. 1996).

Solubility

Solubility is the amount of protein in a sample that goes into solution or into colloidal dispersion under specific conditions and is not sedimented by moderate centrifugal force (Kinsella 1984). The solubility of proteins depends on many factors like the inherent properties of proteins, environmental conditions, pH and temperature etc. (Damodaran & Kinsella 1982). Due to the differences in inherent properties, various proteins were reported to respond differently to different processing conditions, such as, caseins are highly heat stable whereas β-lactoglobulin is very susceptible to heat denaturation. So milk proteins containing heterogenous protein types will behave differently to the processing conditions. The pH affects the charge and electrostatic interactions between proteins. So above or below the isoelectric point of proteins, a net charge enhances solubility whereas at isoelectric point, where net charge is zero, molecules tends to coagulate and become insoluble (Charalambous & Doxastakis 1989). Increasing temperature causes the protein unfolding, protein-protein interaction and results in aggregation and precipitation. Caseins are less soluble compared to sodium and potassium caseinate as caseinates form complexes with calcium which enhances the solubility (Muller 1982, Morr 1982, Muller 1971 & Ishiro and Okamoto 1975).
The most common method involves the determination of nitrogen in the supernatant by the Kjeldahl method (International Standard 1995). However, Vane & Zayas (1995) used Dumas method for the determination of protein solubility using a nitrogen determiner LECO for various dried powders.

**Water holding and fat absorption capacity**

Water holding capacity of proteins is the weight in grams of water per gram of protein (Charalambous & Doxastakis 1989) which can be measured using farinograph (Dunkerley & Hayas 1980, Short 1980) or American Association of Cereal Chemists method (AACC 1994). Fat absorption capacity is the weight in grams of oil per gram of protein (Chakraborty 1986). Both AACC method and Chakraborty used centrifugation method for determining water and fat absorption capacities. Caseinates tend to absorb more water than the casein micelles (Ruegg, Lusher & Blanc 1974), however, the addition of salt can enhance the hydration of both caseins and caseinates at water activity higher than 0.65 (Berlin, Anderson & Pallansch 1968).

**Emulsion capacity and emulsion stability**

McWatters and Holmes (1979a) reported emulsion capacity as the maximum amount of oil emulsified by a standard amount of protein. The residual oil evident in the final phase of this process gives an indication of emulsion capacity. However, the accuracy of this method is found to be dependent on various factors such as mixer speed, rate of oil addition and temperature etc. However, it is a simple and economical method as long as experiment is performed uniformly. It is also one of the most widely used industrial methods for measuring emulsion capacity of complex food emulsions. Crenwelge et al. 1974 reported to measure emulsion capacity through the change in electrical resistance of the material. Other indicators could be a sudden drop in emulsion viscosity or conductivity. This is a reliable method and
can be used mainly for novel protein ingredients. The most recent method is the use of interfacial tension rheometer found by Nagarajan, Chung & Wasan (1999), however, it is more apt to study the viscoelastic properties of the emulsions.

Different researchers reported different ways to indicate emulsion stability. One of these methods includes the inspection of emulsion breakdown over several months (Dickinson 1994a) or determination of creaming, flocculation or droplet size etc. The basic method includes change in oil concentration in aqueous phase (Tornberg & Hermansson 1977, Yamachi et al. 1980). Recently Mengual, Meunier, Cayre, Puech, Snabre (1998) found the turbidity measurement using turbiscan or spectrophotometer can indicate the stability of the emulsions.

4.3 MATERIALS AND METHODS

4.3.1 MATERIALS

Two types of IWP were manufactured and provided by Manildra Group namely Gemtec1100 and Gemtec1160 as shown in Table 4.1. Gemtec1100 was produced by acid deamidation of gluten; Gemtec1160 was also made by the same deamidation process followed by a deflavouring process. Deflavouring resulted in removal of around 5% lipids, resulting in an increase in protein content in Gemtec1160 as compared to Gemtec1100. The following were the specifications for both Gemtec1100 and Gemtec1160. NaCAS containing 96.7% protein was purchased from New Zealand Milk Powders and medium heat skim milk powder (33.6% protein) was purchased from Bonlac Foods Limited.
Table 4.1: Specifications of Gemtec1100 and Gemtec1160 (Manufacturer’s specification sheet)

<table>
<thead>
<tr>
<th>SPECIFICATIONS</th>
<th>GEMTEC1100</th>
<th>GEMTEC1160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein content</td>
<td>91.2%</td>
<td>93.7%</td>
</tr>
<tr>
<td>Fat content</td>
<td>5.0%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.8%</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

4.3.1.1 Chemicals for analysis

1 M Sodium hydroxide (NaOH) solution: To make a 1M solution of NaOH, NaOH (40g) was added in deionized water to make up 1000ml volume in a volumetric flask. When all the NaOH pellets are dissolved and the solution is at room temperature, it was diluted to the 1000ml mark and the flask inverted several times to mix.

1M Hydrochloric acid (HCl) solution: To make a 1M solution of HCl, HCl (60.05g) was added in deionized water to make up 1000ml volume in a volumetric flask. The flask was inverted several times to mix.

4.3.2 METHODS

4.3.2.1 Solubility

Protein solubility was determined using Vani and Zayas (1995) method with modifications discussed below. Protein solution (2% w/v) was made with water at 50-60°C temperature from various protein sources and pH was adjusted to 6.5, 7.5, 8.5 using 1M NaOH or HCl. Samples obtained were then centrifuged (model Sorvall RC-5B, DuPont Instruments,
Newtown, CT) at 12000g for 15 min at 25°C. Supernatant liquid was analysed in duplicate for nitrogen content using a FP-428 LECO nitrogen determinator (LECO Corp., St. Joseph, MI).

4.3.2.2 Water holding and fat absorption capacity

Water holding capacity was determined using the American Association of Cereal Chemists method (AACC 1994). Each protein ingredient containing constant protein content (5g) was weighed into 50ml centrifuge tubes and distilled water was added in small increments. After the mixture was thoroughly wetted, the stirring rod was wiped on the side of the centrifuge tube to minimize losses of protein materials and samples were centrifuged (model Sorvall RC-5B, DuPont Instruments) at 2000g for 10 minutes. Water holding capacity (grams of water per gram of protein) was calculated as

$$\text{WHC} = \frac{(W_2 - W_1)}{W_0},$$

where $W_0$ is the weight of the dry sample (g), $W_1$ is the weight of the tube plus the dry sample (g), and $W_2$ is the weight of the tube plus the sediment (g). Samples were analysed in triplicates for each protein ingredient.

Fat absorption capacity was determined by the Chakraborty (1986) method with modifications. Each protein ingredient with constant protein mass (1g) was weighed into 50ml centrifuge tubes and was thoroughly mixed with canola oil (10ml). The protein-oil mixtures were then centrifuged at 1600g (model Sorvall RC-5B, DuPont Instruments) for 10 minutes. After centrifugation, supernatant was drained off and tubes were weighed. Fat absorption capacity (grams of oil per gram of protein) was calculated as

$$\text{FAC} = \frac{(W_2 - W_1)}{W_0},$$

where $W_0$ is the weight of the dry sample (g), $W_1$ is the weight of the tube plus the dry sample (g), and $W_2$ is the weight of the tube plus the sediment (g). Samples were analysed in triplicate for each protein ingredient.
4.3.2.3 Emulsifying capacity and stability

Emulsifying capacity (EC) and stability (ES) were determined according to the method described by Yasumatsu et al. (1972) with modifications. Each protein ingredient with constant protein mass (8g) was weighed and mixed with distilled water (100ml) using an overhead stirrer followed by addition of canola oil (100ml) at high speed for 1 minute to form an emulsion. For each protein ingredient, a fixed quantity (40ml, \(V_T\)) of emulsion was poured into a 50ml centrifuge tube and centrifuged (model Sorvall RC-5B, DuPont Instruments) at 1475g for 5 minutes, and the volume of emulsified fraction (\(V_{F1}\)) was recorded. The tubes containing the oil-in water emulsified fraction were heated in a water bath at 80°C for 30 minutes and cooled to room temperature (25°C). Upon cooling, these tubes were centrifuged at 1475g for 5 minutes, and the volume of the remaining emulsified fraction (\(V_{F2}\)) was recorded. EC and ES were, respectively, reported as

\[
EC \text{ } (\%) = \left( \frac{V_{F1}}{V_T} \right) \times 100 \text{ and }
\]

\[
ES \text{ } (\%) = \left( \frac{V_{F2}}{V_T} \right) \times 100
\]

4.3.2.4 Statistical analysis

The data obtained on the functional properties of different proteins was analysed using one-way analysis of variance (ANOVA) with \(p<0.05\).

4.4 RESULTS AND DISCUSSION

4.4.1 Solubility

The solubility profile for both NaCAS and IWP was investigated in the pH range 6.5-8.5. This pH range was chosen on the basis of pH of dairy-type products selected for investigation. The solubility of IWP (90%) was found to be comparable to NaCAS (95%) in this pH range. The similar trends were reported and the study was carried out for pH range 2.5-8 (Manildra Group, 2006) as shown in Figure 4.1. The solubility of NaCAS increased sharply in the pH
range 4.5-6 that is, increased (20 times) from 5% at pH 4.5 to 100% at pH 6 whereas solubility increased (6 times) from 15% at pH 4.5 to 97% at pH 6 for IWP. However, the least solubility was found near the isoelectric point (pH 4.3) for IWP (Mimouni et al. 1994) and 4.6 for NaCAS. The evident solubility differences exhibited by NaCAS and IWP can proposedly be attributed to the distribution and extent of hydrophilic and hydrophobic patches. Also, this solubility profile of IWP was similar to the deamidated wheat gluten (Mimouni et al. 1994) and wheat germ protein (Hettiarachchy et al. 1996) which indicates its usage in the products requiring emulsifying properties at neutral to slightly alkaline pH.

![Figure 4.1: Solubility profile of IWP (■) and NaCAS (▪) over pH range from 2.5-8 (Pearce 2006)](image)

**4.4.2 Water holding and fat absorption capacity**

The water holding and fat absorption capacity of NaCAS, IWP and SMP based on constant protein mass were compared as shown in Figure 4.2. It was found that the water holding capacity of IWP (2.00g/g) was significantly (p< 0.05) higher than that of SMP (0.75g/g) and NaCAS (1.20g/g). This likely reflects the significant differences in hydrophilic/hydrophobic amino acid composition among these proteins. Also it can be attributed to the presence of
Other ingredients like starch in IWP that absorbs three times more water (termed as “swelling”) than other proteins. However, fat absorption capacity of IWP (2.6g/g) was insignificantly different (p<0.05) to NaCAS (2.3g/g) but significantly different from SMP (1.3g/g). The fat absorption capacity of IWP (2.6g/g) was slightly higher than water binding capacity (2.0g/g). The improved ability of IWP to bind fat, like other plant proteins, is likely due to more non-polar side chains available to bind hydrocarbon chains (Lin and Zayas 1987). The ability of IWP to absorb and retain water and fat may help improve binding of the structure of the end product. These results were in agreement to previous work (Ahmedra et al. 1999).

![Water and Fat Binding Capacity](image)

**Figure 4.2:** Water holding (■) and fat absorption (□) capacities of NaCAS, IWP and SMP

### 4.4.3 Emulsifying capacity and stability

The EC and ES of NaCAS, IWP and SMP based on constant protein mass were compared as shown in Figure 4.3. It was found that EC of IWP (97%) was higher than NaCAS (80%) followed by SMP (56%). The similar trends were noticed in ES of various protein ingredients.
NaCAS is a disordered form of protein whereas SMP is an aggregated form of protein. The superior ability of NaCAS to emulsify the oil and protein suspension relative to SMP may be attributed to the proteins in NaCAS which are more flexible and can unfold easily at the interface (Euston & Hirst 1999). However SMP being an aggregated form of protein, is less flexible (structure is held by calcium bridges) and cannot unfold easily at the interface. IWP is found to have emulsifying capacity comparable to NaCAS but significantly better than SMP because of structural differences which offer IWP with more flexibility for adsorption at the interface.

4.5 CONCLUSIONS

Based on experimentation at laboratory-scale on water holding/fat binding capacity and emulsifying capacity/stability, IWP exhibited superior functional properties relative to commercially available dairy protein sources. These results showed the potential for use of IWP in various dairy-type emulsion systems.
CHAPTER 5.0 LIQUID EMULSION SYSTEM: RECOMBINED SWEETENED CONDENSED MILK AND IMITATION SWEETENED CREAMER

As a result of the work in the previous chapter, it was evident that further study was needed to investigate the feasibility of replacing casein with IWP in both liquid and spray-dried dairy-type products. Four liquid and spray-dried dairy-type emulsion products were selected for study namely Recombined Sweetened Condensed Milk, Recombined Full Cream Milk Powder, Recombined High Fat Powder and Coffee Creamer. The basis of choosing these dairy products were

- the simplicity of the system,
- the availability of ingredients and
- the potential markets for these products

The term RSCM refers to a product derived from milk. The term Imitation Sweetened Creamer (ISC) refers to formulations of this type in which milk protein is wholly or partially replaced by IWP. Similarly the term RFCMP is reserved for product derived entirely from milk. For this thesis the term Recombined Creamer Powder (RCP) is used to refer to formulations of this type where milk protein is wholly or partially replaced by IWP.

5.1 INTRODUCTION AND LITERATURE REVIEW

This section summarises the processing, structure and evaluation of Recombined Sweetened Condensed Milk (RSCM) and the recent studies on the properties of RSCM made using SMP.

5.1.1 RSCM processing

RSCM was one of the first recombined products successfully made using SMP (Clarke 1999). It is a liquid emulsion system and the initial viscosity is considered to be the most critical attribute because it gives the best estimate of consumer’s perception of the thickness of RSCM in a freshly opened container (Samel and Muers 1962).
RSCM manufacturing varies with country, manufacturer, raw materials used and operation size (Davidson 1999). The general RSCM manufacturing process could be identified as shown on Figure 5.1.

SMP, sugar and fat added to warm water at 40-50°C

Homogenisation (50-65°C at 5 and 1 MPa)

Heat treatment (92°C / 30mins)

Vacuum cooling

Packaging

**Figure 5.1:** Outline of RSCM Production (Clarke 1999)

The most critical factor in the manufacture of RSCM is the selection of milk powder in order to achieve desirable viscosity (2-3 Pa.s) of RSCM (Lawrence 1963). Several methods have been proposed for powder suitability depending upon the degree of heat treatment during manufacturing termed as “whey protein nitrogen index (WPNI)” (Brady 1972). The selection of powders based on WPNI was found to be a good indicator, but the range of viscosity values obtained from powders with similar WPNI values limited its use as a predictive tool for RSCM viscosity (Clarke 1999). To overcome these limitations, the laboratory assessment of viscosity was made in order to predict RSCM viscosity behaviour on the pilot-scale (Kieseker & Southby 1965). However, there were issues related to the RSCM processing such as the reliability and repeatability of RSCM viscosity at laboratory scale. Kieseker and Southby (1965) reported a good correlation between laboratory-scale assessment method and pilot-scale manufacture. However, Clarke (1999) stated that these test methods do not have any correlation between them. So there was a lack of any universally accepted method for
selecting milk powders for manufacturing RSCM. However, powders with WPNI of 1.5-5.99 were the most acceptable for RSCM.

Apart from the selection of milk powder, season of powder manufacture, RSCM processing conditions, particularly homogenisation and pasteurisation regimes, and storage conditions also influences the final RSCM viscosity. However, the extent of seasonal variation in powder manufacture can be controlled using combination of process parameters at the pilot-scale level (Muller and Kieseker 1962).

5.1.2 RSCM structure

The contribution of heat treatment to the structure of RCSM has been related to the heat induced interaction between denatured whey proteins and casein micelles. It has been long recognised that whey proteins undergo denaturation when heated above 70°C and resulting in complex formation with casein (Singh 1995). The complex formation between β-lactoglobulin and κ-casein at the micelle surface resulted in intermolecular disulphide bonding, in turn resulting in RCSM structure (Jang & Swaisgood 1990). The electron microscopy study identified its presence in the form of appendages and filaments on the micelle surface (Davies et al. 1978).

Semenova, Antipova & Belyakova (2002) reviewed the impact of presence of sugar on the thermal stability of protein gelation. It was stated that sugars can not only alter the gelation mechanism by increasing its gelation temperature but also slows the rate of gelation and increases protein gel rigidity. Kulmyrzaev, Bryant & McClements (2000) reported the increase in protein gelation temperature due to the slower unfolding of the proteins and alteration in its intramolecular interactions leading to intense folding of protein structure, hence requiring higher temperature to unfold. Moreover, sugar acts as a cross-linking agent and has a pronounced strengthening of proteins on cooling. Also, the slower gelation rate was because of the increase in the viscosity of the continuous phase.
During storage, RSCM was reportedly gelled because of the change in the molecular structure of the proteins and the formation of weak network structure between the protein micelles (Sone 1972). So it might be safe to assume the role of sugar in strengthening networking during RSCM storage.

5.1.3 Rheological analysis

This section will identify RSCM behaviour including time dependent and time independent behaviours as shown in Figure 5.2 and different approaches to evaluate RSCM quality etc.

**Time independent flow behaviour**

Ideally, very few fluids fall in the category where shear stress is directly proportional to the shear rate termed “Newtonian fluids” and the viscosity is independent of the shear rate. Newtonian fluids are water, tea, coffee, beer and milk etc. Non-Newtonian fluids are those in which shear rate is not directly proportional to shear stress and hence deviates from the Newtonian behaviour. Most foodstuffs fall into this category. The “apparent viscosity” is the term used to define the viscosity of non-Newtonian fluid.

Non-Newtonian fluids can fall into one of three classes:

First, pseudoplasticity is the type of flow where an increasing shear rate gives a less than proportional increase in shear stress. In other words, the apparent viscosity decreases with shear rate. A typical product example of this type of fluid is honey. Second, dilatant fluids are those in which the shear stress-shear rate plot of this type of flow begins but is characterised by equal increments in the shear stress giving less than equal increments in the shear rate. Examples include chocolate syrup.

Third, plastic fluids refers to the materials that exhibits yield stress. Yield stress is defined as the minimum stress that needs to be exceeded before flow begins. Shear rate is also directly proportional to shear stress in these materials. Typical examples include tomato ketchup, whipped cream, mayonnaise etc.
Figure 5.2: Types of viscous behaviour (Bourne 1990)

Time dependent flow behaviour

Different non-Newtonian food systems can ideally fall into two behavioural patterns. These properties are all time dependent.

Thixotropic fluids are those in which apparent viscosity decreases with the time of shearing but the change is reversible; that is, the fluid will revert to its original state on standing. Some starch paste gels are in this class.

Rheopectic fluids are those where apparent viscosity increases with time of shearing and the change is reversible; that is, after resting, the product returns to its original apparent viscosity. It is rare to find this type of behaviour in a food system.

RSCM was characterised as non-Newtonian and thixotropic in nature (Rohm 1988). A common sensor system used for evaluating RSCM is concentric cylinder (also known as cup and bob) geometry (Samel & Muers 1962b). During analysis, a sample is subjected to shear and the sample response was categorised in terms of apparent viscosity. Two types of rheological measurements are commonly applied: apparent viscosity as a function of a shear rate and apparent viscosity as a function of time.

Apparent viscosity as a function of shear rate has been generally reported to be conducted over a range of shear rates. Haque, Richardson & Morris (2001) choose shear rate of 0.1-
100/s in order to prevent the slippage effect between the measuring surfaces during the measurement. However, the use of high shear rate (1000/s) used by Teggatz & Morris (1990), Bhattacharya (1999), Hassan et al. (1996) reported severe damage of the sample structure limiting differentiation of flow behaviour between samples.

The measurement of apparent viscosity as a function of shear rate can determine the hysteresis loop area and permanent viscosity of the material (Samel & Muers 1962), however, its practical application in any of the food systems was not reported. The hysteresis loop area represented the structural breakdown of structure during shearing, as described by Halmos & Tiu (1981), Ramaswamy and Basak (1991) & Hassan et al. (1996a) whereas the permanent viscosity of the material determines the inherent viscosity of the material after the highest application of shear and can be obtained from the slope of the downward curve of hysteresis loop area (Samel & Muers 1962).

Another important physical phenomenon studied in RSCM was age thickening. It has been attributed to the increase in viscosity during storage as a result of colloidal swelling and hydration of the proteins during storage (Hunziker 1949). Samel & Muers (1962), Flippe (1991) & Patil and Patel (1992) concluded that RSCM showed thixotropic behaviour due to the molecular aggregation. Sone (1972) suggested the age thickening of RSCM as the slow irreversible change occurring in the size and the shape of the casein micelles which produced a permanent rise in the viscosity. These micelles orient themselves in such a way as to form a loose network enclosing some of the dispersed phase, therefore producing an increase in the initial viscosity. Salzberg & Georgevits (1956) and Hayes & Muller (1961) showed relation between casein concentration and its contribution to viscosity to the solution. That is, the higher is the casein concentration, the greater is the viscosity of the product. Samel & Muers (1962) reported an insignificant role for whey proteins in the age thickening process. However, Flippe et al. (1991) contradicted these outcomes and did an in-depth study of the structure of RSCM during storage. The presence of filamentous appendages within SCM
structure was correlated to the raw milk study where the profound association was a result of presence of denaturated whey proteins. So it was agreed that the whey proteins indirectly help in building RSCM structure during storage.

Another most significant rheological parameter in RSCM is “yield stress”. Bingham and Green in 1920 first introduced the concept of a yield stress in fluid like materials. They defined yield stress as the minimum shear stress corresponding to the first evidence of flow e.g. the value of shear stress at zero velocity gradient. There had been many techniques devised for measuring yield stress. The most common technique was the indirect measurement made by conventional methods, by extrapolating the shear stress–shear rate data to zero shear rate followed by application of some rheological models. However, accuracy was the limiting factor. So an alternative approach found was its direct measurement by Nguyen and Boger (1985) using vane geometry. This geometry consists of 2-8 blades and can measure yield stress by applying constant shear rate /or shear stress or as a function of time. Dzuy & Boger (1983) identified typical torque-time curve obtained using a vane geometry as shown by \( T_m \) in Figure 5.3. As the vane rotates from rest, the region of the suspension close to the edges deforms elastically as shown by the linear part of the curve. Such linear behaviour might be attributed to the stretching of the network bonds interconnecting structural elements. Since more bonds would be stretched and the resistance to more deformation increased as the vane’s rotation continues, the torque required to keep the motion constant must also rise. The speed of the vane was 0.1 rpm. At excessive rotational speed, significant viscous resistance, together with instrument inertia and insufficient damping, may introduce errors to the measured maximum torque and hence to the calculated yield stress. The various advantages associated using a vane geometry include:

The disturbance caused by the introduction of the vane into the sample could be kept to a minimum. This is particularly of relevance while evaluating thixotropic materials like RSCM that are sensitive to past shear application. And it made it possible to quantify the structural
recovery of RSCM. Also it minimises the disadvantage of apparent slip during yield stress measurement for RSCM.

![Graph of yield stress curve](image)

**Figure 5.3:** Typical yield stress curve (Dzuy & Boger 1983)

Lidell & Boger (1996) reported an insight on the various factors affecting these measurements such as vane dimensions, measuring system stiffness etc. Extension of Lidell and Boger’s work was undertaken by Christopher et al. (2004) where they successfully completed the instrumental and sensory tests to quantify the various texture attributes of various fluid food products. Various conclusions emerged from these studies including:

- Fresh SCM showed a slight divergence from Newtonian behaviour (Samel and Muers 1962)
- During age thickening, SCM showed thixotropy, due to the aggregation of casein micelles and network formation and was enhanced by storage time (Samel and Muers 1962)
- No appreciable yield stress is detected in fresh SCM (Higgs and Norrington 1971)
- Processing notably affected the viscosity (Newstead, Baldwin and Hughes 1978)
- The thickening rate was much more rapid at 40°C than 30°C (Newstead, Baldwin & Hughes 1978)
• Fluid consistency coefficient increased during storage time (Flippe et al. 1991)
• Viscosity increased exponentially during storage time up to 39 days (Patil & Patel 1992)

Another important aspect of rheology is the study of the flow behaviour properties of the finished product. Bloore & Boag (1981), Flippe et al. (1991), Velez-Ruiz & Barbosa – Canovas (1998) stated that flow behaviour properties of RSCM were greatly influenced by factors such as concentration, temperature and storage time among others and were correlated with one of the three above mentioned variables, however, they mainly focused on skim milk products. To analyse the rheological behaviour of RSCM, its shear thinning behaviour was fitted well in a power law model (Velez Ruiz & Barbosa- Cannovas 1998); the effect of storage time on the power law model was determined. Power law model (Oswald de Waele) is given by:

\[ \zeta = \kappa \gamma^n \]

where
\[ \zeta = \text{shear stress} \]
\[ \gamma = \text{shear rate} \]
\[ n = \text{flow behaviour index} \]
\[ \kappa = \text{consistency index} \]

So based on the above literature, our research work chose to use SMP with WPNI of 1.51-5.99 for the manufacture of RSCM. The stated problem of reliability and repeatability of RSCM viscosity was addressed by carrying out a sufficient number of experiments in order to achieve reliable results and to control the processing conditions at laboratory-scale. The casein from RSCM was partially and fully replaced using IWP and the finished product containing IWP was termed “Imitation Sweetened Creamer (ISC)”. The formulations were then short listed based on the most critical attribute “viscosity” at 2.2 rpm shear rate and were
subjected to rheological analysis, age-thickening behaviour during storage and yield stress measurements.

5.2 MATERIAL AND METHODS

5.2.1 Materials

Commercial RSCM product (Nestle) was bought from a supermarket. Medium Heat SMP (containing 36% protein) was purchased from Bonlac Foods Limited. Anhydrous Milkfat (AMF) and lactose were manufactured by NZMP. Ground sugar was also supplied by Manildra Group, Sydney.

5.2.1.1 Chemicals for Analysis

Petroleum ether was bought from Sigma Aldrich (Castle Hill, NSW)

5.2.2 Methods

5.2.2.1 Product development

The original RSCM formulation was modified through the following stages in order to develop the new product ISC.

- Imitating the commercial RSCM manufacturing process at laboratory-scale
- Using the same RSCM formulation as a base, partially or fully replace casein with IWP on a laboratory-scale
- Short-listing the desirable ISC formulations based on the critical attribute “viscosity”
- Carrying out pilot-scale trials for short-listed formulations
- Conduct quality assessment of both RSCM and ISC.
5.2.2.2 Laboratory-scale production of RSCM / ISC

SMP and IWP were added to warm water at 40-50ºC and was mixed for five minutes (as shown in Table 5.1, 5.2 and 5.3 respectively). The sugar was added to the above prepared mix while the temperature was maintained at 50ºC and blended for further five minutes using an ultra-turrax mixer (model T45). The speed of the mixing was increased so as to maintain temperature to 60ºC. Then melted AMF was added to the mix and the temperature was allowed to reach 92ºC in eight minutes by increasing speed of mixing. The solution was maintained at the same temperature for 30 seconds, then cooled in an ice water bath for six minutes and vacuum applied for 10 minutes. The final product was packed and incubated in the oven at 32ºC for 18 hours. Both RSCM and ISC obtained was then analysed for its initial viscosity.

**Table 5.1:** Experimental design for the direct replacement of casein with IWP in RSCM formulations

<table>
<thead>
<tr>
<th>REPLACEMENT (%)</th>
<th>SMP (g)</th>
<th>IWP (g)</th>
<th>WHEY PROTEIN POWDER (g)</th>
<th>TOTAL PROTEIN CONTENT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>125.0</td>
<td>0.0</td>
<td>0.0</td>
<td>42.0</td>
</tr>
<tr>
<td>10</td>
<td>112.5</td>
<td>3.6</td>
<td>6.3</td>
<td>42.0</td>
</tr>
<tr>
<td>15</td>
<td>106.2</td>
<td>5.5</td>
<td>9.5</td>
<td>42.0</td>
</tr>
<tr>
<td>18</td>
<td>102.5</td>
<td>6.6</td>
<td>11.4</td>
<td>42.0</td>
</tr>
<tr>
<td>20</td>
<td>100.0</td>
<td>7.3</td>
<td>12.7</td>
<td>42.0</td>
</tr>
<tr>
<td>25</td>
<td>93.7</td>
<td>9.2</td>
<td>15.6</td>
<td>42.0</td>
</tr>
<tr>
<td>50</td>
<td>62.5</td>
<td>18.4</td>
<td>31.8</td>
<td>42.0</td>
</tr>
<tr>
<td>75</td>
<td>31.2</td>
<td>27.6</td>
<td>47.7</td>
<td>42.0</td>
</tr>
<tr>
<td>100</td>
<td>0.0</td>
<td>36.8</td>
<td>63.6</td>
<td>42.0</td>
</tr>
</tbody>
</table>

*AMF (48g), sugar (276g) and water (151g) in the above formulations were kept constant
Table 5.2: Experimental design for decreased overall protein content compensated with water content in 50% ISC formulation

<table>
<thead>
<tr>
<th>PROTEIN CONTENT (%)</th>
<th>SMP (g)</th>
<th>IWP (g)</th>
<th>WHEY PROTEIN POWDER (g)</th>
<th>TOTAL PROTEIN POWDER (g)</th>
<th>WATER (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>62.5</td>
<td>18.4</td>
<td>31.8</td>
<td>42.0</td>
<td>151.0</td>
</tr>
<tr>
<td>6</td>
<td>53.5</td>
<td>15.7</td>
<td>27.2</td>
<td>36.0</td>
<td>168.0</td>
</tr>
<tr>
<td>5</td>
<td>44.6</td>
<td>13.1</td>
<td>22.7</td>
<td>30.0</td>
<td>184.0</td>
</tr>
</tbody>
</table>

AMF (48g) and sugar (276g) were kept constant in the above formulations

Table 5.3: Experimental design for decreased overall protein content compensated with sugar content in 50% ISC formulation

<table>
<thead>
<tr>
<th>PROTEIN CONTENT (%)</th>
<th>SMP (g)</th>
<th>IWP (g)</th>
<th>WHEY PROTEIN POWDER (g)</th>
<th>TOTAL PROTEIN POWDER (g)</th>
<th>SUGAR (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>62.5</td>
<td>18.4</td>
<td>31.8</td>
<td>42.0</td>
<td>276.0</td>
</tr>
<tr>
<td>6</td>
<td>53.5</td>
<td>15.7</td>
<td>27.2</td>
<td>36.0</td>
<td>293.0</td>
</tr>
<tr>
<td>5</td>
<td>44.6</td>
<td>13.1</td>
<td>22.7</td>
<td>30.0</td>
<td>309.0</td>
</tr>
</tbody>
</table>

AMF (48g) and water (151g) were kept constant in the above formulations

Pilot-scale production of RSCM/ISC

The initial mix was prepared in the pilot plant as per formulation enlisted in Table 5.4. The water at 40-50ºC was added to the mix vessel and the milk powder added over a few minutes with continuous agitation. The sugar was then added progressively, the temperature being maintained at 50ºC until addition of sugar was completed. Then liquid fat was added and the
temperature was allowed to increase to 60°C with the energy input from the high shear mixer. The mix was homogenised using a two-stage homogeniser at 5 and 1MPa pressures respectively followed by pasteurisation at 92°C with a holding time of 30 seconds and was then vacuum cooled. The final product was packed at 33°C.

**Table 5.4:** Pilot-scale composition of RSCM

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>QUANTITY (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMP</td>
<td>15.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>33.0</td>
</tr>
<tr>
<td>AMF</td>
<td>6.25</td>
</tr>
<tr>
<td>Water</td>
<td>19.9</td>
</tr>
</tbody>
</table>

**5.2.2.3 RSCM/ISC quality assessment**

**Compositional analysis**

RSCM was thoroughly mixed in order to get a homogenous product before analysis. The following compositional analysis was carried out:

**Protein content determination**

Protein contents were determined by the Dumas method using a LECO FP-2000 (Leco Corporation, MI, USA) which measures the nitrogen content of the sample. Accurately weighed samples (0.2g±0.001g) were placed in a sample holder and dried using a hot plate at a temperature low enough to prevent the sugar from burning. Dried samples were inserted into the combustion chamber and the instrument displayed the results as nitrogen content. Protein content was calculated as N X 6.25 for samples containing IWP and for samples containing milk proteins.

**Total fat content determination**
The total fat content was determined using the Rose-Gottlieb method. Accurately weighed samples (2.0±0.001g) were delivered into the lower bulb of the extraction flask. The water at 30°C was added to the test portion to obtain a total volume of 10-11ml and the product was completely dispersed. Next, ammonia solution (2ml) was mixed thoroughly with the diluted test portion. The ethanol solution (10ml) was allowed to mix gently with the contents of the flask followed by the addition of two drops of congo red solution. The flask was corked after the addition of diethyl ether (25ml) and was shaked vigorously for one minute with the flask in a horizontal position and the small bulb extending upwards allowing the liquid to flow from the large bulb into the small bulb. Then the light petroleum (25ml) was added and the flask was closed and centrifuged for one to five minutes at 500 to 600/ minute. The supernatant was decanted and the second extraction was carried out similarly.

The fat content, expressed as a percentage by mass, is equal to

\[
\frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100
\]

where,

- \(m_0\) is the mass, in grams, of the test portion
- \(m_1\) is the mass, in grams, of the fat collecting vessel and extracted matter
- \(m_2\) is the mass, in grams, of the prepared fat-collecting vessel
- \(m_3\) is the mass, in grams, of the fat-collecting vessel used in the blank test
- \(m_4\) is the mass, in grams, of the prepared fat-collecting vessel used in the blank test

**Total solids determination**

A petri-dish containing approximately sand (25g), with lid and a stirring rod was placed in the drying oven for at least one hour. The covered dish (with lid) along with a stirring rod was cooled in the desiccator for at least 45 minutes. Dish, lid and rod were weighed to three decimal places. Sand was tilted to one side of the prepared dish and the prepared sample (2g) was placed and hence weighing the dish with lid and stirring rod to the nearest 0.1mg.
Water (5ml) was added to the test portion in the dish and was thoroughly mixed with the stirring rod. The mixture was evenly spread over the bottom of the dish. The stirring rod was left in the mixture with the other end resting on the rim of the dish.

The dish was heated on a boiling water bath with bottom of the dish sufficiently exposed to steam for approx. 30 minutes. The mixture was frequently mixed in the early stages of drying so that it was well aerated and became crumbly.

These dishes along with lid and stirring rod were placed in the drying oven for two hours and were cooled in the desiccator. This was repeated until the difference in mass between two successive weightings was not more than 1mg. The total solids were calculated as:

$$\text{Total solids content} = \frac{(m_2-m_0)}{(m_1-m_0)} \times 100$$

where

$m_0$ – is the mass, in grams of the dish (including sand), lid and stirring rod

$m_1$ – is the mass, in grams of the dish (including sand), lid, stirring rod and test portion

$m_2$ – is the mass, in grams of the dish, lid, stirring rod and dried test portion including sand)

5.2.2.4 Rheological analysis

The rheological properties of RSCM and ISC were measured using a Paar physica rheometer (Modular Compact Rheometer MCR 300, Anton Paar, Austria) using cup and bob geometry as shown in Figure 5.4. Each sample was measured in triplicates to determine the initial apparent viscosity of the finished product allowing standard deviation of 10%. All the samples were measured at 25°C.
Figure 5.4: Paar physica rheometer

**Apparent viscosity:**

The apparent viscosity of RSCM was measured after loading samples for 5 minutes in Paar physica rheometer with geometry as shown in Figure 5.5.

**Apparent viscosity measurements as a function of shear rate:** The measurements were carried out at increasing shear rate (0.1-100/s) and decreasing shear rate (100-0.1/s) and the apparent viscosity was measured. The duration of the measurement was 10 minutes.

**Apparent viscosity measurements as a function of time:** The samples were sheared at constant shear rate at 2.2/s and the apparent viscosity was measured as a function of the shearing time for 10 minutes.
Figure 5.5: Cup and bob geometry (CC27 AND TEZ150) used for measuring rheological properties of RSCM

Yield stress measurement:

Yield stress measurement was carried out using a vane geometry as shown in Figure 5.6. A vane test was carried out by gently introducing the vane spindle into a sample until the vane was fully immersed. The depth and the diameter of the container needed to be at least twice as great as the length and diameter of the vane to minimise any effects caused by the rigid boundaries. The vane was slowly rotated at a constant rotational speed of 0.1 rpm and the torsional moment required maintaining the constant motion of the vane was measured as a function of the time.
**Figure 5.6:** Paar physica rheometer with vane geometry (FL100) for measuring RSCM yield stress

**Shelf-life study:**

The shelf-life study of the finished product (at room temperature) was based on stability of viscosity. Measurements were made daily over one week at laboratory-scale and up to six months for pilot scale trials. The finished product was tested for its viscosity at 2.2 rpm daily up to seven days.

**Water activity**

The term water activity ($a_w$) refers to unbound water in the food system and is significant in studying the microbiological stability of the product. It was measured using a water activity meter (Model CX-2, Aqua lab, Washington, USA). The following measurement method was used to measure the water activity of pilot-scale RSCM.

An instrument was switched on approx. one hour prior to measurement to warm up the instrument and to equilibrate to the temperature of its surroundings. Initially water activity was measured for distilled water chosen as a standard. The cup was only half filled with sample to increase the surface area and to avoid chamber contamination. The half filled cup
was placed in the instrument and the knob was turned to READ position. This caused the sample cup to be pushed up to form a vapour seal with the sensor block. The water activity reading was automatically made and displayed on the screen. Once measurement was made, the knob was moved to LOAD position for measuring next sample.

5.3 RESULTS AND DISCUSSION:

5.3.1 PRODUCT DEVELOPMENT:

Commercial RSCM products were found to have viscosities in the range of 3.2-4.4 Pa.s; the viscosity was different for different brands as shown in Table 5.5.

Table 5.5: Measured viscosity for various commercial brands

<table>
<thead>
<tr>
<th>BRAND</th>
<th>MEASURED VISCOSITY (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home brand</td>
<td>4.4±0.08</td>
</tr>
<tr>
<td>Carnation</td>
<td>4.0±0.05</td>
</tr>
<tr>
<td>Golden</td>
<td>3.7±0.12</td>
</tr>
<tr>
<td>Nestle</td>
<td>3.2±0.11</td>
</tr>
</tbody>
</table>

The average laboratory-scale RSCM viscosity was found 3.3 Pa.s. The laboratory-scale prepared RSCM was considered to be the standard for further formulations, and an attempt was made to achieve similar viscosity using IWP (both Gemtec1100 and Gemtec1160).

5.3.2 LABORATORY-SCALE TRIALS

The standard formulation for RSCM contained 7% protein, 8% fat, 46% sugar and 25% water with 14% minor components (Clarke 1999). In order to compare the functionality of IWP with casein, various levels of replacements of casein were evaluated ranging from 0-100% replacement. All the laboratory-scale trials were carried out using Gemtec1100.
5.3.2.1 Rheological analysis

Direct replacement of casein with IWP in RSCM formulation

Assumptions

SMP used for manufacturing RSCM contain typical levels of major components, that is, 33.6% proteins, 55% carbohydrates, 7.8% minerals, 3.6% moisture and 0.8% fat. The following assumptions were made:

- The mass balance was based on the maintenance of protein content.
- For direct replacement of casein in SMP, it was not possible to separate casein from whey proteins; therefore the whey protein level was maintained by including additional whey powder. The presence of other ingredients was allowed to change.

Figure 5.7 illustrates average laboratory-scale RSCM viscosity (3.3 P) prior to any casein replacement. So it was considered the optimal and desired viscosity for ISC product. Also the increase in replacement level of casein with IWP resulted in increase in viscosity of the finished product. A one-way analysis of variance (ANOVA) with p<0.05 showed insignificant differences in viscosities from 0-25% replacement level of casein.

The initial viscosities of ISC containing IWP ranging from 0-25% replacement level were insignificantly different from RSCM, whereas above 25% replacement resulted in significantly higher viscosities than the control RSCM. This may be attributed to network interactions between the ingredients that resulted in binding more water (refer to section 4.3) and so resulted in higher viscosity of the dispersion. During the mixing process of the ingredients, it was observed that the increase in wheat protein content resulted in the formation of a gel which may also have contributed in the increase of viscosity of the mix. Therefore, the results showed the feasibility of up to 25% replacement of casein with IWP without any significant change in viscosity. It was noted that 50% replacement might be used in further trials by varying the other ingredients such as sugar and water content in the overall
formulation in order to obtain similar viscosity as RSCM. Also, there wasn’t any relationship found between casein to whey protein ratio and viscosity of RSCM.

Figure 5.7: Measured viscosity of RSCM and ISC at laboratory-scale

**Effect of protein reduction on functionality**

**a. Functionality of 50% ISC with protein-water compensation**

The superior functional properties (section 4.4) shown by IWP compared with casein suggested use of a lower protein content than in the standard formulation. Therefore, to compare the functional efficiency of wheat proteins with that of casein in RSCM the overall wheat protein content was decreased. The potential advantage of this behaviour might include greater efficiency by using IWP not only for its lower cost but also from its reduced usage rate.

Figure 5.8 showed that based on a standard formulation of 50% replacement with 7% protein content, it was found that final viscosity of the finished product decreased from 6.44 to 1.9 Pa.s, that is, by 72% when the overall protein content was decreased from 7 to 6%. A further decrease of up to 0.62 Pa.s, that is, viscosity by 91% when protein content was further decreased to 5%. As protein content was compensated for with water it implies that the higher water content can act as a lubricant and hence lower the final viscosity of the finished product.
So decreasing the total protein content from 7% did not result in achieving the laboratory-scale standard RSCM of 3.3 Pa.s.

Figure 5.8: Measured viscosity of 50% ISC with varying protein content

b. Functionality of 50% ISC with protein-sugar compensation

Decreasing the total protein content with a corresponding increase in the sugar content also resulted in achieving the desired viscosity of the finished product. As sugar is known to have a protective effect over proteins i.e. allows only restricted denaturation of proteins during heating (refer to section 2.2), it follows that it helps proteins in maintaining the desired viscosity.

Figure 5.9 showed that based on the standard formulation with 7% protein content, it was found that the final viscosity of the finished product decreased from 6.44 to 3.59 Pa.s, that is, by 44% as the overall protein content was decreased from 7 to 6%. An insignificant decrease in viscosity from 3.59 to 3.42 Pa.s was reported as protein content was further decreased to 5%. Decrease in protein content compensated by sugar implies the protective effect of sugar to maintain the desired viscosity. So incrementally decreasing the protein content from 7% to 5% still resulted in achieving the laboratory-scale standard RSCM viscosity of 3.3 Pa.s.
In summary, a short list of desirable formulations based on the critical attribute “viscosity” has been determined as follows:

1. 25% direct replacement of casein with IWP with overall protein content 7%, termed “25% ISC”
2. 50% replacement of casein with total protein content decreased from 7% to 6% followed by corresponding increase in sugar content, termed “50% ISC with overall protein content 6%, i.e. 50% ISC (6% protein)”
3. 50% replacement of casein with total protein content decreased from 7% to 5% followed by corresponding increase in sugar content, termed as “50% ISC with overall protein content 5%, i.e. 50% ISC (5% protein)”

5.3.2.2 Shelf-life study

Longer shelf-life study of RSCM and ISC was not feasible at laboratory-scale because of the crystallisation of sugar, which ultimately occurred due to the lack of efficient homogenisation achievable at laboratory-scale.
Table 5.6: Percentage decrease in viscosity for short-listed samples during a one week storage

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>INITIAL VISCOSITY (Pa.s)</th>
<th>FINAL VISCOSITY (Pa.s)</th>
<th>% DECREASE IN VISCOSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control RSCM</td>
<td>3.3</td>
<td>2.1</td>
<td>36.0</td>
</tr>
<tr>
<td>25%ISC</td>
<td>3.8</td>
<td>2.5</td>
<td>33.0</td>
</tr>
<tr>
<td>50% ISC (6% protein)</td>
<td>3.5</td>
<td>2.3</td>
<td>35.7</td>
</tr>
<tr>
<td>50% ISC (5% protein)</td>
<td>3.4</td>
<td>0.7</td>
<td>79.5</td>
</tr>
</tbody>
</table>

The fall in viscosity in 50% ISC (5% protein) as shown in Table 5.6 was approximately twice compared with other formulations. So it was concluded that viscosity declined for all the formulations’ comparable to the decline in control RSCM except 50% ISC (5% proteins). The graphs representing this shelf life stability are shown in Appendix I.

5.3.2.3 Short-listing of formulations

A statistical evaluation was undertaken for standard RSCM, 25% ISC and 50% ISC (6% protein) in order to shortlist desired formulations on the basis of viscosity. The above formulations were compared using one-way ANOVA with p<0.05 and they were insignificantly different from each other.

5.3.3 PILOT-SCALE TRIALS

Pilot-scale formulations were manufactured using Gemtec1100 at first and then using Gemtec1160 as soon as it became available; these products were analysed for compositional analysis and rheological properties.
5.3.3.1 Compositional analysis

Table 5.7 showed an interesting observation that protein content was unexpectedly less than 7% (as calculated) for all pilot-scale prepared formulations. This can be attributed to the different batches of raw ingredients used during RSCM manufacturing which are not guaranteed to contain the precise protein content often claimed in the specification sheets. It should have been dealt by using raw ingredients having same batch number.

Table 5.7: Pilot-scale formulations manufactured using Gemtec1100 and Gemtec1160

<table>
<thead>
<tr>
<th>COMPOSITIONAL ANALYSIS</th>
<th>RSCM (CONTROL)</th>
<th>25% ISC</th>
<th>50% ISC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1100</td>
<td>1160</td>
<td>1100</td>
</tr>
<tr>
<td>Total Solids</td>
<td>73.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.6±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.8±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein content</td>
<td>6.0±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.93±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.16±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat content</td>
<td>9.1±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>2.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b</sup> Values followed by the same letter in each column within each set are not significantly different at (p<0.05)

5.3.3.2 Rheological analysis

The freshly prepared Recombined Sweetened Condensed Milk (RSCM) showed a divergence from Newtonian behaviour such that the measured shear rate was not directly proportional to shear stress.
Apparent viscosity

Figure 5.10 showed that the RSCM replicates were found to be different in their initial apparent viscosities. This was likely to be due to the small differences in initial setup of the recombining plant, the effect of processing conditions and differing batches of raw materials (such as SMP, whey protein powder etc.) used for RSCM manufacturing.

Figure 5.11 shows that RSCM, 25% ISC and 50% ISC (6%) prepared using Gemtec1100 were similar (p<0.05) in their initial apparent viscosities. Similarly, 25% ISC and 50% ISC (6% protein) made using Gemtec1160 were similar, but were unexpectedly different from RSCM. These differences were due to higher protein content of Gemtec1160 obtained during its manufacture. Also, differences between formulations manufactured using Gemtec1100 and Gemtec1160 might be due to

1. Differences in the protein content of Gemtec1100 and Gemtec1160
2. Processing variations in the manufacture of these two proteins (Table 5.2)
3. The higher fat content and fat-protein relationship in Gemtec1100

As initial viscosities between RSCM, 25% and 50% ISC (6% protein) made using Gemtec1100 were found to be similar (p<0.05), further analysis of the results such as permanent viscosity, hysteresis loop area and yield stress can indicate any differences in RSCM and ISC structure. However, the same was not achieved using Gemtec1160 but attempts were made to identify the trends.
Figure 5.10: Measured viscosity of various pilot-scale control RSCM trials

Figure 5.11: Measured viscosity versus type of formulation; RSCM ( ), ISC using Gemtec1100 ( ) and Gemtec1160 ( )

**Apparent viscosity as a function of time**

RSCM and ISC products exhibited thixotropic behaviour as apparent viscosity decreased with time and partially recovered its original viscosity after resting. This behaviour was expected due to their weak physical bonds, electrostatic and hydrophobic interactions between different
ingredients. The destruction of these interactions resulted in its loss of viscosity. But given resting time, the structure was partially recovered to regain its original viscosity.

**Apparent viscosity as a function of shear rate**

The apparent viscosity of RSCM was found to decrease with the corresponding increase in the shear rate (Appendix II). The phase of decreasing apparent viscosity can be divided into two broad sections. In the initial phase, when the shear rate was low, the rate of viscosity decrease was greater. With prolonged stirring, the rate of viscosity decrease became slower ultimately attaining constant viscosity. These changes indicate a continuous breakdown of structure or emulsion aggregation at higher speeds resulting in less resistance to flow. These results are in agreement with the previous work of Rha & Pradipasena (1978). Also RSCM showed pseudo plastic behaviour as shown in Appendix III.

Also it was found that RSCM was more shear stable than ISC formulations made using Gemtec1100. This implies that RSCM interactions between ingredients were stronger than interactions in ISC formulations. However, the trend was reversed as both ISC formulations were found to be stronger in interactions than RSCM. The reason is attributed to:

1. Differences in the protein content of Gemtec1100 and Gemtec1160
2. Processing variations in the manufacture of these two proteins (Table 5.2)

**Permanent viscosity**

Figure 5.12 shows the lower permanent viscosity for ISC formulations relative to RSCM made using Gemtec1100. It can be attributed to the weaker interactions formed in ISC formulations; hence, the molecular network is destroyed to a greater extent than in RSCM during mixing. However, Figure 5.13 shows the reverse trend for the permanent viscosity of ISC made using Gemtec1160 compared with RSCM. The reason might be the presence of extra proteins (section 5.1), hence more the protein content the more the build up of structure.
Also the close fat-protein relationship in Gemtec1100 may be important, which is lacking in Gemtec1160.

**Figure 5.13:** Changes in permanent viscosity of RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) made using Gemtec1100 during storage of three months

**Figure 5.14:** Changes in permanent viscosity of RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) made using Gemtec1160 during storage of three months
Also, the permanent viscosities of both RSCM and ISC (Gemtec1100 and Gemtec1160) increased with storage time. It might be attributed that during storage, irreversible changes occur in the shape and size of the casein micelles, probably by aggregation, which increased interactions between ingredients. So it resulted in the increase of the permanent viscosity of the end product. These results are in agreement with research undertaken by Hostettler & Imhof (1953).

Following the same trend, predicted permanent viscosities of ISC (made using Gemtec1100) after six months storage showed an increase of 58%. The corresponding increase in permanent viscosities for 25% ISC and 50% ISC (6% protein) was found to be three times and five times higher than in RSCM. So this implies that inherent structure build up in ISC samples is greater compared to RSCM during storage. The same trend of increased permanent viscosity was shown by ISC made using Gemtec1160. The reason might be that with storage more of the network is being established between protein and non-protein ingredients.

**Hysteresis loop area**

The research found that ISC formulations such as 25%ISC and 50% ISC (6% protein) made using typical type of IWP, that is, Gemtec1100 showed lesser hysteresis loop area than casein (Figure 5.14). That indicates that not only the networking within the ingredients is weaker but also that it takes more time to rebuild its structure. However, the reverse trends were found for ISC made using Gemtec1160 as shown in Figure 5.16. This might be due to more protein denaturation in Gemtec1160 as compared to Gemtec1100 during processing or due to differences in fat-protein interactions (refer to section 5.1).
Figure 5.14: Hysteresis loop area of RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) made using Gemtec1100.

Also, there was insignificant difference in rate of increase of these areas either for RSCM or ISC using Gemtec1100. However, these differences were significant between RSCM and ISC.
using Gemtec1160 (12 times in 25% ISC and around eight times in 50% ISC (6%) as compared to RSCM).

So it appears that ISC made using Gemtec1100 behaves similarly to RSCM made using casein. However the difference in hysteresis loop area resulting from using Gemtec1160 can be compensated for by using less protein in the overall formulation (i.e. by considering 5% extra proteins in Gemtec1160).

**Yield stress measurement**

Fresh RSCM did not show any significant yield stress using vane geometry. The findings are in agreement to Higgs and Norrington (1971). Figure 5.16 and Figure 5.17 showed that comparison of the yield stress measurement for RSCM, 25% ISC and 50% (6% protein) ISC made using Gemtec1100 and Gemtec1160. It was found that 50% (6% protein) ISC followed the parallel trend to RSCM, however, 25% ISC is significantly different from other formulations.

![Figure 5.16: Yield stress measurement for RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) made using Gemtec1100 after storage of 3 months](image-url)
Also it was noted that during storage time, yield stress started to build up in RSCM but both 25% ISC and 50% ISC (6% protein) showed no particular trends. This difference in behaviours is not fully understood.

### 5.3.3.3 Shelf-life study

Figure 5.18 shows that apparent viscosity increased during storage time possibly as a result of slow irreversible changes that occur in the shape and size of casein micelles. These micelles may orient themselves in such a way that they form a loose network enclosing some of the dispersion medium so producing an increase in the initial viscosity. Research found that the same pattern is observed in ISC formulations.
5.3.3.4 Water activity

The water activity was significantly different (p<0.05) for RSCM, 25%ISC and 50%ISC (6% protein) as shown in Table 5.8. This can be attributed to the higher water holding capacity of wheat proteins, resulting in more complex structure during its interactions between different ingredients within the system, when compared with casein (refer to section 4.4). It was also observed that during mixing, the more the wheat protein content the higher the tendency of the mix to gel. It may result in imparting higher viscosity to the solution in the presence of wheat proteins.
Table 5.8: Water activity of RSCM and ISC formulations

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>% WHEAT PROTEIN CONTENT</th>
<th>WATER ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSCM</td>
<td>0</td>
<td>0.847</td>
</tr>
<tr>
<td>25% ISC</td>
<td>20</td>
<td>0.844</td>
</tr>
<tr>
<td>50% ISC (6% protein)</td>
<td>35</td>
<td>0.822</td>
</tr>
</tbody>
</table>

However, both ISC formulations were within the recommended water activity range for RSCM (<0.85).

5.3.3.5 Flow properties of RSCM / ISC

Applying the power law model, it was found that $n$ was less than 1 so RSCM has a shear thinning nature with $r^2$ values ranging from 0.9994 to 0.9998 which is in agreement with the previous work.

Flow behaviour index increased from forward (0.1-100/s) to backward (100-0.1/s) shear rate for both RSCM and ISC. This is because of the material being already sufficiently sheared. As the shear rate starts to decrease, it flows easily. However, the consistency index decreased from forward (0.1-100/s) to backward (100-0.1/s) shear rate. It may be supported by the fact that the structure of the materials was fully destroyed and caused irregular structure which decreased its consistency.

Also the rheological behaviour study of RSCM and ISC was carried out while in storage. It was found that the flow behaviour index tends to decrease and consistency index tends to increase with a larger number of possible interactions occurring between proteins and other ingredients. These findings are in agreement with a study carried out by Alvarez, Melcon and Zapico (1991).

5.4 CONCLUSIONS
The following conclusions were made about RSCM made from milk proteins, and ISC formulations made using both milk proteins and IWP:

**Functional properties comparison between RSCM and ISC made using Gemtec1100:**
- Initial permanent viscosity of ISC was lower than RSCM. However, it increased during storage for both RSCM and ISC
- ISC showed more hysteresis loop area than RSCM; it also increased with storage
- Fresh RSCM and ISC showed no yield stress; this continued to build up during storage
- No trend was noticed in ISC formulation.

**Functional properties comparison between RSCM and ISC made using Gemtec1160:**
- Initial permanent viscosity of ISC was higher than for RSCM, and increased with storage time
- ISC showed smaller hysteresis loop area than RSCM; this increased during storage
- Yield Stress for 50% ISC (6% protein) was comparable to RSCM, however, no trend was noticed in 25%ISC

**The above conclusions relating to RSCM and ISC can be summarised thus:**
- IWP can be used to partially replace casein while maintaining all the functional properties of the end product within acceptable limits.
- No drastic changes to functional properties were found. Two feasible replacements may be:
  1. Direct replacement of up to 25% of casein at 7% protein content, or
  2. 50% ISC (6% protein) resulted in a desirable product
- Emulsifying properties were comparable for RSCM and ISC with Gemtec1100.
- Rheologically both RSCM and ISC were found to be similar so the functional properties of casein and IWP were also comparable
• Cost savings of around 28% can be attained by using IWP in an Imitation Sweetened Creamer compared with all dairy RSCM
CHAPTER 6: SPRAY-DRIED EMULSIONS

This chapter describes the laboratory-scale and pilot-scale production trials of spray-dried emulsions developed into different dairy-type powders. These were Recombined Full Cream Milk Powder equivalent and Recombined High Fat Powder equivalent each called “Recombined Creamer Powder” and the coffee creamer equivalent “Imitation Creamer”. The aim of these trials was to assess the functional properties of IWP in these powdered emulsion products and hence the suitability of IWP for the production of these dairy-type products. However, it should be noted that powders produced at pilot-scale may have different physical characteristics to those made in a full-scale plant due to the influence of plant and equipment.

6.1 INTRODUCTION AND LITERATURE REVIEW

Many dairy products are manufactured based on Full Cream Milk Powder (FCMP), High Fat Powder (HFP) or Coffee Creamer (CC). FCMP, also known as whole milk powder (WMP), is manufactured typically with a fat content of 28% and protein content of 24%. HFP contains fat at a content greater than 70% and protein content typically about 2-3%. However, dairy fat and protein is expensive so researchers have sought fat and protein alternatives in order to make cheaper products.

“Imitation creamer” is the term used for dairy-type powders in which non-dairy protein is used as the main fat emulsifier. It may be made from cheaper sources of fat and replacement of both fat and proteins. For example, whey protein concentrates, sourced from acid whey, were used to replace caseinates in coffee whiteners (Gruetzmatcher and Bardley 1991).

Manufacturing process

The process of manufacturing spray-dried emulsion products consist of the following steps (see Figure 6.1):

- Preparation of the initial mix by reconstituting dry ingredients with warm water followed by the addition of melted fat. Heat treatment is applied to improve
microbiological, physical and chemical properties of milk products. Typical conditions are 85-115°C for 2-3 minutes (Early 1992), 75-120°C for several seconds (Webby 1994)

- Initial mix is then evaporated under vacuum to concentrate solids up to 48-50% solids (Robinson 1994). Typical evaporation temperatures range from 45 to 75°C (King, Sanderson & Woodhams 1974). The minimum temperature is limited by the capacity of vacuum producing equipment.

- Homogenisation is performed to reduce the fat globule size, establish a stable emulsion and so reduce the tendency for fat separation from the aqueous phase

- Two-stage drying using a spray drying chamber followed by a fluidised bed is applied to produce an agglomerated, powdered product. The concentrate is preheated to 70-75°C before spraying through a fine high pressure nozzle into the drying chamber. The inlet and outlet air temperatures may range from 150-200°C (Early 1992) and 65-98°C (Masters 1991). The moisture content of the powdered product obtained is typically 5-6% (Pisecky 1980).

**Figure 6.1:** Manufacturing process for dairy-type powders

Preparation of initial mix by mixing dry ingredients, fat in warm water

↓

Homogenisation

↓

Evaporation

↓

Drying

↓

Packaging
**Heat Treatment**

Heat treatment affects milk components as follows:

- Denaturation of whey proteins and subsequent association with casein micelles
- Inactivation of enzymes
- pH decrease due to production of organic acids from lactose, precipitation of phosphates and hydrolysis of casein phosphate

Heat treatment affects the milk powder functionality:

- Severe heat treatment increases the insolubility index (Kudo, Hols & Van Mil 1990).
- The best wettability was achieved by lowest heat treatment temperatures (Zbikowski, Zbikowska & Ziajka 1993).
- Baldwin & Ackland (1991) reported the effect on insolubility index due to holding time and temperature.

**Evaporation**

Evaporation affects the milk components as follows:

- Concentration of solids
- Increase in casein micelle size
- Decrease in pH
- Disruption of fat globules
- Adsorption of casein micelles on to fat globules

During evaporation, the increase in solids increases the viscosity of the concentrate (King, Sanderson 1970; de Vilder et al. 1979). However this increase of concentrate viscosity is also affected by milk composition, preheating conditions (Baucke & Sanderson 1970) and concentrate temperature and holding time (Beeby 1966). Bloore & Bloag (1982) reported the effect of concentrate viscosity on the degree of atomisation of the concentrate and the resultant particle size distribution of powder obtained. The increase in concentrate viscosity
increased the overall particle size and bulk density, decreased the solubility but no consistent change in moisture content was observed.

**Homogenisation**

The milk concentrates containing fat are homogenised prior to drying. This establishes the stable emulsion and reduces the free fat content of the finished product, which can otherwise be detrimental to finished product properties including solubility (Robinson 1994). The type of homogenisation affects functionality; one-stage homogenisation is sufficient to emulsify effectively gives a higher insolubility index (de Vilder, Martens & Naudts 1979) whereas in two-stage homogenisation, the second stage may break up fat droplet clusters formed during first stage as a result of proteins adsorbed onto the interface and achieve the desired emulsification without adversely affecting the solubility index.

**Drying**

Milk powders containing fat are usually manufactured using two-stage drying. The first drying stage results in powder with 2-3% higher moisture content than required in the final powder. This is then removed using a second stage fluidised bed dryer, which also achieves agglomeration. Spray-drying involves:

- atomisation into a spray of droplets leading to large surface area droplets for evaporation
- evaporation from droplets in a hot air stream
- agglomeration, the aggregation of fine particles to material units of larger sizes (Schubert 1981).

Inlet and outlet temperature affects the powder functionality; increase in inlet temperature may lower the moisture content of the powder but increase heat damage to the product (Bloore & Boag 1982). Also, increased temperatures may increase the “ballooning” of
powdered particles, increasing the mean particle size but reducing bulk density (Amundson 1960).

A specific and important storage quality parameter for milk powders is stability. It may be influenced by factors including manufacturing conditions, powder composition, storage conditions, raw ingredient quality and microbiological contamination of powder.

Dairy-type, powdered emulsion products formulated with IWP to wholly or partially replace the casein or sodium caseinate of milk powders may be susceptible physically or chemically to the effects of processing in ways previously observed in the production of milk powders. Such observations will be reviewed and may be directly relevant to similar products formulated with IWP.

6.1.1 RECOMBINED FULL CREAM MILK POWDER

In 2004-05, Australia exported 150 thousand tonnes of Full Cream Milk Powder (FCMP) (Dairy Australia 2005). The main uses of this powder are:

- Reconstitution in water to give the equivalent of liquid milk
- Direct addition to hot tea or coffee as a whitener
- As an ingredient in foods, for example, soups, sauces, bakery, confectionery, dairy beverages, ice cream etc
- As a milk supply for the catering industry where it is difficult to manage large amounts of fresh milk

Traditionally, FCMP is manufactured from fresh whole milk. The composition of FCMP made from fresh milk or recombined is given in Table 6.1.
Table 6.1: Composition of FCMP (Walstra & Jenness, 1984)

<table>
<thead>
<tr>
<th>COMPOSITION</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>36.0-38.0</td>
</tr>
<tr>
<td>Fat</td>
<td>25.0-28.0</td>
</tr>
<tr>
<td>Protein</td>
<td>25.0-27.0</td>
</tr>
<tr>
<td>Ash</td>
<td>6.0-7.0</td>
</tr>
<tr>
<td>Moisture</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.1-1.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.9-1.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.7-0.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.7-0.7</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.3-0.4</td>
</tr>
</tbody>
</table>

FCMP may also be obtained by rehydrating milk powder, NaCAS, whey powder and vegetable fat followed by concentration and spray drying (Walstra & Jenness, 1984). It needs to be a soluble powder containing 25-29% fat with a moisture content of 2.7-3.0% (Pisecky, 1990). In this form the product is referred to as a recombined product.

Recombined full cream milk powder (RFCMP) is reserved for product derived entirely from dairy ingredients. For this thesis the term recombined creamer powder (RCP) is used to refer to formulations of this type where milk protein is wholly or partially replaced by IWP and the milk fat wholly or partially replaced by non-dairy fat.

6.1.2 RECOMBINED HIGH FAT POWDER

Foster, Bronlund, Paterson (2005) stated that HFPs are susceptible to sticking and caking problems during processing and storage due to the high fat content. Peleg (1977) suggested that viscous liquid bridges formed within fat globules due to high temperature may have
caused sticking followed by resolidifying on reducing the temperature again. At ambient temperature, 77% of the total milk fat was in a fluid-like state and was able to flow over the particles and resulted in caking when the temperature was reduced. In other studies, presence of lactose was suggested to be the reason for caking of powders with conditions favouring its crystallisation. Also, it was proposed that a loss of ordered structure in which proteins, minerals and fat were excluded and hence resulted in increase in free fat of the stored powder. So it can be concluded that not only crystallisation of lactose but also the increase in free fat content resulted in caking of powders. Foster, Bronlund & Paterson (2005) found that caking during storage was related to the amount of crystallised surface fat present on the surface of the powder but no measurable increase in cohesiveness was reported. Fitzpatrick et al.(2004) demonstrated that free fat and particle size of high fat powders were related to the protein content and solid fat content of the milk and were not affected by the lactose content. The term Recombined High Fat Powder (RHFP) is reserved for product derived entirely from milk.

6.1.3 COFFEE CREAMER

Coffee is frequently consumed with coffee creamer or lightener (Pordy 1994). Since the 1950s there have been alternatives available for creamers which typically consist of NaCAS, vegetable fat, stabilizers, sweeteners, emulsifiers, flavour and colour (Ellinger 1972). NaCAS is commonly used in coffee whiteners to serve as an emulsifier, to impart body and whitening ability and to contribute dairy like flavour (Knightly 1969; Abdullah et al 1993). Coffee creamers may be manufactured by rehydrating dry ingredients and mixing with melted fat followed by concentration and drying. It should be a soluble powder with 35% fat and protein content ranging from 1.8-4%. A composition table for typical commercial coffee creamer is shown in Table 6.2.
Table 6.2: Typical composition of coffee creamer

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Syrup</td>
<td>55.4</td>
</tr>
<tr>
<td>Palm Oil</td>
<td>34.0</td>
</tr>
<tr>
<td>Sodium Caseinate</td>
<td>3.5</td>
</tr>
<tr>
<td>Mono &amp; diglycerides</td>
<td>1.3</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.0</td>
</tr>
<tr>
<td>Dipotassium orthophosphate</td>
<td>2.1</td>
</tr>
<tr>
<td>Sodium stearoyl lactylate</td>
<td>0.1</td>
</tr>
</tbody>
</table>

An important feature related to stability of coffee creamers is the tendency to flocculate once poured into the hot coffee. This defect is called feathering and is defined as the coagulation of creamer protein in coffee, which in turn may decrease the appeal of the coffee. Various reasons have been suggested for feathering in coffee such as changes in the structure of milk proteins brought about by processing, acid content and high temperature of coffee (Kelly et al. 1999).

Literature also reported that cream stability towards heat was greatly altered by the degree of preheating and by homogenisation pressure. Homogenisation of the cream resulted in the lowering of the heat coagulation point unlike in evaporated milk in which homogenisation pressure does not affect the stability of the cream. Geyer & Kessler (1989) found the optimum combination of processing conditions to improve the cream stability. They also reported the effect of individual milk components on feathering, both increase in fat content and protein level resulted in more flocculation. Also, whey proteins in their native state were more liable to cause flocculation than the casein fraction of milk proteins.

The literature provides evidence for the following regarding the stability of coffee creamers:
• Increasing homogenisation pressure destabilises cream towards heat and increases fat clumping
• Preheating at lower temperatures such as 62.8°C/30 minutes destabilises product towards heat than at higher temperatures
• Use of hard water in coffee making results in feathering
• Increases in viscosity of creamer results in increasing feathering and fat clumping
• Addition of sodium citrate prior to homogenisation stabilizes the product

Previous study carried out by Teehan and Kelly (1996) studied the effect of various process parameters, effect of physical powder characterisation on the stability of milk powder when added to coffee based beverages. The research work found that the physical characteristics of the powder have a major bearing on the coffee stability.

Golde and Schmidt (2005) studied the functionality of four liquid coffee creamers two of which were made up of plant proteins (soy protein isolate and wheat protein isolate) and others from dairy proteins (NaCAS and whey protein concentrate). It was reported that the viscosity of the liquid emulsions made with plant proteins was significantly higher than those made with dairy proteins. Also the addition of NaCAS decreased the apparent viscosity of the plant proteins. However feathering was observed in coffee creamers made with soy protein isolate which supported the results from Decker (1999). It might be caused by the larger calcium and magnesium contents (313 ppm) used in their study. Kelly, Oldfield & Kennedy (1999) and Golde and Schmidt (2005) found that spray-dried coffee creamers made with soluble wheat proteins were exceptionally stable. Few researchers have reported incorporating alternative sources of protein in spray-dried coffee creamer. Acid whey protein was successfully used to replace NaCAS in spray dried coffee whiteners (Gruetzmacher & Bradley 1991). Kelly et.al.(1999) replaced NaCAS with several alternatives; solubilized wheat protein manufactured by Amlyum, Belgium was found to be exceptionally stable. The list of methods was reported in literature to quantify the amount of feathering in coffee
including visual feathering inspection, both mass and volume based coffee stability, Babcock method and ultraviolet spectroscopy.

The term Coffee Creamer (CC) is reserved for product derived entirely from dairy ingredients. For this work the term Imitation Creamer (IC) is used to refer to formulations of this type where milk protein is wholly or partially replaced by IWP and milk fat is wholly or partially replaced by non-dairy fat.

6.2 MATERIALS AND METHODS

6.2.1 MATERIALS

Additionally, palm oil was bought from a local supplier “Tim & Terry” produce, Footscray, Victoria). A Niro FSD4 at Food Science Australia, Werribee was used for spray drying (inlet temperature 190°C and outlet temperature 80°C). The chemical reagent such as petroleum ether mentioned in this section was analytical grade and purchased from Sigma-Aldrich (Castle Hill, NSW) unless otherwise stated.

6.2.2 METHODS

The trials were carried out at the pilot-scale (Figure 6.1) in a manner similar to that for RSCM (section 5.2.2.2). All the chemicals and food ingredients used in these trials were of food grade quality.

6.2.2.1 Manufacturing process

Various dairy-type powders (RFCMP, RHFP and CC) were produced at pilot-scale as in Figure 6.1:

- Initial mix was prepared by dispersing dry ingredients including proteins, lactose in warm water
- Melted fat and non-protein emulsifiers added in the mix
• The above mix was evaporated with pilot-scale falling film evaporator to total solids contents about 45%
• The mix was homogenised using a two-stage homogeniser at 170 and 85 bar pressures respectively
• It was then dried in a Niro FSD4 spray-drier with inlet air temperature of 190°C and outlet air temperature of 90°C.

6.2.2.2 Compositional analysis
Protein, fat, moisture and ash content analysis were performed as described in section 5.2.

6.2.2.3 Free fat determination
An accurately weighed sample (10g) was placed into a 250 ml erlenmeyer flask and 50ml of petroleum ether was added in the flask. The stoppered flask was agitated using a shaking device at speed 2.5 rpm for 15 minutes. The sample was allowed to settle for 2-3 minutes before pouring the solvent through the fluted filter paper into a pre-weighed 250 ml round bottomed flask. The flask was swirled slightly to suspend the remaining powder and was passed through filter paper. Solvent was evaporated from the sample using a rotary evaporator attached with water bath at 60°C. The dried sample was placed in an oven maintained at 102.3°C for one hour followed by cooling in desiccator for 90 minutes. The flask and residue was weighed and percentage free fat was calculated as:

\[
\% \text{ Free fat of powder} = \left( \frac{\text{weight of residue}}{\text{weight of powder}} \right) \times 100
\]

6.2.2.4 Particle size determination
A powder sample (25g) was dispersed in distilled water to make up volume 200ml such as RHFP and CC and the droplet size was measured using the laser diffraction technique (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). However,
powdered samples can also be measured for particle size in iso-butanol such as in case of RFCMP. All measurements were run in duplicates. The refractive index of water was 1.330 with absorption of 0.1. The emulsion was transferred into instrument’s dispersion tank containing distilled water and its size was measured. Particle size distribution was expressed both as median diameter D and volume percentage. Particle size < 10µm were considered as small particles and those 10-45µm were considered as large particles (Lineback & Rasper 1988; Hermmasson & Svegmark 1996).

6.2.2.5 Product stability
A powder sample (25g) was reconstituted with warm distilled water (175g) using an ultra-turrax homogeniser. The solution (12.5% solids) was mixed for five minutes and was checked for visual inspection of any emulsion breakdown or synersis.

6.2.2.6 Emulsion capacity and stability
Refer to section 4.3.2.3

6.3 RECOMBINED FULL CREAM MILK POWDER AND RECOMBINED CREAMER POWDER
Recombined Full Cream Milk Powder (RFCMP) and Recombined Creamer Powders (RCP) emulsions were prepared at laboratory-scale using two types of proteins namely caseins (in form of SMP) and IWP. The two casein replacement levels used were 50% and 100% with both using dairy and non-dairy fats.
6.3.1 EXPERIMENTAL DESIGN

Table 6.3 shows the six formulations (A to F) of RFCMP and RCP manufactured at pilot-scale. Set 1 refers to the formulations made using AMF and Set 2 refers to those made with palm oil.

6.3.2 RESULTS AND DISCUSSION

6.3.2.1 Pilot-scale trials

**Compositional analyses of RFCMP and RCP powders:** The compositional analysis of RFCMP and RCP on dry solid basis is shown in Table 6.4. The calculated composition of the RFCMP and RCP was made and their averages are given in the columns labelled “calc”. These calculations assume no loss of components during processing.

**Moisture content determination:**

Figure 6.2 shows that the moisture content of powders A to F (see table 6.3) is insignificantly different (p<0.05) from each other. Also, the moisture contents of all powders were within the recommended limits (<3%) (Jensen 1988). However, during experimentation, it was noted that the spray drying time for powders containing IWP took 1.5 times longer than for powders prepared using milk proteins. It might be attributed to the higher water binding capacity of IWP which resulted in slower removal of water during spray-drying. And its implications could be the higher cost incurred by the manufacturers.
Protein content determination:

Protein content for all six formulations was in the range 22.9-24.2; however, were not significantly different from the calculated protein content of 24%.

Fat content determination

The fat content of all the powders varied from 28.6 to 30.3%, however, the calculated fat content for these powders were 28%. This may be attributed to the different batches of raw materials used for each trial.

Ash content determination

The ash content varied from 3.90 to 4.70 for all the samples as shown in Table 6.4
Table 6.3: Batches of RFCMP and RCP at pilot-scale

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>SET 1</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL (A)</td>
<td>50% CASEIN REPLACEMENT (B)</td>
</tr>
<tr>
<td>SMP</td>
<td>16.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Whey powder</td>
<td>N.A.</td>
<td>4.1</td>
</tr>
<tr>
<td>Gemtec1160</td>
<td>N.A.</td>
<td>2.4</td>
</tr>
<tr>
<td>Lactose</td>
<td>N.A.</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Water</td>
<td>27.0</td>
<td>37.0</td>
</tr>
<tr>
<td>AMF</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Palm oil</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Total solids</td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td>% Solids before drying</td>
<td>45.2</td>
<td>37.5</td>
</tr>
</tbody>
</table>
### Table 6.4: Compositional analysis of RFCMP and RCP powders

<table>
<thead>
<tr>
<th>POWDER ANALYSIS</th>
<th>CALCULATED VALUE (%)</th>
<th>SET 1</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL (A)</td>
<td>50% CASEIN REPLACEMENT (B)</td>
<td>100% CASEIN REPLACEMENT (C)</td>
</tr>
<tr>
<td>MOISTURE</td>
<td>N.A.</td>
<td>2.00</td>
<td>2.11</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>24</td>
<td>22.9</td>
<td>23.8</td>
</tr>
<tr>
<td>TOTAL FAT</td>
<td>28</td>
<td>28.9</td>
<td>28.8</td>
</tr>
<tr>
<td>FREE FAT</td>
<td>&lt;3%</td>
<td>0.96</td>
<td>2.85</td>
</tr>
<tr>
<td>ASH</td>
<td>N.A.</td>
<td>4.70</td>
<td>4.19</td>
</tr>
</tbody>
</table>
**Free fat content determination**

The free fat content for powders A, B and C (Table 6.4) containing AMF were found to be lowest in powder A (0.96%) followed by C (2.17%) and B (2.85%) as shown in Figure 6.3. However, powder E (2.49%) and F (2.50%) containing palm oil were insignificantly different (p<0.05) from each other, whereas, significantly different from powder D (1.05%) in set 2. It showed that full cream milk powder made using 100% casein such as powder A and D can emulsify more fat than 100% IWP such as powders C and F. Hereby, the presence of free fat content is lower in powders A and D compared with powders C and F. It may be attributed to the difference in functionality of proteins in the presence of other ingredients within this food system. However, all powders were within recommended limits (<3%) of free fat content.

![Figure 6.3: Free fat content versus type of powder](image)

**Particle size determination**

Particle size distribution of powders in Set 1 and 2 exhibited a bimodal distribution. The median particle size of powders is given in Table 6.5. The median particle size, D (v, 0.5) was 139.8, 175 and 141.7µm for powder A (control), powder B (50% replacement of casein) and
powder C (100% replacement of casein) in set 1 respectively. It was found that these samples have a significant effect on the volume median diameter of the powders. Powder B has 50% volume of the particles less than 175.8µm and was significantly different compared to powder A (139.8 µm) and C (141.7 µm).

**Table 6.5:** Particle size distribution for RFCMP and RCP powders

<table>
<thead>
<tr>
<th>Powders</th>
<th>VMD d (0.5)(a) µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1 b</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>139.8(^{a*})</td>
</tr>
<tr>
<td>B</td>
<td>175.8(^{b})</td>
</tr>
<tr>
<td>C</td>
<td>141.7(^{a})</td>
</tr>
<tr>
<td><strong>SET 2 c</strong></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>117.0(^{c})</td>
</tr>
<tr>
<td>E</td>
<td>124.9(^{c})</td>
</tr>
<tr>
<td>F</td>
<td>137.7(^{c})</td>
</tr>
</tbody>
</table>

\(^{a}\) Value median diameter with 50% of particle size distribution above the values and 50% below

\(*\) Values followed by the same letter in each column are not significantly different at \(p< 0.05\)

b Powders A, B, C were made with 0%, 50% and 100% replacement of casein respectively
c Powders D, E, F were made with 0%, 50% and 100% replacement of casein respectively using palm oil

In set 1, powder C (50% replacement of casein) has the highest percentage of small particles (0.98%) and powder A (control) has the lowest portion of small particles which was 0.16% (Figure 6.4). On the other hand, powder C has the highest percentage of large particles (13.19%) followed by powder B (9.28%) and powder A (5.62%) as shown in Table 6.6.

Set 2 samples didn’t show any significant effect on the volume median diameter (Figure 6.5 and Table 6.6). The volume percentage of small particles in Set 2 samples were 1.40, 1.74 and 1.42%. However, it was 117, 124.9 and 137.7µm for the control, 50% replacement of casein
and 100% replacement of casein along with full replacement of AMF with palm oil. So particle size determination being a measure of emulsification efficiency showed that powder A is comparable with powder C and also powder D, E, F are not significantly different to each other.

Figure 6.4: Particle size distribution of RFCMP and RCP powders
(I) Set 1 Powder A ( ), B ( ) and C ( )
(II) Set 2 Powder E ( ), F ( ) and G ( )
Table 6.6: Volume percentage of RFCMP and RCP powders

<table>
<thead>
<tr>
<th>Powders</th>
<th>&lt;10 µm (%)</th>
<th>10-45 µm (%)</th>
<th>&gt;45 µm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.16 c*</td>
<td>5.62 a</td>
<td>94.22 a</td>
</tr>
<tr>
<td>B</td>
<td>0.86 b</td>
<td>9.28 a</td>
<td>89.86 a</td>
</tr>
<tr>
<td>C</td>
<td>0.98 b</td>
<td>13.19 b</td>
<td>85.83 b</td>
</tr>
<tr>
<td>SET 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.40 c</td>
<td>16.54 b</td>
<td>82.06 b</td>
</tr>
<tr>
<td>E</td>
<td>1.74 d</td>
<td>17.19 b</td>
<td>81.07 b</td>
</tr>
<tr>
<td>F</td>
<td>1.42 c</td>
<td>13.99 b</td>
<td>84.59 b</td>
</tr>
</tbody>
</table>

* Values followed by the same letter in each column within each set are not significantly different at (p<0.05)

*a Powders A, B, C are made with 0%, 50% and 100% replacement of casein respectively

*b Powders D, E, F are made with 0%, 50% and 100% replacement of casein respectively using palm oil

Product stability

All the reconstituted emulsions formed from spray dried powders were found to be stable at six hours after preparation.

6.3.3 CONCLUSIONS

The RFCMP and RCP powders prepared using casein and IWP respectively were found to behave similarly with regards to their emulsifying properties. The particle size distribution, an indicator of emulsification, was insignificantly different in terms of volume mean diameter of powders made using casein and IWP. However, it was significantly different for powder B, which is 50% casein and 50% IWP in Set 1. This is attributed to their likely interactions between casein and IWP, the mechanism of which is not fully understood. However, the compositional analysis of these powders such as moisture, protein, fat and ash content were also found similar.
On the other hand, Set 2 did not show significant differences in emulsifying properties and compositional analysis for powders, which additionally include the replacement of milk fat with palm oil.

RCP powders made using IWP and palm oil would result in cost savings of up to 58% as compared to original RFCMP.

6.4 RECOMBINED HIGH FAT POWDER AND RECOMBINED CREAMER POWDER

Recombined High Fat Powder (RHFP) and Recombined Creamer Powder (RCP) emulsions were made with two types of proteins namely sodium caseinate and IWP with two levels of protein contents 3.5 and 2% using two fat contents 70 and 80%.

6.4.1 EXPERIMENTAL DESIGN

Table 6.7 and Table 6.8 list the batches of trials conducted at both laboratory-and pilot-scale.
Table 6.7: Batches of RHFP and RCP emulsions at laboratory-scale

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>SET 1</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCAS</td>
<td>SET 1</td>
<td></td>
</tr>
<tr>
<td>A (2.2% PROTEIN AND 70% AMF)</td>
<td>NaCAS</td>
<td>2.2</td>
</tr>
<tr>
<td>B (2.2% PROTEIN AND 70% AMF)</td>
<td>100% IWP</td>
<td>N.A.</td>
</tr>
<tr>
<td>C (2.2% PROTEIN AND 80% AMF)</td>
<td>NaCAS</td>
<td>2.2</td>
</tr>
<tr>
<td>D (2.2% PROTEIN AND 80% AMF)</td>
<td>100% IWP</td>
<td>N.A.</td>
</tr>
<tr>
<td>NaCAS</td>
<td>3.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>Gemtec1160</td>
<td>2.2</td>
<td>N.A.</td>
</tr>
<tr>
<td>17DE Maltodextrin</td>
<td>27.8</td>
<td>27.8</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Water</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>AMF</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Total solids</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>% Solids before drying</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>
### 6.4.2.2 PILOT SCALE TRIALS

Table 6.8: Batches of RHFP and RCP powders at pilot-scale

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>SET 1</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% IWP (2.2% PROTEIN AND 70% AMF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemtec1160</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>17 DE Maltodextrin</td>
<td>6.6</td>
<td>4.1</td>
</tr>
<tr>
<td><strong>Total solids before fat</strong></td>
<td><strong>7.5</strong></td>
<td><strong>5.0</strong></td>
</tr>
<tr>
<td>Water</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>AMF</td>
<td>17.5</td>
<td>20</td>
</tr>
<tr>
<td>Hydrogenated coconut oil</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td><strong>Total solids</strong></td>
<td><strong>25</strong></td>
<td><strong>25</strong></td>
</tr>
<tr>
<td>% Solids before drying</td>
<td>40.3</td>
<td>40.3</td>
</tr>
</tbody>
</table>
Table 6.9: Compositional analysis of RHFP and RCP powders

<table>
<thead>
<tr>
<th>POWDER ANALYSIS</th>
<th>CALCULATED VALUE (%)</th>
<th>SET 1</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% IWP (2.2% PROTEIN AND 70% AMF) A</td>
<td>100% IWP (2.2% PROTEIN AND 80% AMF) B</td>
<td>100% IWP (2.2% PROTEIN AND 70% COCONUT OIL) C</td>
</tr>
<tr>
<td>MOISTURE</td>
<td>2</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>3.2</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td>TOTAL FAT</td>
<td>70 &amp; 80</td>
<td>70.4</td>
<td>79.5</td>
</tr>
<tr>
<td>FREE FAT</td>
<td>&lt;7%</td>
<td>3.89</td>
<td>3.84</td>
</tr>
<tr>
<td>ASH</td>
<td>N.A.</td>
<td>0.17</td>
<td>0.13</td>
</tr>
</tbody>
</table>
6.4.2 RESULTS AND DISCUSSION

Firstly, the particle size distribution of RHFP and RCP formulations were conducted at laboratory-scale in order to study the emulsifying capacity and stability of these emulsions. Only the short-listed formulations were made at the pilot-scale and were checked for their compositional analysis, and emulsifying properties.

6.4.2.1 LABORATORY-SCALE TRIALS

Particle size determination

Set 1 particle size distribution of laboratory-scale emulsions was found to have a significant effect on the volume median diameter as shown in Figure 6.6 and Table 6.10. Emulsion D has 50% volume of the particles less than 7.63 µm and was significantly different compared with emulsion A (1.29%), B (1.15%) and C (1.22%). It means that the emulsion D containing 2.2% IWP and 80% fat have median volume significantly higher than emulsions containing 2.2% protein and 70% fat (emulsion A, B) and 2.2% casein and 80% fat (emulsion C).

![Particle Size Distribution](image)

**Figure 6.5:** Particle size distribution of laboratory-scale RHFP and RCP emulsions

(I) Emulsions A & B: 2.2% protein content and 70% AMF (—) caseinates and (−) IWP

(II) Emulsions C & D: 2.2% protein content and 80% AMF (−) IWP and (—) caseinates
Set 2 samples did not show any significant effect on the volume median diameter except for emulsion H as shown in Figure 6.7 and Table 6.11. The significantly larger volume median diameter of both emulsion D and H were previously reported to be due to the presence of the larger particles in Gemtec1160 powder itself and had nothing to do with its efficiency of forming emulsion (Xi 2005). The volume percentage of small particles in Set 2 samples was 100, 97.56, 100 and 77.31% respectively. Also emulsions with protein content 2.2% and 3.5% NaCAS with 70% fat were insignificantly different from each other. That implies that 2.2% NaCAS is sufficient to provide the required emulsification. However, IWP provided lesser emulsification at 2.2% protein content but comparable at 3.5% protein content. So to have similar emulsifying properties from both casein and IWP, the required protein contents are 2.2% and 3.5% respectively. Also the emulsions tabulated in Table 6.9 were the only ones short listed for pilot-scale production.
Table 6.10: Particle size distribution for RHFP and RCP emulsions

<table>
<thead>
<tr>
<th>Powders</th>
<th>VMD d (0.5) µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1b</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>1.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>1.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>7.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>SET 2c</strong></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>1.98&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>G</td>
<td>1.99&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>H</td>
<td>5.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Value median diameter with 50% of particle size distribution above the values and 50% below

* Values followed by the same letter in each column are not significantly different (p<0.05) with standard deviation is up to 10% for all the samples

b Emulsions A, B are made with 0%, 100% replacement of casein respectively containing 70% AMF and powder C, D with 0 and 100% replacement of casein containing 80% AMF, with total protein content of 2.2%

c Emulsions E, F are made with 0%, 100% replacement of casein respectively containing 70% AMF and G, H with 0 and 100% replacement of casein containing 80% AMF, with total protein content of 3.5%
Table 6.11: Volume percentage of RHFP and RCP emulsions

<table>
<thead>
<tr>
<th>Powders</th>
<th>&lt;10 μm (%)</th>
<th>10-45 μm (%)</th>
<th>&gt;45 μm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET 1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>99.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>65.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SET 2b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>97.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>H</td>
<td>77.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values followed by the same letter in each column within each set are not significantly different (p<0.05)

a Emulsions A, B are made with 0%, 100% replacement of casein respectively containing 70% AMF and emulsions C, D with 0 and 100% replacement of casein containing 80% AMF, with total protein content of 2.2%

b Emulsions E, F are made with 0%, 100% replacement of casein respectively containing 70% AMF and emulsion G, H with 0 and 100% replacement of casein containing 80% AMF, with total protein content of 3.5%

Product stability

All the emulsions except the ones containing 3.5% protein and 80% fat were stable overnight.

6.4.2.2 PILOT-SCALE TRIALS

Compositional Analysis of RHFP and RCP

The moisture content for all the powders was less than 2%, that is, within recommended range.

However, the measured total fat content was found different than calculated total fat content. This can be attributed to the different batches of raw ingredients used during manufacturing.
which might not contain specified amount of protein content as mentioned in the manufacturers’ specification sheet.

**Free fat determination**

The free fat content for powders A and B (see Table 6.8) containing AMF were found to be have insignificant difference in the free fat content as shown in Figure 6.7. However, powder C (3.58%) and powder D (4.19%) containing coconut oil were significantly different (p<0.05) from each other. It showed that high fat powder made using 100% casein with coconut oil can emulsify more fat than 100% casein using AMF. However, all powders were within recommended limits (<7%) of free fat content.

![Figure 6.7: Free fat content versus type](image)

**Particle size determination**

The particle size distribution did not report any significant differences in using 2.2% or 3.5% IWP in terms of emulsion capacity.
**Table 6.12:** Particle size distribution for RHFP and RCP reconstituted emulsions

<table>
<thead>
<tr>
<th>Powders</th>
<th>VMD d (0.5) $^a$ µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1b</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.23$^a$</td>
</tr>
<tr>
<td>B</td>
<td>1.33$^a$</td>
</tr>
<tr>
<td><strong>SET 2 c</strong></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.80$^b$</td>
</tr>
<tr>
<td>D</td>
<td>1.60$^b$</td>
</tr>
</tbody>
</table>

$^a$ Value median diameter with 50% of particle size distribution above the values and 50% below

* Values followed by the same letter in each column are not significantly different at P ($< 0.05$)

b Reconstituted emulsions A, B are made with 100% replacement of casein containing 70% and 80% AMF, with total protein content of 2.2%
c Reconstituted D, E are made with 100% replacement of casein containing 70% and containing 80% coconut oil, with total protein content of 2.2%

**Table 6.13:** Volume percentage of RHFP and RCP reconstituted emulsions

<table>
<thead>
<tr>
<th>Powders</th>
<th>&lt;10 µm (%)</th>
<th>10–45 µm (%)</th>
<th>&gt;45 µm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1 a</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>93.88$^a$</td>
<td>6.12$^a$</td>
<td>0$^a$</td>
</tr>
<tr>
<td>B</td>
<td>91.97$^a$</td>
<td>8.03$^a$</td>
<td>0$^a$</td>
</tr>
<tr>
<td><strong>SET 2 b</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>88.77$^b$</td>
<td>11.23$^b$</td>
<td>0$^a$</td>
</tr>
<tr>
<td>D</td>
<td>89.23$^b$</td>
<td>10.77$^b$</td>
<td>0$^a$</td>
</tr>
</tbody>
</table>

* Values followed by the same letter in each column within each set are not significantly different at p < 0.05

a Reconstituted emulsions A, B are made with 100% replacement of casein containing 70% and 80% AMF, with total protein content of 2.2%
b Reconstituted emulsions D, E are made with 100% replacement of casein containing 70% and containing 80% coconut oil, with total protein content of 2.2%
Figure 6.8: Particle size distribution of pilot-scale RHFP and RCP reconstituted emulsions
(I) 2.2% protein content and 70% AMF (—) and 80% AMF (—)
(II) 2.2% protein content and 70% coconut oil (—) and 80% coconut oil (—)

Product stability

All five RCP reconstituted emulsions were stable overnight.

6.4.3 CONCLUSIONS

The laboratory-scale results showed that the particle size determination of IWP at 3.5% protein content can behave as efficiently to casein at 2.2% protein content. However, when scaled up at pilot-scale, there were no significant differences reported in using either 2.2% or 3.5% IWP content.

6.5 COFFEE CREAMER AND IMITATION CREAMER

6.5.1 EXPERIMENTAL DESIGN

Table 6.14 and Table 6.15 list the batches carried out at the laboratory-scale
6.5.2 RESULTS AND DISCUSSION

Firstly, the particle size distribution of CC and IC formulations were conducted at laboratory-scale in order to study the emulsifying properties of these emulsions. The short-listed stable emulsions were made at the pilot-scale and were checked for their compositional analysis.

6.5.2.1 LABORATORY-SCALE TRIALS

Particle size determination

The particle size distribution of emulsions were examined for three different protein contents, that is, 0.9, 1.8 and 3.6% as shown in Figure 6.9 and Table 6.14. The protein content was varied from 0.9 to 3.6%, it was found that control CC with 0.9% protein content was most stable as compared to control made using 1.8% and 3.6% protein contents. Hence 0.9% protein content was found optimal.
Figure 6.9: Particle size distribution of laboratory-scale CC and IC emulsions

(I) 3.6% total protein content; NaCAS (___) and 50% IWP (___) and 100% IWP (___)

(II) 1.8% total protein content; NaCAS (___) and 50% IWP (___) and 100% IWP (___)

(III) 0.9% total protein content; NaCAS (___) and 50% IWP (___) and 100% IWP (___)
### Table 6.14: Particle size distribution for CC and IC emulsions

<table>
<thead>
<tr>
<th>Powders</th>
<th>VMD d (0.5) μm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1b</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>13.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>14.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>10.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>SET 2c</strong></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>0.95&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>14.81&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>SET 3d</strong></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>H</td>
<td>1.26&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>I</td>
<td>11.9&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d,e,f</sup> Value median diameter with 50% of particle size distribution above the values and 50% below

* Values followed by the same letter in each column are not significantly different (p< 0.05)

b Emulsions A, B, C are made with 0%, 50% and 100% replacement of casein with total protein content of 3.6%

c Emulsions D, E, F are made with 0%, 50% and 100% replacement of casein with total protein content of 1.8%

d Emulsions G, H, I are made with 0%, 50% and 100% replacement of casein with total protein content of 0.9%
Table 6.15: Batches for CC and IC emulsions prepared at laboratory-scale (expressed in percentage)

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>3.6% PROTEINS</th>
<th>1.8% PROTEINS</th>
<th>0.9% PROTEINS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% CASEIN</td>
<td>100% CASEIN</td>
<td>50% CASEIN</td>
</tr>
<tr>
<td></td>
<td>REPLACEMENT</td>
<td>REPLACEMENT</td>
<td>REPLACEMENT</td>
</tr>
<tr>
<td>CONTROL A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>NaCAS</td>
<td>4</td>
<td>2</td>
<td>N.A.</td>
</tr>
<tr>
<td>Gemtec 1160</td>
<td>N.A.</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Corn Syrup</td>
<td>57.4</td>
<td>57.4</td>
<td>57.4</td>
</tr>
<tr>
<td>Mono &amp; di</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>glycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipotassium</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>orthophosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSL</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Water</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Coconut Oil</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 6.16: Batches for CC and IC powders prepared at pilot-scale (expressed in percentage)

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>CONTROL A</th>
<th>SET 1</th>
<th>CONTROL D</th>
<th>SET 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% CASEIN REPLACEMENT (0.5% total protein)</td>
<td>100% CASEIN REPLACEMENT (1.0% total protein)</td>
<td>100% CASEIN REPLACEMENT (0.5% total protein)</td>
<td>100% CASEIN REPLACEMENT (1.0% total protein)</td>
</tr>
<tr>
<td>NaCAS</td>
<td>0.5</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.5</td>
</tr>
<tr>
<td>Gemtec1160</td>
<td>N.A.</td>
<td>0.5</td>
<td>1</td>
<td>N.A.</td>
</tr>
<tr>
<td>Corn syrup / mylose syrup</td>
<td>14.8</td>
<td>14.8</td>
<td>14.3</td>
<td>14.8</td>
</tr>
<tr>
<td>Mono &amp; diglycerides</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Dipotassium orthophosphate</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Solids before adding fat</td>
<td>16.2</td>
<td>16.2</td>
<td>16.2</td>
<td>16.2</td>
</tr>
<tr>
<td>Water</td>
<td>37.5</td>
<td>37.5</td>
<td>37.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>8.7</td>
</tr>
<tr>
<td>Hydrogenated palm oil</td>
<td>8.7</td>
<td>8.7</td>
<td>8.7</td>
<td>N.A.</td>
</tr>
<tr>
<td>% solids before drying</td>
<td><strong>40</strong></td>
<td><strong>40</strong></td>
<td><strong>40</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>
Table 6.17: Compositional analysis of CC and IC powders prepared at pilot-scale (expressed in percentage)

<table>
<thead>
<tr>
<th>POWDER ANALYSIS</th>
<th>CALCULATED VALUE (%)</th>
<th>CONTROL A</th>
<th>100% CASEIN REPLACEMENT (0.5% total protein) B</th>
<th>100% CASEIN REPLACEMENT (1.0% total protein) C</th>
<th>CONTROL D</th>
<th>100% CASEIN REPLACEMENT (0.5% total protein) E</th>
<th>100% CASEIN REPLACEMENT (1.0% total protein) F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOISTURE</td>
<td>N.A.</td>
<td>1.8</td>
<td>1.6</td>
<td>1.5</td>
<td>1.7</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>1.8 (A, B, D, E) &amp; 3.6 (C, F)</td>
<td>2.2</td>
<td>2.1</td>
<td>3.4</td>
<td>2.2</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td>FAT</td>
<td>35</td>
<td>36.8</td>
<td>36.8</td>
<td>35.9</td>
<td>36.5</td>
<td>36.4</td>
<td>36.4</td>
</tr>
<tr>
<td>ASH</td>
<td>N.A.</td>
<td>2.29</td>
<td>1.88</td>
<td>4.01</td>
<td>0.14</td>
<td>2.27</td>
<td>2.28</td>
</tr>
</tbody>
</table>
6.5.2.2 PILOT SCALE TRIALS

Compositional Analysis of CC and IC

The moisture content for all the powders was within the recommended range. However, the measured total protein and fat content were found different than calculated values. This can be attributed to the different batches of raw ingredients used during manufacturing which might not contain specified amount of protein content as mentioned in the manufacturers’ specification sheet.

Free fat determination

The free fat content of the powders A to F are shown in Figure 6.9 (Also refer to Table 6.16). It was observed that the powders made using both coconut oil (powders A, B and C) and palm oil (powders D, E and F) did not show any significant difference in emulsification. So powders C and F showed optimal level of protein that should be used to get proper emulsification.

Figure 6.10: Free fat content versus type of powder
**Particle size determination**

Particle size distribution of reconstituted emulsions formed from powders in set 1 and 2 exhibited a bimodal distribution. The median particle size is given in Table 6.5. The median particle size, $D_{(v, 0.5)}$ was 0.270, 0.439 and 0.333µm for 0%, 100% and 200% replacement of casein in set 1. It was found that set 1 samples have a significant effect on the volume median diameter. Reconstituted emulsion B has 50% volume of the particles less than 0.439µm and was significantly different compared to A (0.270%) and C (0.333%).

**Table 6.18: Particle size distribution for CC and IC reconstituted emulsion**

<table>
<thead>
<tr>
<th>Powders</th>
<th>VMD $d_{(0.5)}$ $\mu$m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SET 1b</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.270&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>0.439&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>0.333&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>SET 2c</strong></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.250&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>0.495&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>0.299&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Value median diameter with 50% of particle size distribution above the values and 50% below

<sup>b</sup> Values followed by the same letter in each column are not significantly different at P (< 0.05)

<sup>c</sup> Reconstituted emulsions A,B,C are made with 0%, 100% and 200% replacement of casein respectively using hydrogenated coconut oil

<sup>c</sup> Reconstituted emulsions D,E,F are made with 0%, 100% and 200% replacement of casein respectively using palm oil
In Set 1, reconstituted emulsion A (0% replacement of casein) has the highest percentage of small particles (99.90%) and C (200% replacement of casein) has the lowest portion of small particles which was 98.05% (Figure 6.4). However, there is no significant effect of samples on volume median diameter. On the other hand, reconstituted emulsion C has the highest percentage of large particles (1.95%) followed by B (0.42%) and A (0.10%) as shown in Table 6.6.

Set 2 samples didn’t show any significant effect on the volume median diameter (Figure 6.5 and Table 6.6). The volume percentage of small particles in Set 2 samples were 99.79, 98.91 and 99.03%.

**Table 6.19: Volume percentage of CC and IC reconstituted emulsions**

<table>
<thead>
<tr>
<th>Powders</th>
<th>&lt;10 μm (%)</th>
<th>10-45 μm (%)</th>
<th>&gt;45 μm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET 1 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>99.90a</td>
<td>0.10a</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>99.58a</td>
<td>0.42a</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>98.05a</td>
<td>1.95a</td>
<td>0</td>
</tr>
<tr>
<td>SET 2 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>99.79a</td>
<td>0.21a</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>98.91a</td>
<td>1.09a</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>99.03a</td>
<td>0.97a</td>
<td>0</td>
</tr>
</tbody>
</table>

* Values followed by the same letter in each column within each set are not significantly different at (p<0.05)

a Reconstituted emulsions A,B,C are made with 0%, 100% and 200% replacement of casein respectively

b Reconstituted emulsions D,E,F are made with 0%, 100% and 200% replacement of casein respectively using palm oil
**Product stability**

All five CC and IC reconstituted emulsions made from powders at pilot-scale were stable overnight.

**6.5.3 CONCLUSIONS**

The laboratory-scale formulations made using different three levels of protein (0.9%, 1.8% and 3.6%) indicated 0.9% protein in the overall formulation as optimal based on the desirable emulsifying properties of the coffee creamers. These results were similarly achievable in pilot-scale products containing 1.0% protein. It was also found that the total replacement of casein with IWP is feasible using non-dairy fats in coffee creamers.
CHAPTER 7: SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

From the research that I have done to compare the emulsifying properties of Isolated Wheat Protein with those of both casein/caseinates and skim milk powder in dairy-type emulsions, I can conclude that:

Isolated Wheat Protein (IWP) was functionally as effective as casein/caseinates and Skim Milk Product when incorporated in dairy-type emulsion products. The various functional properties of IWP which the research revealed together with high protein content (>90%) proved its potential in fortifying food systems and facilitating new product development.

IWP was found to perform better than casein in Recombined Sweetened Condensed Milk in terms of viscosity, the most critical parameter for this product. The Recombined Creamer Powder and Recombined Full Cream Milk Powder made using IWP and casein respectively were essentially the same in terms of both compositional analysis and emulsifying properties. Similar products made using both dairy and non-dairy fats showed that casein and IWP can effectively stabilise emulsions regardless of type of fat/oil used. Similarly, it was found that IWP could be used to fully replace caseinate in both Recombined High Fat Powder and Coffee Creamer.

The study showed that casein/caseinate could successfully be replaced by IWP as primary emulsifier partially in a selected dairy-type liquid emulsion product equivalent to Recombined Sweetened Condensed Milk, and totally in spray-dried emulsion products equivalent to Recombined Full Cream Milk Powder, Recombined High Fat Powder and Coffee Creamer.

FUTURE DIRECTIONS
The detailed study of the structure and interfacial properties of wheat proteins needs to be investigated as compared to casein in order to extensively explain its similar/dissimilar behaviour in a wide range of dairy products. It will lead to the different interactions arising between milk and wheat proteins in the presence of other non-protein ingredients.

RECOMMENDATIONS

This study identified a need to investigate further the molecular structural interactions between casein/caseinates and wheat proteins in various food systems, specifically in the presence of other ingredients like sugar, maltodextrins etc. Furthermore, the time span of the project permitted the product stability to be studied for only six months in storage. It still needs to be established that the market requirement for storage stability for up to one year is attainable for these products.
APPENDIX I: Measured viscosity of RSCM/ISC versus storage

a. Measured viscosity of control RSCM versus storage

b. Measured viscosity of 25% ISC versus storage
c. Measured viscosity of 50% ISC (6% protein) versus storage

![Graph](image1)

d. Measured viscosity of 50% ISC (5% protein) versus storage

![Graph](image2)
APPENDIX II: Measured viscosity versus shear rate for RSCM/ISC

(a) Measured viscosity versus shear rate for freshly prepared RSCM (■), 25% ISC (□) and 50% ISC (6% protein) (▲) manufactured using Gemtec1100
(b) log (viscosity) versus log (shear rate)

(a) Measure viscosity versus shear rate for freshly prepared RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) manufactured using Gemtec1160

(b) log (viscosity) versus log (shear rate)
APPENDIX III: Pseudo plastic behaviour of RSCM and ISC

Shear stress versus shear rate for RSCM ( ), 25% ISC ( ) and 50% ISC (6% protein) ( ) with Gemtec1100 (a) and Gemtec1160 (b)
CHAPTER 9: BIBLIOGRAPHY


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