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# **Effect of Starch Addition on Rheological Properties of Processed Cheese**

**A thesis presented in partial fulfilment of the  
requirements for the degree of  
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## Abstract

The main objective of this study was to determine the effect of starches on the rheological and functional properties of processed cheese. The key challenge of this project was to succeed in developing a reduced protein processed cheese. To do so, a fundamental understanding was needed of the textural and functional contributions of the starches in a dynamic processed cheese environment. A collection of different techniques, namely rheology, confocal microscopy, particle size measurement and functionality tests (firmness, stress and strain, melt) were used. The effects of various starches were studied in two formulations: model rennet casein-based processed cheese spread and an Individual Wrapped Slice (IWS) processed cheese.

A range of experimental cheeses was produced by addition of starches at different rates. Samples were prepared on three different pieces of equipment: a Paar-Physica Rheometer (starch cell), a Rapid Visco Analyzer (RVA), and a Blentech cooker (pilot-plant). Ten different starches (Cornstarch, Waxy cornstarch, High amylose cornstarch (HACS), Rice starch, Waxy rice starch, Potato starch, Wheat starch, Resistant starch, Acid converted starch and Perfectamyl (Di-starch phosphate of potato starch) were used for the study. These showed differences in rheological, microstructural and functional properties of the final products. The differences arising in the samples prepared on the various equipment might be due to the physio-chemical properties of the food components and/or to differences in processing conditions.

The rennet casein-based processed cheese spreads were prepared on the Paar-Physica and the RVA with 9.7% protein level. Addition of starch (ten starches) to processed cheese spread increases the rheological attributes, such as complex modulus ( $G^*$ ), strain and viscosity. However the extent of this increase is totally dependent on the type and physio-chemical properties of the starch. For better understanding of the effect of starch addition, microstructural evaluation and particle size distribution were required in addition to rheological behaviour. The microstructure showed marked differences, which were attributed to different processing conditions and also the physio-chemical properties of the food components (protein, fat and starch). This was also observed in the particle size distribution, where the RVA tended to produce smaller fat droplets than the Paar-Physica.

The protein level in the rennet casein-based processed cheese spread was decreased from 9.7% to 8.5% and 7.5%. Six different starches (Waxy cornstarch, HACS, Rice starch, Potato starch, Wheat starch and Acid converted starch) were investigated in

these reduced protein systems. It was found that reducing the level of protein in the processed cheese influenced the rheological properties, the mean particle size and the microstructural properties of model processed cheese spreads. The emulsifying properties and gel strength of the gel network decrease with reduction in the protein level, thereby decreasing the elasticity of the processed cheese.

Validation of the effects of starch on rennet casein-based processed cheese spreads was carried out in an IWS processed cheese system using the RVA. The impact of four different starches on the meltability and textural properties of IWS processed cheese was dependent on the type of starch. Potato starch, wheat starch and rice starch increased the firmness and viscosity of the processed cheese. However, as the firmness increased the melt decreased. HACS contributed slightly to the firmness and viscosity of the IWS processed cheese. Strain values were largely unaffected by starch addition suggesting that the processed cheese structure remained a continuous protein network. Among all the starches, potato starch gave the best results in terms of textural properties of the processed cheese, especially firmness. Protein substitution of 1 and 2 wt% was achieved by replacement with 1 and 2 wt% potato starch respectively. The low protein and high starch levels introduced some stickiness into the product.

On a pilot-plant scale, when potato starch was used to replace protein at 1 and 2 wt% levels, the firmness of the control was retained in the resulting reduced protein processed cheeses and the flavour of the starch containing products was found to be satisfactory. Although the melt characteristics decreased, they remain within the commercially acceptable limits. However stickiness was again an issue that needs to be addressed before commercialisation.

Processed cheese is a very complex system involving protein-protein, protein-fat and protein-water interactions. Addition of starch increases the number of different interactions. Differences in the processing conditions along with physio-chemical properties of the food components lead to differences in the rheological and functional behaviour of the processed cheese. Despite all these complexities, this work provides useful information on the effects of adding starch on the rheological, microstructural and functional properties of processed cheese. This work demonstrates reduced protein IWS processed cheese manufacture is possible although some further work is needed on the stickiness issue.

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# 1. Introduction

## 1.1 Background

Cheese is one of the most widely used ingredients in prepared foods for imparting taste, texture and nutritional qualities. Processed cheese is made by mixing, heating and shearing of natural cheeses, butter, protein powders, salt, water and emulsifying salts. This produces an emulsion that gels on cooling, to form a slice, block, spread etc. Young natural cheese is used to provide the strength and texture to the product while mature cheese provides the cheesy, cheddar flavour. Among all the ingredients, cheese and protein powders are the most expensive ingredients. As a consequence of a constant industrial focus on reducing the cost of processed cheese formulations, there is always a drive to reduce the protein content and increase the moisture content. Because of both the high cost of caseins (proteins) and certain functionality limitations, a number of researchers have investigated low-cost casein substitutes such as vegetable proteins: peanut, cotton-seed and soy protein isolates, but with limited success. The attempts to reduce the cost by the means of replacing the protein with a cheap ingredient like starch have led to this research work.

Proteins provide the structural backbone for processed cheese. Reducing protein can seriously affect the product in terms of softening and loss of functional properties of the processed cheese. Removal of protein must be complemented by addition of another agent capable of both contributing to the structure and maintaining the functionality of the product. Polysaccharides can be used in this capacity, and many processed foods are multi-component systems, containing protein-polysaccharide-fat mixtures. Polysaccharides have the ability to bind water, improve viscosity and aid in gelling and are water-soluble. Polysaccharides are widely used to fulfil different roles in a variety of manufactured dairy products. One class of polysaccharides that has generated much interest in the food industry is starch. There are several patents relating to the use of starches as casein substitutes in imitation cheese. However, there has been no detailed study reported of the influence of native starches on the textural properties of processed cheese.

In addition to formulation cost reduction, starches may offer the opportunity to make an ambient-stable processed cheese, i.e. a product that can withstand non-refrigerated storage. Addition of starch to a processed cheese formulation may provide that extra body required to resist softening at room temperature without compromising the functionality of the product.

Although extensive research has been carried out on processed cheese, there still remains a great deal to be understood on the complex interactions of protein, fat and minerals during processed cheese making and also during storage. The main aim of this research work was to study the effect of different starches on the rheological, melt and microstructural properties of processed cheese.

## **1.2 Thesis outline**

This thesis aims to provide an understanding of the effects of different starches on the rheological properties of processed cheese.

A review of literature on starch and processed cheese is presented in Chapter 2.

Details of raw materials used in this study are given in Chapter 3, along with a description of the methods used.

Chapter 4 contains the results of a preliminary study on the impact of ten starches on a model-processed cheese spreads. The model was a rennet casein-based spread and rheological testing was used to assess the impact.

A subset of the starches in Chapter 4 was studied in more detail in Chapter 5. In addition to rheological testing, confocal microscopy was used to study the microstructure of the processed cheese containing starches.

The same model processed cheese was used as the basis of the first investigation into protein reduction via starch addition. The results are presented in Chapter 6.

In chapter 7, the focus changed from model systems to a commercial processed cheese slice (IWS) formulation. The impact of four different starches on IWS was assessed through rheological and melt testing along with microstructural evaluation.

Native potato starch was selected for protein reduction studies in Chapter 8. The results of this chapter were used to plan pilot-plant studies in which up to 12% of the protein was replaced with starch. The pilot-plant investigation is presented in Chapter 9.

Chapter 10 is a general discussion of the results presented in Chapters 4-9. Chapter 11 summarises conclusions and recommendations for future work.

## **2. Literature Review**

### **2.1 Processed cheese**

#### **2.1.1 Introduction**

Processed cheese and processed cheese products are produced by comminuting, blending and melting one or more cheeses and optional ingredients into a smooth homogeneous blend with the aid of heat, mechanical shear and emulsifying salts (Guinee, 2002).

The main advantages of processed cheese are (Guinee, 2002; Chambre and Daurelles, 2000):

- 1- Their nutritive value. These types of products are good source of nutrition.
- 2- Their cost is comparatively less than the natural cheeses. This is due to the use of non-cheese solids in its production and higher moisture content.
- 3- Their versatility as foods as they offer wide range of foods with different texture, flavour, functionality (melt), size and shape of final product.
- 4- Their convenience to use at home due to their excellent preservation, consistent tailor made functionality, convenient packaging size etc.

#### **2.1.2 Types of processed cheese**

According to the composition and ingredients, processed cheese products can be classified into processed cheese blocks, processed cheese spreads, processed cheese foods and processed cheese analogues (Caric & Kalab, 1993). The regulations might vary from country to country. Table 2.1 summarises the types and characteristics of different processed cheese products.

**Table 2.1. Different types of processed cheese and its characteristics (Caric & Kalab, 1993)**

Type of product	Ingredients	Cooking temperatures	Composition	pH
Processed cheese block	Natural cheese, emulsifiers, sodium chloride, colouring	71-80°C	Moisture and fat correspond to the legal limit for natural cheese	5.6-5.8
Processed cheese foods	Same as above, plus optional ingredients such as milk, skim milk, whey, cream, albumin, skim-milk cheese, organic acids	79-85°C	≤44% moisture <23% fat	5.2-5.6
Processed cheese spread	Same as processed cheese foods plus gums for water retention	88-91°C 85-98°C 90-95°C	≥ 44% and ≤ 60% moisture ≤ 55% moisture	5.2 5.7-5.9 5.8-6.0
Processed cheese analogue	Rennet casein, Sodium caseinate, calcium caseinate, suitable vegetable fats, emulsifying agent, sodium chloride, artificial flavouring material	As for processed cheese foods	As for processed cheese foods	5.8-5.9

### 2.1.3 Role of ingredients

Processed cheese can contain a large number of ingredients. Optional dairy and non-dairy ingredients can be added depending on the type of product required. The role played by each ingredient can be summarised in Table 2.2.

**Table 2.2. Different ingredients of processed cheese and their main contribution (Guinee, 2002)**

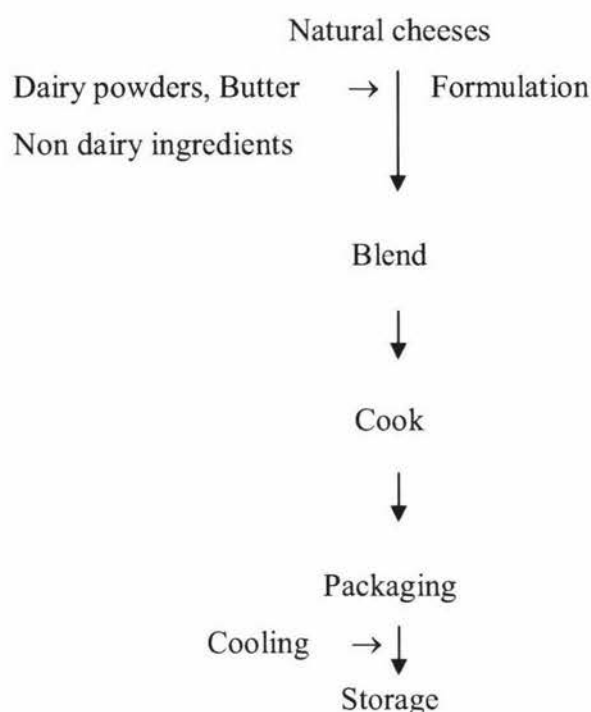
<b>Ingredient</b>	<b>Main function/role</b>	<b>Source</b>
Milk fat	Provides required composition, provides structure i.e. backbone, texture and melting properties	Butter, anhydrous milk fat
Milk protein	Provides compositional specifications, texture and meltability and assists in preparation of a physicochemical stable product	Casein, caseinate, whey proteins, milk proteinates
Lactose	Low cost filler may have adverse properties	Whey powder
Emulsifying salts	Assist in physicochemical stable product, gives desired melting, binds water and provides textural properties	Emulsifying salts, sodium and phosphate salts of citric acid
Stabilizers	Bind together finely dispersed casein particles	Hydrocolloids
Acidifying agents	Assist in pH control of the final product	Organic acids like fumaric acid, lactic acid, citric acid

<b>Ingredient</b>	<b>Main function/role</b>	<b>Source</b>
Flavours	Imparts flavour	Enzyme modified cheese, starter distillate, flavour concentrates, sodium chloride
Sweetening agent	Increase sweetness especially for product intended for children	Sucrose, dextrose, corn syrup, lactose
Colours	Provides desired colour	Annatto, artificial colour
Preservatives	Prolongs shelf life	Nisin, potassium sorbate, propionate
Condiments	Provides variety of products with respect to appearance, texture	Preparations of meat, vegetable, fruits etc
Water (Berger et al, 1996)	To maintain the moisture content, provide a good dispersion and complete emulsion	Cheese, added water

#### **2.1.4 Manufacture of processed cheese**

Selection of natural cheese is the first and most important step in processed cheese manufacture. Good raw material leads to good finished product. Along with good natural cheese, heating, time and temperature effect, shear rate applied, type and amount of emulsifying salt are equally important to obtain a good final product (Caric & Kalab, 1993).

Following is the process flow for processed cheese manufacture.



Manufacturing involves the following steps (Fox et al, 1996, Guinee, 1987):

- 1) Formulation of blend, which involves selection of correct type and quality of raw cheese, emulsifying salts, water and other optional ingredients.
- 2) Shredding of cheese and dry mixing of all the ingredients including the optional ones.
- 3) Processing of the whole blend.
- 4) Packaging of the blend and cooling.

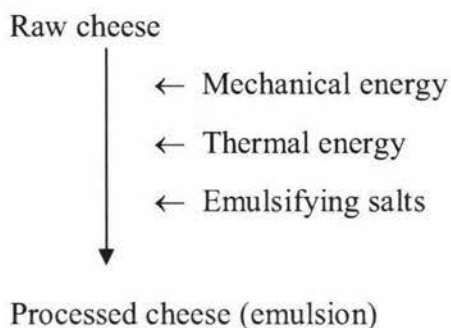
### 2.1.5 Principles of processed cheese

Natural cheese is a three dimensional network of protein with fat globules and moisture entrapped in it (Guinee, 1987). On application of heat and mechanical shearing in the absence of emulsifying salts, a gummy pudding-like mass with oiling off and moisture exudation is obtained on cooling. This defect arises due to shearing of fat globule membrane and partial dehydration of protein leading to free moisture exudation through the porous structure (Fox et al, 1996).

Addition of emulsifying salts, during processing promotes emulsification of fat and rehydration of protein. This leads to a smooth, homogeneous, stable product.

The figure below gives an overview of the principles involved in process cheese manufacture (Fox et al, 1996).

### A) Overall reaction



### B) Process Responsible for transition

Process	Main Causative agent
Calcium Sequestration	Emulsifying salts
pH buffering	Emulsifying salts
Protein dispersion	Emulsifying salts Thermal and mechanical energy
Protein hydration	Emulsifying salt Thermal energy
Emulsification	Dispersed hydrated protein Mechanical energy
Structure formation	Protein-protein interactions Emulsified fat globules (Pseudo-protein particles)

During processing, the natural cheese, consisting mainly of insoluble calcium paracaseinate and fat globules, is finely dispersed, homogenised and converted into a gel in which fat is emulsified. By chelating the calcium from the protein structure, the emulsifying salts contribute to emulsifying properties. The structure of the processed

cheese depends on the type of cheese used, the fat ratio, the dry matter and the ability of the emulsifying salt to sequester the calcium (Schar & Bosset, 2002).

The physicochemical changes taking place within the cheese during processing are:

### ***Calcium sequestering***

Monovalent sodium from the emulsifying salt replaces the divalent calcium in the para-caseinate network. This leads to formation of soluble sodium para-caseinate instead of insoluble calcium para-caseinate (Guinee, 1987; Fox et al, 1996).

### ***pH displacement and stabilization***

Use of correct blend of emulsifying salts and acids usually shifts the pH of natural cheese (~5.0-5.5) to 5.5-5.9 in processed cheese. This leads to increase in the calcium sequestering ability of the emulsifying salt. The correct pH aids disintegration of the calcium para-caseinate network leading to better water binding and emulsification properties (Guinee, 1987).

### ***Peptization***

Conversion of calcium para-caseinates into charged sodium para-caseinates by the presence of emulsifying salts in presence of mechanical and thermal energy is called peptization. This is a major factor affecting the hydration of protein (Guinee, 1987; Fox et al, 1996).

### ***Emulsification***

Sodium para-caseinate contributes to emulsification, by coating the free fat droplets, formation of fat globule membranes and also maintains emulsion stability by immobilization of large amount of free water (Guinee, 2003).

## **2.1.6 Structure formation on cooling**

On cooling the molten mass sets to form a body that depends on the blend formulation, processing conditions and cooling rate. Factors affecting structure formation during cooling are: fat crystallisation, protein-protein interaction, interactions between the para-caseinate coated fat globules and para-caseinate. The structure consists of emulsified fat globules dispersed in the protein matrix. Fat globules are evenly distributed and the size of fat globules depends on degree of emulsification, ingredients used (emulsifying salts, other ingredients) and processing conditions. The diameter of the fat globule decreases with increase in the processing time. Increase in the quantity

of emulsifying salts and processing time decreases the size of the fat globule considerably (Guinee, 2003).

### **2.1.7 Emulsifying salts**

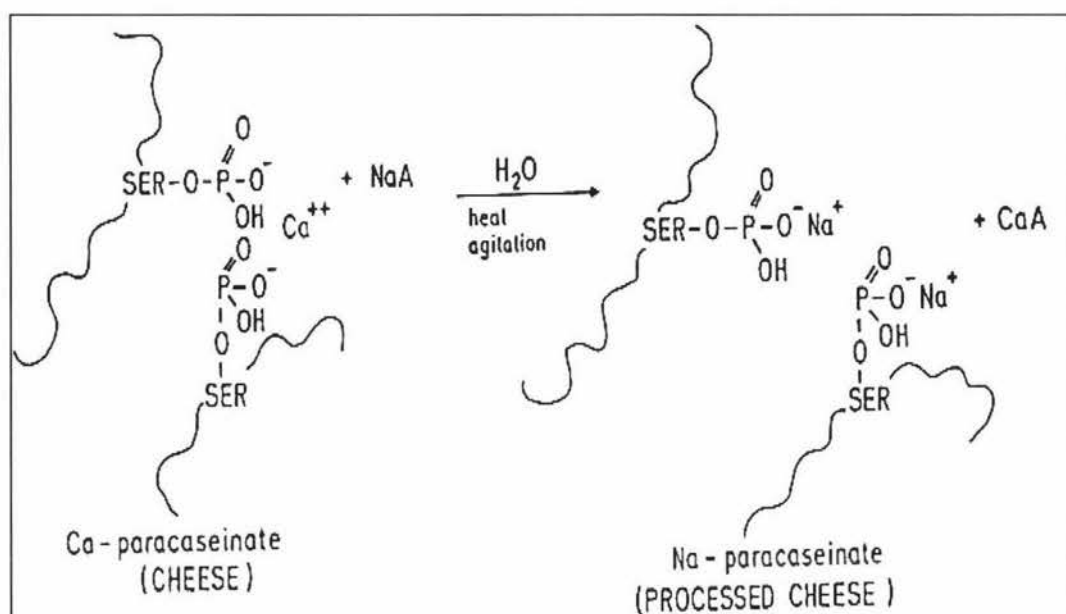
The emulsifying salts most commonly used are citrates, phosphates, polyphosphates and sodium aluminium phosphate. Other possible emulsifying salts include gluconates, lactates, malates, ammonium salts, glucono lactones and tartrates. Citrates are usually used as sodium or potassium salts. These salts are not fat-soluble and thus have no direct interaction with fat-soluble components. As a result, they interact with water-soluble groups of the protein and not fat-soluble groups (Fox et al, 1996).

The main role of emulsifying salt is to aid in the emulsifying capacity of proteins. This is done by (Caric & Kalab, 1993; Caric et al, 1985),

- 1) Replacing calcium from the proteins.
- 2) Peptizing, solubilizing and dispersing the proteins.
- 3) Hydrating and swelling the protein.
- 4) Emulsifying fat and thereby stabilizing the emulsion.
- 5) Controlling the pH and stabilizing it.
- 6) Formation of a structure on cooling.

An ideal emulsifying salt is one, which has both monovalent cation and a polyvalent anion (Caric et al, 1985). Use of biological substances in place of emulsifying salts have been studied. By using these substances, it has been found possible to avoid changes in the calcium to phosphorous and potassium to sodium ratios and the formation of heavy metals salts, which may occur when emulsifying salts, are used. Use of vegetable oil/whey emulsions in processed cheese improves the nutritive value of the product, providing both a well-balanced amino acid composition and higher linoleic acid amount than in conventional processed cheese (Mann, 1997).

Figure 2.1 shows the chemical changes occurring as a result of presence of emulsifying salts.



**Figure 2.1. Chemical changes during processed cheese manufacture in presence of emulsifying salt (Caric & Kalab, 1993).**

Processing affects the natural emulsifying properties of proteins. Generally, emulsifiers like potassium polyphosphates are used for emulsifying the fat in manufacture of process cheese. However, when sodium is included as a part of these emulsifiers, there are increasing concerns over the use of such emulsifiers. In addition, the intake of phosphates has a negative effect on the nutrient metabolism, because high intake of phosphate results in a shift in the salt balance in the body (Kwak, 2002).

Among proteins, caseins or its fragments have an important emulsifying property, with  $\alpha$ -casein having the maximum emulsifying properties among all the casein fragments, especially in acidic conditions. Therefore,  $\alpha$ -casein has a very important role to play in food applications. Casein hydrolysates can be used as emulsifiers in place of the traditionally used citrates and phosphates without affecting the quality of the final processed cheese (Kwak, 2002). In general, addition of phosphate during manufacture of processed cheese produces firm and low-melting cheeses, while citrates make processed cheese with good melting properties (Mizuno & Lucey, 2005).

Properties of major emulsifying salts are tabulated in Table 2.3.

**Table 2.3. Properties of major emulsifying salt used for processed cheese manufacture (Shimp, 1985; Caric et al, 1985)**

Emulsifying salts	Properties
Trisodium phosphate	Raise the pH. Cannot be used alone. Some other salt has to be used to adjust the pH
Monosodium phosphate	Binds calcium weakly. Brings down pH to unacceptably low level. Rarely used
Sodium citrate and sodium aluminium phosphate	Do not bind much calcium, requires to be used in higher concentration. This produces weak emulsification, giving weak, soft easily melted cheese and cheese sauces
Tetrasodium Pyrophosphate	Highest calcium binding properties. Give very good emulsification and a hard and low melting variety of cheese
Orthophosphates	Capable of suppressing growth of <i>Clostridium botulinum</i>

### 2.1.8 Factors affecting processing

#### *Effect of Emulsifying Salts*

Use of emulsifying salts presents the most effective way of controlling processed cheese properties. The commonly used salts are sodium citrate, sodium aluminium phosphate (SALP), monosodium phosphate (MSP), disodium phosphate (DSP), trisodium phosphate (TSP), tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP), and insoluble metaphosphate (IMP) (Shimp, 1985).

Defects that can arise during processing are 1) physical damage to the NMFGM (Non Milk Fat Globule Membrane) resulting in formation of non globular fat, 2) liquefaction and coalescence of non globular fat, 3) aggregation of para-casein and contraction of the matrix at a relatively low pH and 4) partial phase separation as a result of leaking of fat and water from the protein matrix. Adding the right amount and type of emulsifying salts can prevent the above defects. The emulsifying salts contribute to formation of smooth, homogeneous, physicochemically stable product.

The changes brought about by emulsifying salts are as follows:

- 1) Calcium sequestering: basically the process involves exchange of divalent calcium ions of the para-casein for the monovalent sodium ions of the emulsifying salt. This reduces the concentration of the calcium in the casein. This results in partial conversion of the para-casein to sodium para-caseinate.
- 2) Displacement and stabilisation of the pH: when emulsifying salts are used, there is a change of pH in the cheese from 5.0-5.5 to 5.6-5.9. As a result, para-caseinate receives a net negative charge, which in turn promotes further disintegration of the calcium para-caseinate network and a more open structure that has better water binding and emulsifying properties.
- 3) Dispersion of para-casein: several changes occur as the pH changes as more of the calcium becomes sequestered. The changes occurring are
  - Conversion of calcium para-caseinate to a net negatively charged sodium para-caseinate.
  - Disintegration of the matrix and swelling of the para-casein.
  - Enhanced water binding capacity of the sodium para-caseinate.
  - Better emulsification properties.
- 4) Fat emulsification: the hydrated para-caseinates contribute to emulsification by forming a membrane around the dispersed free fat, with resulting stability of the emulsion by increasing the viscosity of the aqueous phase (Fox et al, 1996).

Although emulsifying salts are usually used for the purpose of emulsification they can have other beneficial properties as well. The orthophosphates are capable of suppressing the growth of *Clostridium botulinum* in cheese spreads (Shimp, 1985).

Besides having the correct type of cheese blend composition and technological parameters, selection of the proper type of emulsifying salts and its concentration is crucial in processed cheese manufacture. Excess of emulsifying salts with higher phosphorus content can lead to hydrolysis of casein fractions into low molecular weight nitrogen fractions making the final processed cheese bitter (Mayer, 2001).

### ***Effect of pH on cheese processing***

Change in the pH can alter the protein configuration, protein solubility and the efficiency with which the emulsifying salts can bind calcium. Usually the cheese pH is  $\geq 5.0$  and there is an excess of negative charge on the protein. Decreasing the pH leads to final product with a more crumbly texture because of weakening of the protein-

protein interaction. In addition, fat will start to de-emulsify. If the pH is increased then the protein bonds becomes stronger, and the solubility increases making a more elastic cheese with better emulsification. If the pH is increased to too high a level, this may lead to softening of cheese (Shimp, 1985).

Lee & Klostermeyer (2001) indicated that accurate control of the pH is of utmost importance to obtain desirable rheological properties of the processed cheese. The texture of processed cheese can be manipulated by small shifts in the pH of the cheese.

### ***Effect of age of cheese on cheese processing***

Due to ageing, protein gets broken down into lower molecular weight fragments (peptides and amino acids), which are more soluble in water than the parent molecules. Lower molecular weight proteins interact less strongly with each other and with fat than do high molecular weight ones. This decrease in interaction, results in a higher water solubility. On proteolysis the proteins becomes more of a flavour provider than a structure formation unit. Also proteolysis brings about change in pH of the cheese and thereby also affects the pH of the processed cheese.

## 2.2 Starch

Starch is a primary source of stored energy in grains and provides about 70-80 % of the energy intake of humans worldwide. In addition to this nutritive value starches are used to manipulate the physical properties of foods. They can be used in gelling, thickening, adhesion, moisture-retention, stabilizing, texturizing etc. They are also used extensively in the paper and textile industries. Starch has advantages over other materials; it is renewable and biodegradable and can be modified to produce a vast range of diverse products (Ellis et al, 1998). In virtually all of the applications in which starch can be used, gelatinization, pasting and retrogradation behaviour are the most important functions (Goel et al. 1999).

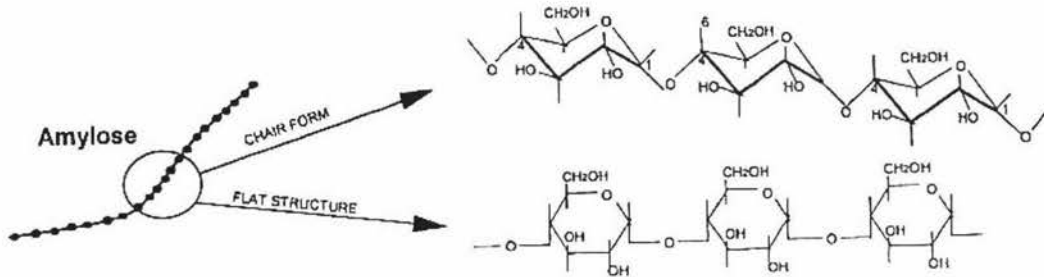
Starch is a carbohydrate, synthesised within plants, and is composed of two types of alpha-glucans, amylose and amylopectin (Tester et al, 2004). Amylose is a linear polymer while amylopectin is a branched polymer. Starch is made up of tiny cells called 'granules', whose size depends upon the botanical source (Whistler & Bemiller, 1997; Ellis et al, 1998).

### 2.2.1 Starch chemistry

The two glucose polymers: amylose and amylopectin, are both made up of D-glucopyranose molecules but there are lots of dissimilarities between the two polymers resulting in major differences in their characteristics. The amylose/amylopectin ratio largely determines the physical properties of starches. Short-term changes in starch crystallinity result from gelation and crystallisation of amylose while long-term changes in starch gels during storage are related to changes in amylopectin (Fredriksson et al, 1998).

#### *Amylose*

Amylose is a linear polymer, but is not defined as a straight chain polymer (Luallen, 2002). It has (1→4)-linked  $\alpha$ -D-glucopyranosyl units. The branches of amylose molecules are either very long or very short. Large distances separate the branch points so the physical properties are essentially those of a linear molecule. Most starches contain around 25% amylose. High amylose cornstarch has an apparent amylose content between 50-75% (Bemiller & Whistler, 1996). Amylose forms opaque, partially crystalline, thermo reversible gels (Morris, 1990). The structure of amylose is shown in Figure 2.2.

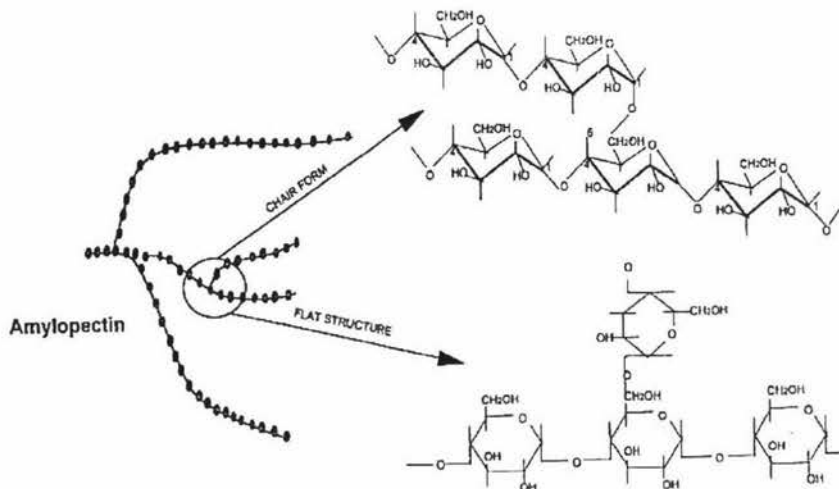


**Figure 2.2. Structure of amylose (Taggart, 2004).**

### *Amylopectin*

Amylopectin is a highly branched polymer. The branch points contain  $\alpha$ -1,6-d-glucose bonds. Due to the large number of chains, amylopectin molecule contains a large number of branch points (Luallen, 2002). There is a great variation in the chain length and in the branching patterns found in amylopectin (Tester et al, 2004). The branches of amylopectin molecules occur in double helices. As a result of these structural occurrences amylopectin is one of the largest molecules found in nature.

Amylopectin is present in all starches contributing about 75% of the mass for most starches. Some starches are made up almost entirely of amylopectin and are known as waxy starches because when the kernel is cut the new surface appears waxy (Bemiller & Whistler, 1996). High amylose starches have amylopectin with a lower degree of branching than is found in the normal or waxy form of starches (Ellis et al, 1998). The structure of amylopectin is shown in Figure 2.3.



**Figure 2.3. Structure of amylopectin (Taggart, 2004).**

Table 2.4 compares different properties of amylose and amylopectin.

**Table 2.4. Different properties of amylose and amylopectin (Thomas & Atwell, 1999)**

Characteristics	Amylose	Amylopectin
Shape	Essentially linear	Branched
Linkage	$\alpha$ -1,4(some $\alpha$ -1,6)	$\alpha$ -1,4 & $\alpha$ -1,6
Molecular weight	<0.5 million	50-500 million
Film forming	Strong, irreversible	Weak, reversible
Gelling	Firm	Soft
Colour with iodine	Blue	Reddish brown
Digestibility, $\beta$ -amylase (Zobel, 1988)	100%	60%
Dilute Solutions (Zobel, 1988)	Unstable	Stable
Degree of polymerisation (Zobel, 1988)	1500-6000	$3 \times 10^5$ - $3 \times 10^6$

**Minor constituents** (Luallen, 2002; Thomas & Atwell, 1999)

Like most ingredients used in the food industry, starch does not exist as a pure entity. Typically starch contains minor constituents such as moisture, lipids, protein, phosphorous and trace elements (Luallen, 2002).

Tuber and root starches contain lower amounts of lipid and protein when compared with the cereal starches (Thomas & Atwell, 1999).

**Lipids:** Usually all the commercially available starches contain less than 1% of lipid material. They are generally bound within the matrix of the starch granule. A higher amount of lipid material is found in cereal starches than in tuber and legume starches (Buleon et al, 1998). The presence of lipid in starches has some adverse effects on the properties that may affect their use in food applications to some extent (Swinkels, 1985).

- Lipid reduces the water binding capacity of starch.
- Oxidation of lipids leads to undesirable flavour.
- Formation of amylose-lipid complexes makes the starch paste cloudy or opaque.

**Protein:** Protein content varies depending on the source of the starch. In general, all the commercially available starches contain less than 1% protein. It is found to be structurally bound to the matrix of the starch granule.

**Phosphorus:** Tuber and legume sources of starch contain esterified phosphorus (phosphate monoesters). Most of the starch sources contain trace amounts of phosphorus that is usually analysed as phospholipids.

**Trace elements:** Collectively these are generally defined as “ash”. The ash content varies depending on the starch type, starch origin and region where the native starch was extracted. The ash content is generally  $\leq 0.5\%$  on a dry basis.

**Moisture:** The moisture content of native starches varies significantly, and is usually around 12-13%.

### 2.2.2 Starch granule

Starch has a complex granular structure. Amylose and amylopectin exist as components of the granules. Amylopectin molecules are arranged radially and the amylose molecules are dispersed among the amylopectin molecules (Jay-lin, 1992). The size, shape and structure vary substantially between starches of different botanical sources (Luallen, 2002; Tester et al, 2004). Although the starch granule is made up of amylose and amylopectin polymers, there is a great variation in the structure and characteristics of native starch granules (Thomas & Atwell, 1999).

The diameters of the granule vary from  $\leq 0.1 \mu\text{m}$  to  $\geq 100 \mu\text{m}$ , and the granule shapes vary from regular (spherical, ovoid, angular) to irregular (Thomas & Atwell, 1999).

The arrangement of amylose and amylopectin within the starch granule is not completely understood. The arrangement of these two polymers in starch granules is not random but is very organised. When heated in water, however the starch granules becomes much less ordered. The loss of order is dependent on the type of native starch. The loss occurs at different temperatures for different types of starches. When starch is heated indefinitely in water, the granule swells until the structure disintegrates wherein amylose and amylopectin are leached out into water (Thomas & Atwell, 1999).

Starch granules are essentially insoluble in cold water. When heated in water, three major changes take place: gelatinisation (increase in viscosity), pasting and retrogradation (Pomeranz, 1985).

### 2.2.3 Gelatinisation

Starch gelatinisation is the collapse of molecular orders within the starch granules manifested in irreversible changes in the properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granular type and heterogeneities within the granule under observation. The temperature at which starch granules begin to swell, lose crystallinity and increase the viscosity of the cooking medium is called gelatinisation temperature (Thomas & Atwell, 1999; Luallen, 2002; Atwell et al, 1988).

This temperature varies greatly with the binding forces within the molecules, which, in turn, vary from species to species ([www.foodstarch.com](http://www.foodstarch.com)). All of the granules don't gelatinise at the same point/temperature, but gelatinisation occurs over a range of temperatures, depending on the species of starch (Pomeranz, 1985).

When a majority of the granules have been gelatinised, the starch is considered to be "pasted" or "cooked-out".

After initial gelatinisation, starch granules keep on swelling with increase in temperature. At some point the starch granules are swollen so much that they absorb all the free water and begin to crowd into one another. This produces a thick, cooked starch paste (Pomeranz, 1985). Excessive heating beyond the gelatinisation temperature leads to an irreversible swelling of the starch granules (Hill et al, 1998)

Gelatinisation is defined as (Thomas & Atwell, 1999):

- Irreversible.
- Increase in the granule size.
- Increase in the solution viscosity.
- Loss of birefringent structure when viewed under a polarising microscope.

It is dependent on:

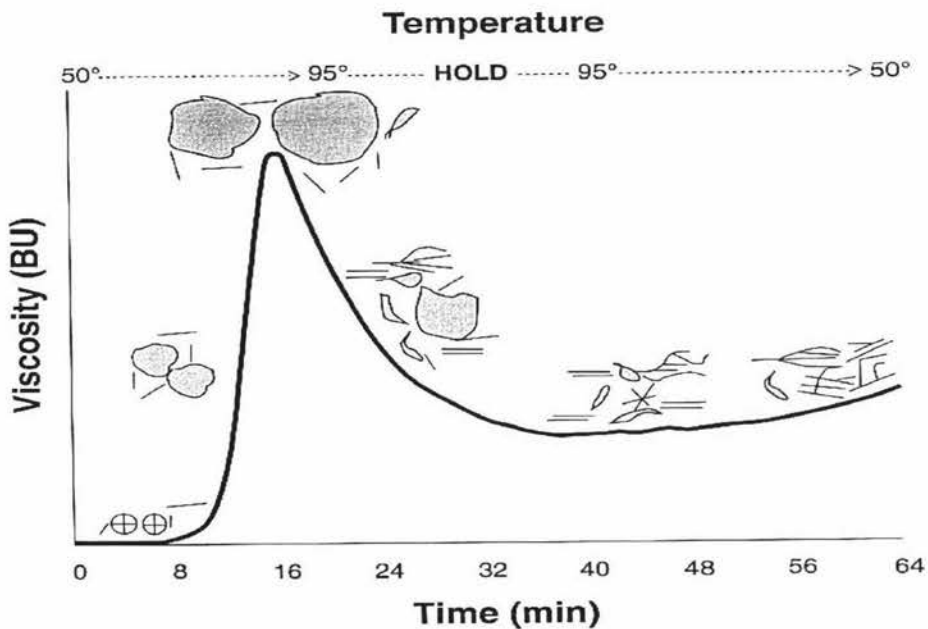
- Temperature and moisture.
- The botanical source of starch.
- The cooking conditions (pH, temperature).

Gelatinisation process involves conversion of weak, temporary network into a strong permanent network (Morris, 1990). To summarize, the physical changes during gelatinisation: the granules swells and lose birefringence, clarity and viscosity increases,

smaller molecules (amylose) dissolve and reassociate to form a gel (www.foodstarch.com).

#### 2.2.4 Pasting

On continuous heating more and more granules swell and the viscosity of the medium increases. The maximum or peak viscosity occurs when largest percentage of swollen granules is present. At this point the starch is said to be fully pasted. Prolonged heating leads to a decrease in the viscosity as a result of granules dissolving (Thomas & Atwell, 1999). Figure 2.4 below indicates the process of gelatinisation and pasting.



**Figure 2.4. Granular changes in relationship to viscosity of starch (Thomas & Atwell, 1999).**

“ Pasting is phenomenon following gelatinisation in the dissolution of starch. It involves granule swelling, exudation of molecular components from the granule, and eventually total disruption of the granules” (Thomas & Atwell, 1999; Atwell et al, 1988).

Pasting is not different from gelatinisation. There is no point where gelatinisation ends and pasting starts. Pasting is a continuation of gelatinisation and is generally related to increase in the viscosity. The viscosity and texture of the paste changes on cooling. The final characteristics, either a viscoelastic paste or a gel is dependent on the amylose content. The higher the amylose content the more are the chances that the paste will set to a firm and cuttable gel. (Thomas & Atwell, 1999)

### 2.2.5 Retrogradation

Retrogradation of gelatinised starch is a reorganisation process involving both amylose and amylopectin, with amylose undergoing retrogradation at a more rapid rate than amylopectin (Jacobson et al, 1997; Thomas & Atwell, 1999).

“Starch retrogradation is a process, which occurs when starch chains begin to reassociate in an ordered structure. In its initial phase, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favourable conditions, a crystalline order appears and precipitation from solution occurs” (Thomas & Atwell, 1999; Atwell et al, 1988).

Retrogradation is more evident in amylose-containing starches. As the process occurs, the starch paste becomes more opaque and forms a cuttable gel. With time this gel becomes more rubbery and has a tendency to loose water in the process of syneresis. Syneresis occurs when heated starch is cooled, as a result it is more evident in frozen or refrigerated products. Starches from different botanical sources retrograde at different rates and to different degrees (Thomas & Atwell, 1999).

### 2.2.6 Effect of pH, shear and other ingredients

*Effect of heat:* Starches do not have a fixed gelatinisation temperature; rather the temperature is extended to a range of temperatures. When a starch solution is heated, the granules start swelling and the viscosity increases. Cooking after attaining the peak viscosity, leads to thinning and a breakdown of viscosity as the granules rupture. Higher temperatures accelerate the breakdown (Cornstarch, 1964).

Rate for heating the system and rate of cooling must be considered. Both of these are important for providing a fixed pattern of conditions. Knowing the rates for both the conditions will help in identifying the equipment for commercialization (Luallen, 2004).

- *Effect of pH:* Most starches gelatinise quickly at both higher and lower pH. At intermediate values of pH (about 4 to 7), gelatinisation is slow. Extreme pH tends to disrupt the molecular integrity of the granule (Cornstarch, 1964; Thomas & Atwell, 1999).

- **Effect of shear:** Shear along with the aid of temperature has a drastic effect on gelatinisation and peak viscosity. Increasing the agitation leads to breakdown of the starch granules (Cornstarch, 1964; Thomas & Atwell, 1999).

At temperature below 57.2°C, starch can be processed with little shear damage at any shear rate. Starch before gelatinisation is very shearing stable (Luallen, 2004).

- **Effect of other ingredients:** Food is a complex system containing many different ingredients. All these ingredients have different effects on the starch functionality. In general, any ingredient that either interacts with the granules or competes with the granules for available water will have a negative effect on the viscosity. Fats, sugar, proteins and salts influence gelatinisation, pasting and retrogradation. Some ingredients have a forward effect on the rate of gelatinisation by altering the pH (Cornstarch, 1964; Thomas & Atwell, 1999). For example acid cleaves hydrogen bonds to bring about faster swelling of the starch granule. Fat and protein delay granule hydration by coating the granule surface thereby lowering the rate of viscosity development. Soluble solids delay the viscosity development by competing for water necessary for hydration (www.foodstarch.com; Whistler & Bemiller, 1997).

1. **Effect of water.** Water as a food ingredient is most essential to make starch functional in a food system (Luallen, 2004). Essentially gelatinization of starch can occur only in presence of water. Therefore all the starch properties are affected by the presence of water.
2. **Effect of sugars.** Effect of sugars on the properties of starch pastes is of considerable importance in industry like pie fillings, various cream types, and puddings and sweet sauces. Sucrose apparently withholds water from the starch granules and thereby impedes swelling of starch granules in hot water. With increasing amount of sugar, there are corresponding greater inhibitions in the normal swelling of starch granules. At high concentrations there is a marked increase in the temperature at which birefringens is lost (Osman, 1967). By exerting an osmotic effect, sucrose inhibits the full development of swelling of starch granules in

hot water, and in sugar solutions of high concentration this is very noticeable (Radley, 1976).

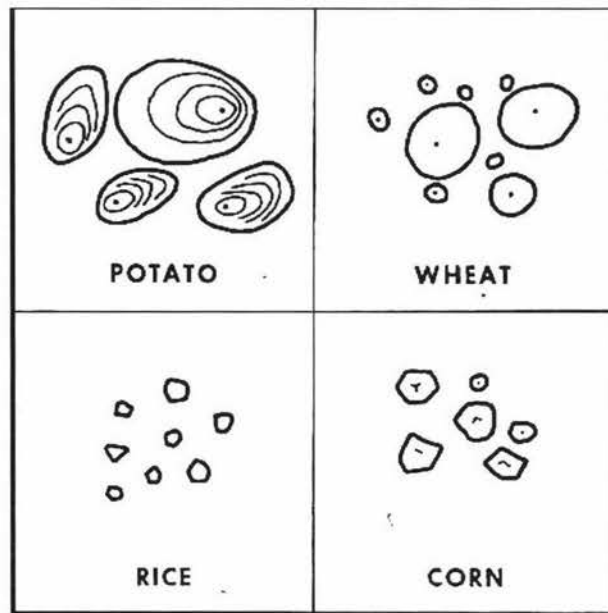
3. **Effect of salts.** The effect of salt on the swelling of starch granules and viscosity is important. The effect of salt on starch is very complex due to effects on other food components like protein (Osman, 1967). Calcium salts usually increase the gel strength and shorten set time, while potassium has the opposite effect. Chloride salts generally produce a distinct unacceptable off-flavour to the final product (Luallen, 2004).
4. **Effect of proteins.** Generally protein-containing foods typically contain carbohydrate, making the system complicated. Protein forms complex very easily with starch. The nature of effects of proteins on starch is not yet fully understood.
5. **Fat:** Fat has a retarding effect on the gelatinisation breakdown of starch granules. Most fat and surfactants raise the temperature at which the maximum viscosity of starch paste occurs (Radley, 1976).

### 2.2.7 Different sources of starch

There are many different sources of starches. A selection are presented below.

#### ***Cornstarch***

Four classes of cornstarch exist. Normal cornstarch is the most common. It has around 25% amylose, compared with waxy cornstarch, which is mostly amylopectin. High amylose cornstarch has 50-55% to 70-75% amylose content. Cornstarch has irregular polyhedron-shaped granules with size ranging from 5-20 microns. Waxy cornstarch also has similar irregular shaped granules. High amylose cornstarch has a narrow size range (Hegenbart, 1996). High amylose starch has unique property of quick setting into stable gels; thereby their use in foods is very important (Hullinger, 1973). Cornstarch is normally not recommended for dairy application as they lack process tolerance and cause problems with poor texture, viscosity instability and syneresis ([www.foodstarch.com](http://www.foodstarch.com)).



**Figure 2.5. Microscopic appearance of common starch granules (Cornstarch, 1964).**

#### ***Potato starch***

Potato starch has about 20% amylose content. Potato starch granules are large with smooth round oval shape (Hegenbart, 1996). Potato starch has the largest granules with the size ranging from 15-75 microns. Potato starch has a relatively high ash content due to the presence of a phosphate group, which improves the thickening power of pastes. Potato starch shows the highest peak viscosity among all the starches (Swinkels, 1985).

#### ***Rice starch***

Normal rice starch contains approximately 20% amylose while waxy rice starch has around 2% amylose content. Rice starch has irregular shaped polygon granules while waxy rice starch exhibits compound granules (Hegenbart, 1996).

#### ***Wheat starch***

Wheat starch contains approximately 25% amylose. Its granules are thick with smooth, round shape ranging from 22-36 microns.

Starch properties are summarised in Table 2.5.

**Table 2.5(A). Properties of different native starches (Murphy, 2000)**

Starch	Type	Diameter ( $\mu\text{m}$ )	Morphology
Corn	Cereal	5-30	Round, polygonal
Waxy corn	Cereal	5-30	Round polygonal
Potato	Tuber	5-100	Oval spherical
Wheat	Cereal	1-45	Round lenticular
Rice	Cereal	3-8	Polygonal spherical compound granules
High amylose corn	Cereal	5-30	Truncated polygonal irregular elongated

**Table 2.5(B). Properties of different native starches (Murphy, 2000)**

Starch	Gelatinisation temperature ( $^{\circ}\text{C}$ )	Pasting temperature ( $^{\circ}\text{C}$ )	Amylose Content (%)	Cooked Properties
Corn	62-72	80	25	Opaque gel
Waxy corn	63-72	74	<1	Clear, Cohesive
Potato	59-68	64	20	Clear, Cohesive, Tendency to gel
Wheat	58-64	77	25	Opaque gel
Rice	68-78	81	19	Opaque gel
High amylose corn	63-92	>90	50-90	Very opaque, Strong gel

### 2.2.8 Modification of starches

Native starches from different sources have been exploited to meet the specific needs unique to that starch. However, native starches lack the versatility to function adequately over the entire range of food products. Modern food processing demand starch to be able to tolerate a wide range of processing techniques and storage conditions. To meet this demand starches are physically or chemically modified (Thomas & Atwell, 1999, Croghan & Mason, 1998).

Main disadvantage of using native starches as food additives are (Croghan & Mason, 1998):

- **Poor mouth feel:** long or gelled texture, unstable texture.
- **Inconsistent viscosity:** vary from plant to plant, region-to-region and year-to-year.
- **Susceptibility to shear:** cooked native starches breakdown and lose viscosity when subjected to mechanical stress.
- **Susceptibility to acid attack:** acidic foods will deteriorate in storage as native starch is degraded.
- **Poor stability:** after cooking, native starches recrystallize or retrograde leading to syneresis. Repeated freezing and thawing accelerate this.
- **Solubility:** for full functionality native starches need improved solubility.

### 2.2.9 Different modification processes for starches

The different starch modification methods (physical and chemical) commonly practiced by the starch manufacturer for the production of modified starches for the food industry are as tabulated in Table 2.5.

**Table 2.6. Modification of starch (Murphy, 2000; Thomas & Atwell, 1999; Pomeranz, 1985; Jay-lin, 1992)**

<b>Modification (Type of modification)</b>	<b>Objective</b>	<b>Benefit to user</b>	<b>Typical uses</b>
Cross-linking (Chemical)	Strengthen starch granule.	Improved process tolerance to heat shear and acid.	Ambient stable products
	Delay viscosity development by retarding granule swelling	Production efficiency: increased heat penetration allowing shorter process time	Bottled sauces
Stabilisation (Chemical)	Prevent shrinkage of starch granule and provide stability at low temperatures	Excellent chill and freeze/thaw stability to extend shelf life	Chilled and frozen processed foods
	Lower gelatinisation temperature.	Easy to cook in high solids system	High brix fillings and toppings
Enzyme conversion (Biochemical)	Produce varied viscosity, gel strength, with thermoreversibility and sweetness	Contribute texture and rheology	Fat mimetic
		Economic dispersant	Flavour carriers Dry mix fillers
Acid thinning (Chemical)	Lower viscosity and increase gel strength	Enhances textural properties at higher usage concentrations of starch	Gums, pastilles, jellies
Lipophilic substitution (Chemical)	Introduce lipophilic groups	Emulsion stabiliser which improves quality of any fat/oil containing product	Beverage, salad dressings
		Reduces rancidity by preventing oxidation	Flavour-encapsulating agents

<b>Modification (Type of modification)</b>	<b>Objective</b>	<b>Benefit to user</b>	<b>Typical uses</b>
Pregelatinisation (Physical)	Pre-cook starch to give cold water thickening properties	Cold water thickening eliminates need to cook, offers convenience and energy savings	Instant soups, sauces, dressings, desserts, bakery mixes
Thermal treatment (Physical)	Strengthen starch granules	Unique functional native starch with 'starch' on label declaration	Ambient stable products
	Delay viscosity development by retarding granule swelling.	Improved process tolerance to heat acid and shear	Bottled sauces
		Production efficiency: increased heat penetration allowing shorter process time	Sterilised soups and sauces

## **2.3 Starch in processed cheese**

Processed cheese is an oil/water type emulsion where milk proteins act as emulsifiers. Cheese is widely used in food preparations in different forms. The high cost of natural cheese has led to development of techniques for replacement of costly dairy ingredients with cheaper non-dairy ingredients. Costly components like fat have been replaced with vegetable fat while casein has been replaced with vegetable proteins. Due to the specific functional role of casein, its replacement with vegetable proteins has not been very successful. Many different vegetable protein source like peanut, cottonseed and soy protein isolates have been used for replacing casein. Starches in modified and native form have been used as a casein replacement. But there has not been a detailed study on use of native starches as a protein replacer (Mounsey & O’Riordan, 2001).

### **2.3.1 Different dairy and non-dairy ingredients in processed cheese**

Different dairy and non-dairy ingredients are used as a source of fat or protein. Thomas (1970) used calcium co-precipitates in processed cheese up to a maximum level of 5% without affecting the overall product. Calcium co-precipitates was also found to reduce the lactose browning reaction in processed cheese (Thomas, 1970). Caseins are the important structural and emulsifying proteins in processed cheese.  $\alpha$ -casein hydrolysis increases the emulsifying properties of protein. The hydrolysates have high emulsifying properties in acidic conditions (pH~5.5). Kwak et al (2002) suggested use of casein hydrolysates in combination with traditional emulsifier. Chang (1979) used partially soluble modified whey solids product in processed cheese products. Laye et al (2003) prepared processed cheese containing casein and whey protein with a ratio of casein to whey protein of from about 50:50 to about 75:25. Lindstorm et al (2004) successfully added up to 9 to 12% soy protein in processed cheese to obtain a homogeneous, pumpable processed cheese that can be formed into sheets, slices or any other desired forms.

### **2.3.2 Fat Substitution**

Processed cheese is normally high in fat. Growing concern over consumption of high fat foods has led to interest in fat replacement (Brummel & Lee, 1990). Reduction of fat in processed cheese has an adverse effect on the texture and functionality of processed cheese. To improve the acceptability of reduced fat processed cheese, different compounds that may partially or fully replace fat and simulate its properties are used (Drake et al, 1999).

Drake et al (1999) suggested use of soy lecithin in reduced-fat processed cheeses. Soy lecithin has been used in processed cheese as an anti-sticking agent. Reduced fat processed cheese with lecithin was comparable with full fat processed cheese. Brummel & Lee (1990) achieved 40-50% fat reduction with different impact on the cheese quality by using soluble hydrocolloids like xanthan,  $\lambda$ -carrageenan, high-methoxy pectin, propylene glycol alginate, low viscosity agar and Zooglan 115 gums at different levels.

Swenson et al (2000) found that hydrocolloids increase the firmness and decrease the meltability of fat free processed cheese compared to processed cheese spreads without hydrocolloids. Cheese spreads with hydrocolloid had uniform and smooth consistency as compared to the one without hydrocolloid.

Finocchiaro (1996) advocated use of high amylose starch produced under specific conditions of temperature, pressure and shear as a texturizing agent to provide fat like attributes in cheese. It helps in replacing fat in foods. Stanley & Harris (1995) employed a fragmented, granular amylose starch for replacing fat in foods. Dunn & Finocchiaro (1997) advocated a novel starch based texturizing agent containing a micro particle, a gum and preferably a pregelatinized starch. This agent can be used in low fat or fat free foods. Finocchiaro (1997) used a starch based texturizing agent for use in fat free or reduced fat processed cheese. The starch is derived from pregelatinized, high amylose starch. Short chain amylose replaces up to 100% of fat in processed cheese. This short chain amylose is obtained by enzymatic debranching of starch (Chiu & Mason, 1998). Eastman (1994) used starch hydrolysates to replace fat.

### **2.3.3 Protein substitution**

Soybeans, soy flour, soy concentrates and soy isolates are an important vegetable source of protein. Other protein source includes cereal, cottonseed, peanut and some single cell proteins (El-Neshawy et al, 1988). El-Neshawy et al (1988) suggested using cheddar cheese, whey protein concentrate and soy protein concentrate powder and chickpea flour in the ratio of 25, 20, 27.5 and 27.5% respectively to obtain a high protein product.

Mounsey & O'Riordan (2001) studied the effect of different native starches like maize, potato, waxy-maize, wheat and rice on the rheology, meltability and microstructure of imitation cheese. Presence of native starch may work as filler at initial stage when the temperature is low, allowing casein to hydrate more and increase its fat emulsifying properties. Rice starch with its smaller globule size, relatively low amylose content has

least negative effect on the properties of the cheese and results in a product with acceptable rheological properties. Rice starch has the potential as a low cost partial casein replacement (Mounsey & O'Riordan, 2001). Zallie et al (1990) advocated up to 100% casein replacement with waxy maize, potato or tapioca starch, which has been partially enzymatically, debranched. Zwiercan et al (1987) suggested use of pregelatinized modified high amylose starches as a partial or full replacement of casein. Carpenter et al (1998) suggested use of granular starch with amylose content less than 30%. Yoder et al (1996) used granular starch instead of pregelatinized starch as granular starch gives better thickening. Zwiercan et al (1985) reported to have successfully used pregelatinized converted starches for up to 80% casein reduction in imitation cheese. The converted starches were prepared by acid- or enzyme conversion or oxidized starched. Merkenich et al (1993) used a modified starch with a high amylopectin content and low amylose content in mixture with emulsifying salt. These starches are used as binding agent and thickeners. This allows the use of traditional emulsifying salts to be halved and thereby crystal formation doesn't occur.

Krumhar et al (1998) suggested use of starch as a source of thickener in cheese products. Using a high starch thickener system and homogenising it before gelatinisation can produce a smooth, long and extensible texture resembling that of processed cheese. This starch-based system is made up of either modified starch or a specific mixture of modified and unmodified starch.

## 3. Materials and Methods

### 3.1 Introduction

This chapter deals with the materials, principles underlining each of the equipment used and the methods used to carry out the experimental work.

In this study, the rheological and functional properties of processed cheese were measured using a Paar Physica Rheometer fitted with a starch cell (Physica, Meßtechnik GmbH, Stuttgart, Germany), and a Rapid Visco Analyzer (RVA-4) (Newport Scientific Ltd, Warriewood, NSW, Australia). The structure of processed cheese products was examined using Confocal Laser Scanning Microscopy (CSLM) (Leica Lasertechnik GmbH, Heidelberg, Germany) and the particle size distribution of the fat droplets was determined using a particle size analyser (Malvern Instruments Limited, Malvern, UK).

The functional properties of the processed cheese slice made on RVA were analyzed using Melt tests, Vane test (stress and strain) and Penetrometry (firmness).

### 3.2 Materials

#### 3.2.1 Rennet casein

Edible rennet casein (ALAREN-799) was purchased from NZMP, New Zealand, and its specifications are summarised in Table 3.1.

**Table 3.1. Rennet Casein specification**

	<b>Protein</b>	<b>Fat</b>	<b>Moisture</b>	<b>Mesh size</b>
	(%)	(%)	(%)	
ALAREN 799	83.4	0.4	9.5	90 (100% through 250µm sieve)

#### 3.2.2 Soya oil

Soya oil (AMCO) used for the study was obtained from Goodman Fielder, Auckland, New Zealand.

#### 3.2.3 Starches

The starches used for the studies and their manufacturers are tabulated in Table 3.2.

**Table 3.2. Starches and suppliers**

Starch	Supplier
Cornstarch	National Starch and Chemical Company
Waxy cornstarch	“
High amylose cornstarch (HACS)	“
Rice starch	“
Waxy rice starch	“
Resistant starch	“
Perfectamyl (Di-starch phosphate of potato starch)	“
Acid converted starch	“
Potato starch	Penford NZ Ltd
Wheat starch	“

### 3.2.4 Lactose

Lactose was obtained from NZMP, New Zealand.

### 3.2.5 Trisodium citrate

Trisodium citrate was obtained from Jungbunzlauer (Basel, Switzerland).

### 3.2.6 Salt

Salt was obtained from Pacific Salt NZ Ltd.

### 3.2.7 Citric acid

Citric acid was obtained from Jungbunzlauer (Basel, Switzerland).

### 3.2.8 Cheese

Cheese, one of the most important ingredients in processed cheese provides functional as well as sensory characteristics to processed cheese. The age of the cheese used affects the structure and flavour characteristics of the processed cheese finally obtained. Details of the cheeses used are given in Table 3.3.

**Table 3.3. Different cheeses used for processed cheese manufacture**

<b>Cheese</b>	<b>Properties imparted</b>	<b>Specification</b>	<b>Cypher</b>	<b>Source</b>	<b>Intact casein (%)</b>
Young	Functional properties	30 900	G004	NZMP	12
Mature	Flavour properties	30 100	GN19	NZMP	4

### 3.2.9 Butter

Butter was obtained from NZMP, New Zealand.

### 3.2.10 Potassium sorbate

Potassium sorbate was obtained from Hawkins Watts Ltd, New Zealand.

## 3.3 Sample preparation-Rennet Casein based Processed Cheese Spreads

### 3.3.1 Formulation

The formulation used for model processed cheese preparation is shown in Table 3.4.

**Table 3.4. Ingredients for rennet casein based processed cheese**

<b>Ingredient</b>	<b>Quantity (g)</b>
Rennet casein	3.52
Soya oil	7.885
Lactose	1.26
Citric acid	0.215
Trisodium citrate	0.85
Salt	0.30
Added Water	15.98
<b>Total Weight</b>	<b>30.0</b>

**Table 3.5. Composition for rennet casein based processed cheese spread (Lee, 2004, Personal Communication)**

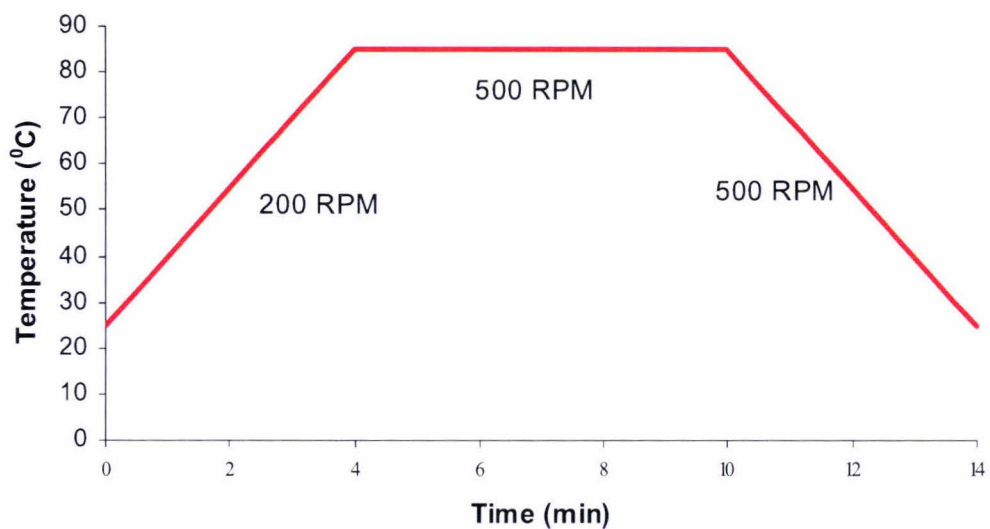
	<b>Target composition (%)</b>
Moisture	54.74
Fat	26.33
Protein	9.79
Intact casein	7.55
Salt	1.0
Lactose	4.19
Target pH	5.70

Starch was added to the processed cheese formulation at addition rates of 0, 1, 2, 3, 4 and 5% of the total weight.

### **3.3.2 Paar Physica**

Model rennet casein based processed cheese sample preparation was carried out in a Starch Cell (ST-24) attached to a UDS 200 Paar-Physica Rheometer (Physica Meßtechnik GmbH, Stuttgart, Germany). The temperature in the rheometer is maintained by a Peltier system and electrical heating elements (cooled by a water bath). The dry ingredients were weighed and then allowed to hydrate in water for 1 h. At this point, oil and starch were added and mixed to give a homogeneous emulsion. This emulsion was then transferred into the stainless steel canister of the starch cell for processed cheese preparation and the canister placed in the rheometer after the emulsion is transferred. A stainless steel paddle is fitted on to the rheometer to provide mixing/shearing. The temperature and shearing profile is as shown in Figure 3.1. Once the processed cheese was cooked, rheological measurements were made in situ, with the temperature being maintained at 25°C. On completion of measurements the cheese was emptied into plastic containers, which were stored at 4°C.

The viscosity was measured throughout the processed cheese manufacture. The data were collected using Physica software US 200 version 2.10 (Physica Meßtechnik GmbH, Stuttgart, Germany).



**Figure 3.1.** Time-temperature profile for Paar Physica sample preparation. The shearing regime (in RPM) at different heating steps is also reported.



**Figure 3.2.** Paar-Physica.

### 3.3.3 RVA

The RVA was used to manufacture rennet casein based processed cheese spread. The RVA can be used to mix and heat a 30 g sample while measuring an apparent viscosity. The sample is weighed into an aluminium can, into which a polycarbonate paddle is inserted prior to loading into the RVA. This aluminium canister is tightly held in an electrically heated jacket and the plastic stirrer stirs the content inside the canister and the measured torque on the stirrer is used to derive an apparent viscosity.

All the dry ingredients (rennet casein, lactose, citric acid, emulsifying salt and salt) are weighed (see the formulation mentioned in 3.3.1) and blended together. Weighed water is added to the dry ingredients. These ingredients are then allowed to hydrate for one hour and the oil or oil and starch are weighed and added to the hydrated dry mixture. This blend is then carefully transferred to the aluminium canister and the canister is inserted into the RVA. The blend is cooked in the RVA for 10 minutes using the heating and shearing profile shown in Figure 3.3.

After cooking the molten processed cheese is transferred to 35 mL plastic vials and cooled immediately in a refrigerator. As mentioned previously the RVA measures the apparent viscosity of the sample during the whole process of processed cheese manufacture and does not allow to perform full rheological measurements as the one performed on the Paar-Physica using cone and plate geometry (40 mm diameter and 4° angle).

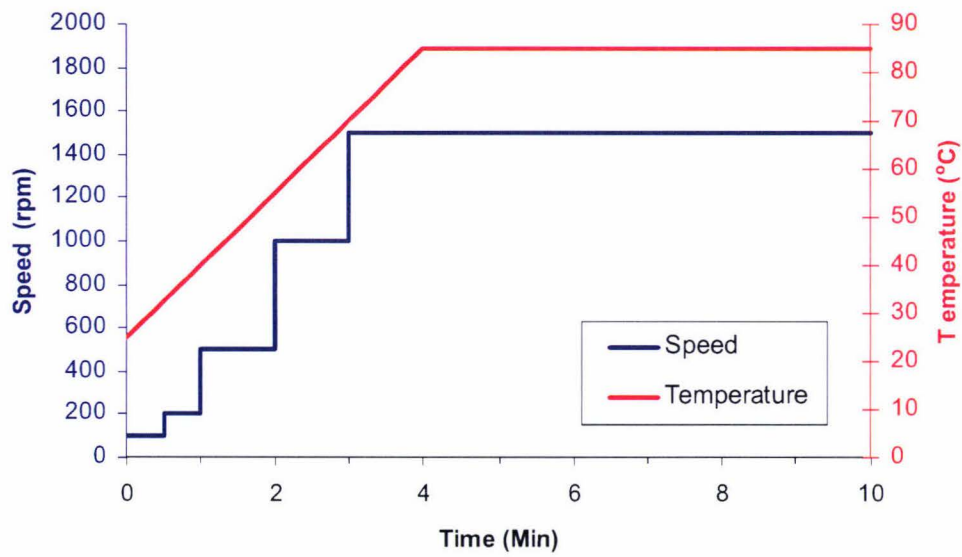


Figure 3.3. Time-temperature and shearing profile used for RVA sample preparation.

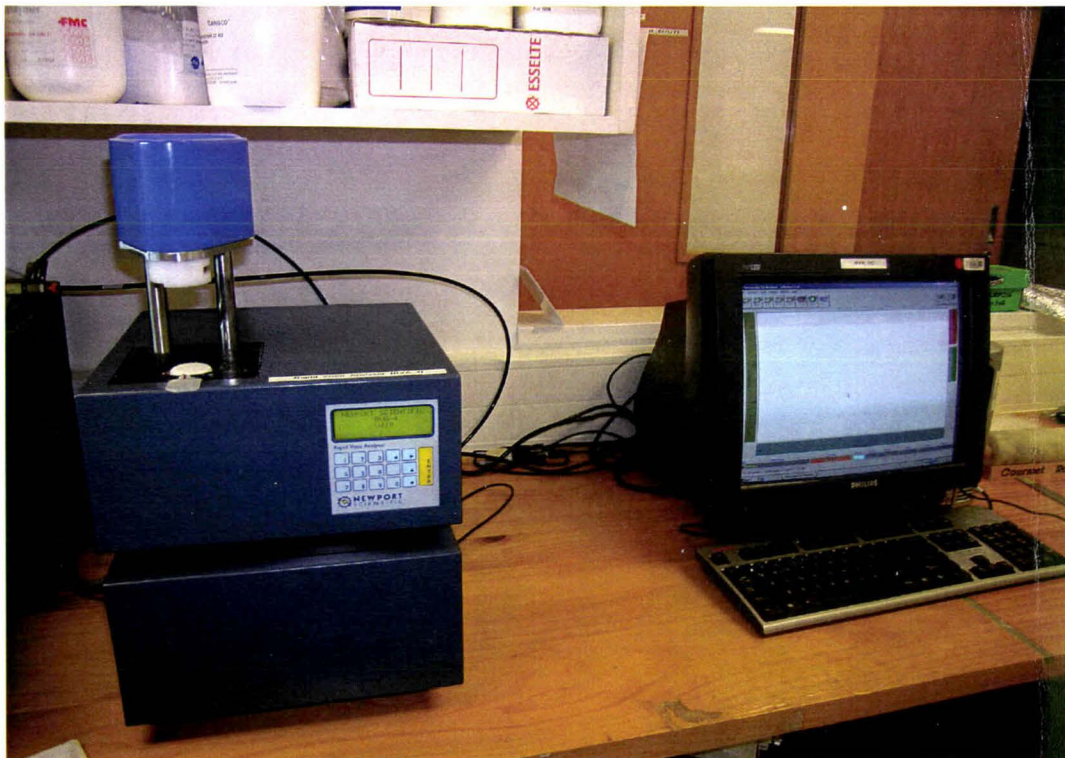


Figure 3.4. Rapid Visco Analyzer (RVA).

### 3.4 Rheological measurements

The samples for rheological measurements were prepared on the starch cell of Paar-Physica rheometer. The rheological properties were determined using low-amplitude dynamic oscillation (stresses or strains were applied in a restricted, sinusoidal manner) with measurement of  $G'$  (storage modulus) and  $G''$  (loss modulus) being the prime objectives.

Stress is the intensity, at a given point in or on the surface of a body, of the components of the force that acts on a given plane through the given point. Strain is the mathematical expression of a change in the size or shape of a body with reference to its original size or shape (Finney, 1972)

The Storage Modulus ( $G'$ ) represents the amount of energy stored per cycle of deformation, and is defined by:

$$G' = (\sigma/\gamma) \cos\delta$$

Where:

$\sigma$  is the maximum stress.

$\gamma$  is the maximum shear strain.

$\delta$  is the phase difference between stress and strain.

Loss Modulus ( $G''$ ) represents the energy lost per cycle of deformation, and is defined by:

$$G'' = (\sigma/\gamma) \sin\delta$$

The Complex Modulus ( $G^*$ ) is a measure of the energy dissipated per cycle of deformation per unit volume and is defined by (Tan, 2003):

$$G^* = [(G')^2 + (G'')^2]^{1/2}.$$

Frequency and Amplitude Sweeps were performed at 25°C. Frequency sweep was followed by amplitude sweep. For the frequency sweep, the frequency was varied from 0.01 to 10 Hz at a constant strain of 1%. The amplitude sweep was performed at a constant frequency of 1 Hz with an applied strain varying from 0.1% to 1000%.

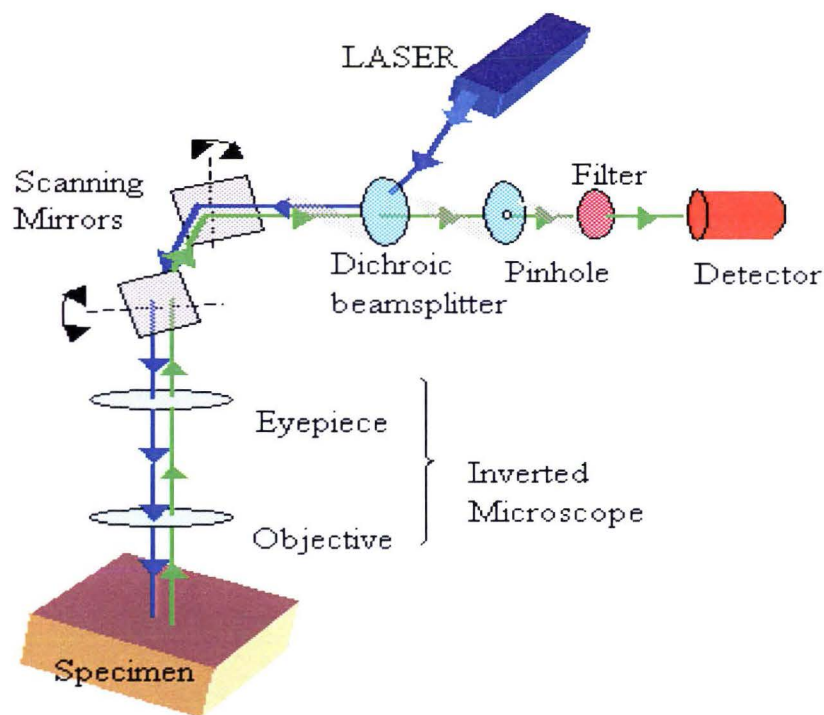
### 3.5 Confocal laser scanning microscopy

Microstructure analysis was conducted at the Institute of Molecular Biosciences, Massey University. Processed cheese samples (approximately 5 mm × 5 mm × 5 mm) were frozen to -20°C for 15-20 minutes in a cryocut machine (Leica Jung Frigocut 2800 E Cryo-Microtome, Leica Instruments, Nussloch, Germany). The cryo-cut allows to section samples to a thickness of about 30 µm. Using the cryo-cut machine, the samples were sectioned into 30µm thickness approximately.

The sections are placed immediately on a glass microscope slide and stained with a solution containing the fluorescent dyes. Fast green (0.33 wt% solution in Citiflour, a glycerol/PBS solution) and Nile Blue (0.33 wt% solution in Citiflour) are specific dyes used for staining of protein and fat respectively. A drop of stain (contains equal amount of Nile Blue and Fast Green) was placed on the section and a cover glass placed on top, which spreads the dye uniformly on the section and prevents drying out of the section. The samples were examined at 25°C with a 40x or 60x objective lens. The micrographs obtained were combined images of fat/oil (red) and protein (green) phases.

Leica Lasertechnik GmbH, Heidelberg, Germany, manufactures the Confocal Laser Scanning Microscope (CLSM). With laser source Ar/Kr 488nm, 568 nm and 647 nm is used to provide the excitation light, laser light is reflected off a dichoric mirror on to 2 further mirrors. The dyes applied to the sample fluoresce and the same mirrors that are used to scan the excitation light from the laser then rescan the emitted light. The emitted light passes through the dichoric mirror and is focused on to the pinhole as shown in Figure 3.5.

The main feature of confocal microscopy is that a detector called photomultiplier tube measures light passing through the pinhole. Single pinhole and illumination pinhole ensures that the image from the focal plane only reaches the detector.



**Figure 3.5. Schematic representation of confocal microscopy** ([http://www.ifr.ac.uk/materials/fractures/images/confocal\\_microscope.gif](http://www.ifr.ac.uk/materials/fractures/images/confocal_microscope.gif)).

Confocal microscopy has certain advantages. The CLSM (Confocal Laser Scanning Microscopy) detects in-focus regions only, the out-of-focus regions appearing black. Therefore the application is not limited to thin samples. Samples can be observed under environmental conditions, which allow the observation of the samples in the hydrated state. A further advantage of CLSM is the possibility to follow in-situ the dynamic processes such as phase separation, coalescence, aggregation, coagulation, solubilization etc. In some cases, only a few preparatory steps are necessary for viewing specimen by CLSM. Also the component in the sample can be labelled using fluorescent dyes as labelling agents (Blonk & Aalst, 1993; Durrenberger et al, 2001).

### 3.6 Particle size measurement

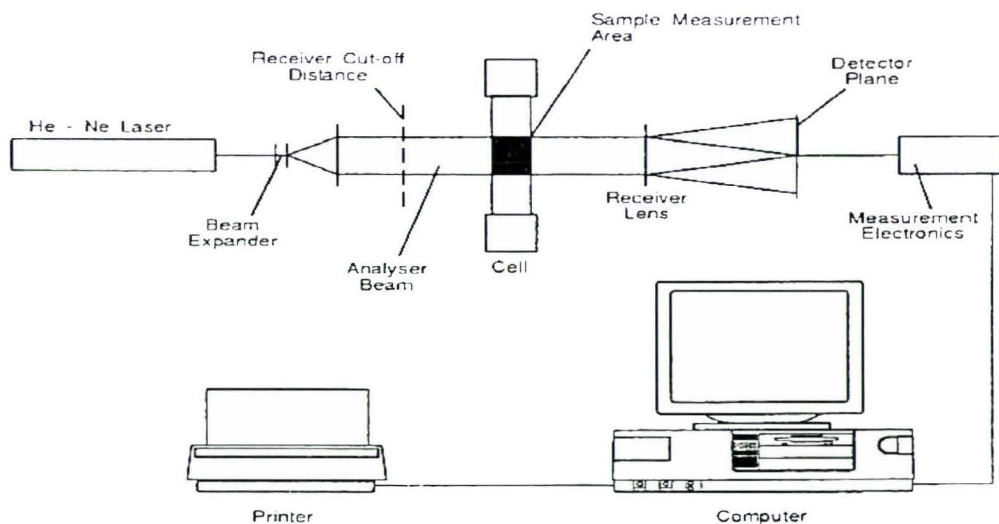
Fat particle size measurement was carried out on a Malvern Mastersizer E (Malvern Instruments Limited, Malvern, UK). Approximately 0.5 g of the processed cheese sample is mixed with 50 mL of Solution A. After allowing it to stand overnight in refrigerated condition, the mixture is then analysed to get the fat particle size.

Solution A is prepared by mixing 3.94 g EDTA (Amersham Biosciences, Sweden) in 800 mL of RO (Reverse Osmosis) water. 1.27 g of Polyoxyethylene Sorbitan Monolaurate (Tween20, Atlas Chemical Company) is added to it. NaOH (0.1 M) is

used to adjust the pH to 10.0. The solution is levelled to 1000 mL using RO water in a volumetric flask.

Alkaline solution of a calcium-complexing agent to which an emulsifier has been added, helps in eliminating the turbidity caused by the caseinate particles. This is achieved by dissolving the casein. Addition of EDTA and Tween20 proves to be very efficient in the disruption of fat globules and destroying the protein network (Walstra, 1965). So that Solution A dissociates casein micelles causing the disruption of the fat globules clusters and allowing the measurement of fat droplets size distribution.

A particle sizer is an instrument wherein optical measuring unit forms the basic part of the particle size sensor and a computer that manages the measurement and performs the result analysis and presentation. Laser light scattering measures the size structure of any one material phase in another. Basically the refractive index of the material to be measured must be different from that of the medium in which it is dispersed.



**Figure 3.6. Schematic representation of particle size analyzer (Malvern Mastersizer E manual).**

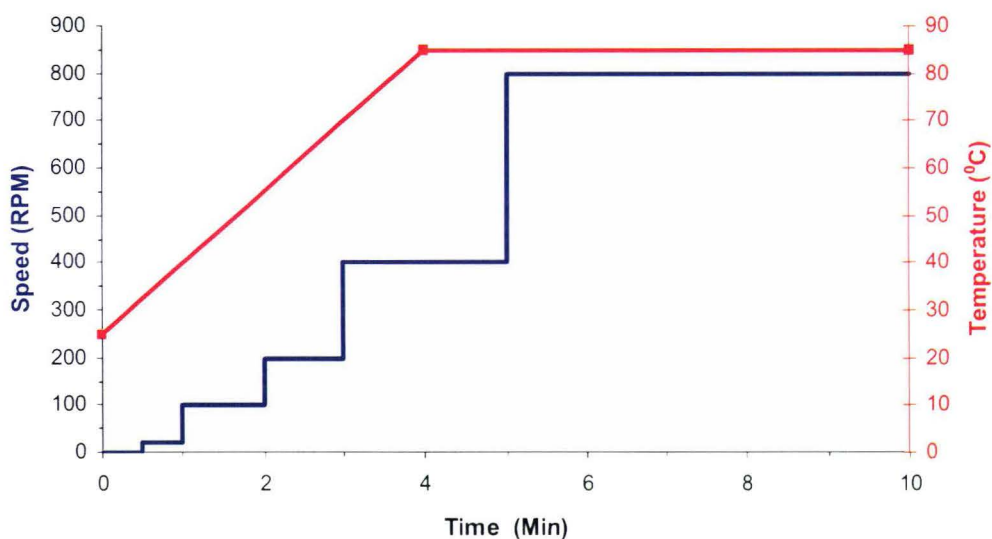
The light from a helium-neon laser is focused to a point in the plane of the detector as shown in the Figure 3.6. All particles present within this beam of light would scatter the laser light. Light scattered by the particles is incident onto a receiver lens. A detector formed by angular sectors, gathers the scattered light. Unscattered light is brought to a focus on the detector and passes through a small aperture in the detector and out of the optical system. The detector measures the diffraction pattern and

provides an electronic outward signal proportional to the light energy measured to the computer. The computer then performs all the calculations to give a particle size.

### 3.7 Sample preparation - Individually Wrapped Slice (IWS) Processed cheese prepared on RVA

Processed cheese slices were prepared on the RVA. The cheese was cut from large blocks, grated and mixed together. The dry ingredients were weighed out, stirred along with the water and then mixed into the cheeses. The blend was then carefully transferred to the RVA canister and then into the RVA.

The cheese blend was cooked in the RVA for 10 minutes. Figure 3.7 shows the time-temperature profile for IWS processed cheese preparation on RVA.



**Figure 3.7. Time temperature profile for IWS processed cheese preparation on RVA.**

Evaporation of moisture during processed cheese manufacture is an issue as it has an adverse impact on the textural properties. To keep record of the moisture loss during the whole process, the canister is weighed before and after the cheese manufacture.

The molten processed cheese from RVA was poured onto a plastic film, covered with another plastic sheet, rolled into a slice of approximately 2 mm thickness and rapidly chilled on an aluminium tray in a refrigerator.

### 3.7.1 Moisture and pH

The moisture content of the slices was measured in duplicate after textural analysis was done. The samples for moisture content measurement were subjected to 105°C for 16 hours.

The pH of the slices was measured after textural analysis by compacting the slice into a 35 mL container and using a Schott M48 pH probe.

### 3.7.2 Viscosity measurement

The viscosity of the processed cheese is measured during the manufacture. It is measured by the RVA. The end viscosity is considered for analysis purpose. Figure 3.8 shows the viscosity profile of processed cheese in a RVA.

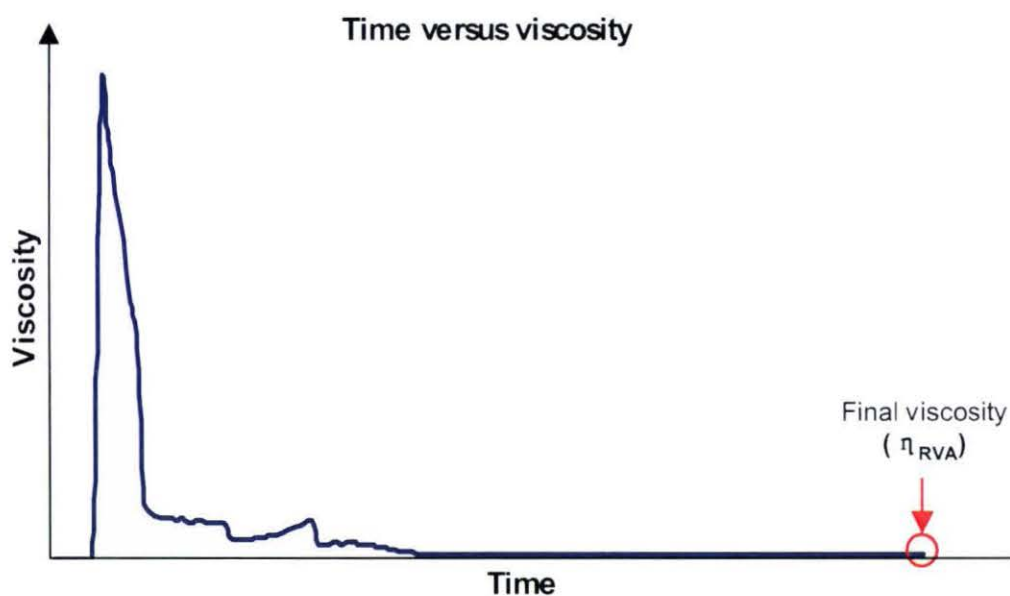
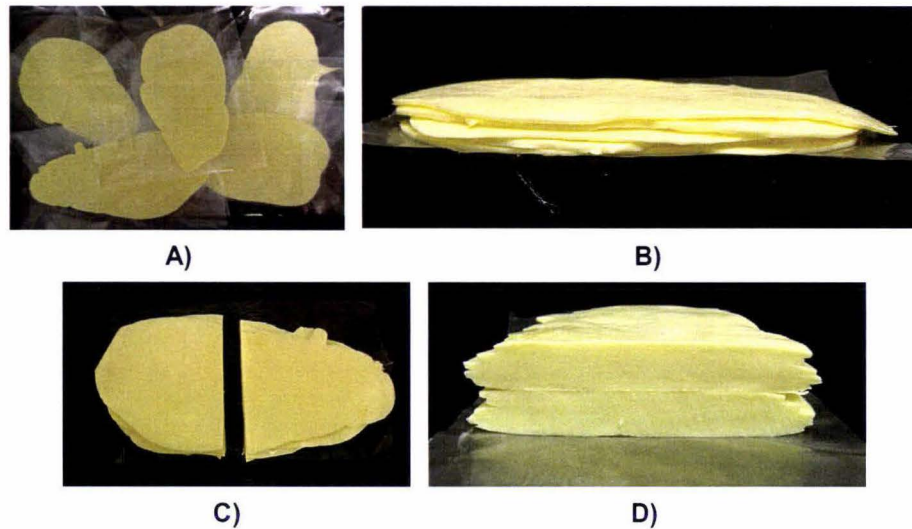


Figure 3.8. Schematic of RVA processed cheese viscosity profile (Hout, 2004).

### 3.7.3 Stack preparation for Penetrometry

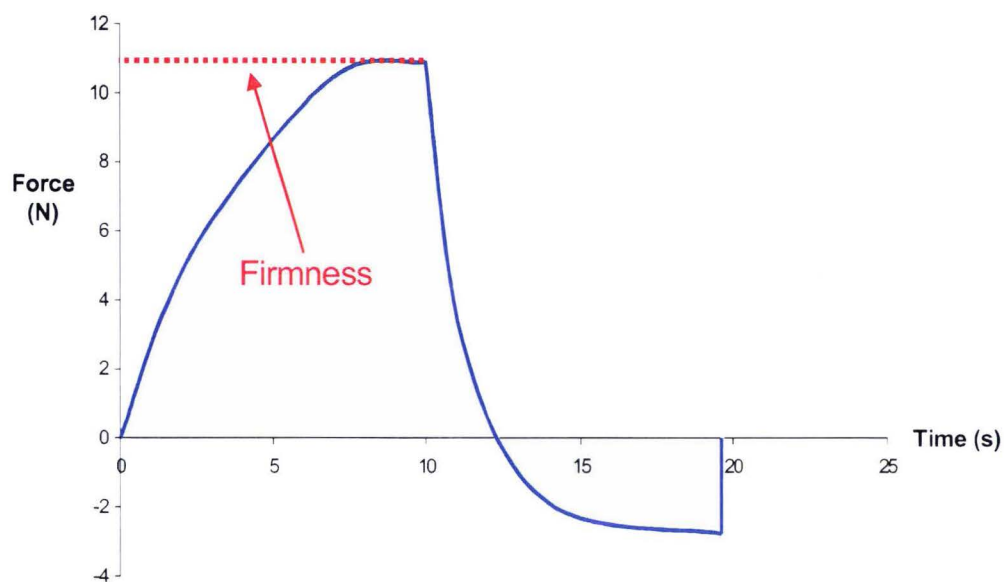
Penetrometry testing requires a minimum stack of 10 slices to conduct the test. Five slices were layered upon each other. The resulting stack was then cut in half and the halves were stacked upon each other to form a new stack, 10 slices thick. Figure 3.9 shows the stack formation process.



**Figure 3.9. Stack formation: A) 5 IWS from RVA manufacture; B) 5 IWS unwrapped and layered; C) IWS layers cut in half; D) two halves stacked for analysis (Hout, 2004).**

#### **3.7.4 Penetrometry texture analysis**

A TA-HD texture analyser (MT-LQ, Stable Micro Systems, England) was used to measure the firmness of the IWS slices. Each set of samples for a particular protein and starch levels from each day were used for the textural analysis. A 6 mm diameter stainless steel cylinder was inserted at  $1 \text{ mm}\cdot\text{s}^{-1}$  speeds to a depth of 10 mm into a 10 slices thick pile. The peak force measured is correlated to the firmness of the product. Each sample was measured 4 times at  $13^\circ\text{C}$ , and they were left to equilibrate for at least 1 h before testing. Figure 3.10 shows a typical penetrometry schematic with peak force measurement.



**Figure 3.10.** Typical penetrometry schematic from the TA-HD texture analyzer, showing peak force measurement.

### 3.7.5 Melt Index

The melt characteristics of each processed cheese sample were assessed using Schreiber Melt test (Kosikowski, 1977). Remaining slices after penetrometry analyses were used for melt testing.

This test involves melting processed cheese circles (39.5 mm diameter, 5 mm thick) at 170°C for 10 min and measuring the melt radius on cooling. Samples were tested in quadruplicate.

### 3.7.6 Vane test

The Vane test consisted of attaching 4-bladed vane to a viscometer, inserting the vane into the sample, and rotating the vane until the sample yielded. The resulting torque/time data were then transformed into stress and strain data and the yield stress and strain values are obtained (Hutt, 2002; Hout, 2004).

The test was performed using a Brookfield Digital Viscometer (Model: 5XHBDTV-II+) and a 6 mm standard vane, in a 13°C room at 0.5 rpm vane speed. Each sample was tested in 4 replicates. 10 slices thick samples prepared for penetrometry analysis is used for the vane test as well.

### **3.8 Sample preparation - Individually Wrapped Slice (IWS) Processed cheese prepared on Blentech cooker**

A Blentech cooker (Blentech Corporation, USA, model CC45) was used for IWS processed cheese preparation in the pilot plant. The cheese was cut from large blocks, ground and mixed together. All of the ingredients were weighed out and placed into the Blentech cooker and then blended at 120 rpm until well mixed. Starch was added on top of the formulation along with water. The mixture was heated to approximately 85°C over 3.5 min, using direct steam injection (DSI). The DSI valve was manually controlled. After cooking, the molten cheese was poured from the Blentech cooker into a clean metal bucket. Half of the molten cheese was passed through a shear pump (colloid mill at 1470 rpm with a 1.08 mm aperture between impeller and body). The function of the shear pump is to homogenize the fat globules in the processed cheese, thereby smoothing the product and supplying a product with consistent viscosity and improved slice separation from the packaging film (Zehren & Nusbaum, 1992).

The sheared and unsheared mixtures were placed into a divided trough over a chilled metal table. The trough was drawn across the table allowing the cheese mixture to drop on to the table (Figure 3.12). By this mean the mixture is cast into a uniform thickness of 2.4 mm approximately. The molten cheese solidifies and is cooled to around 10-12°C within a few minutes. It is then cut into ~80 mm × ~80 mm slices (Figure 3.13).

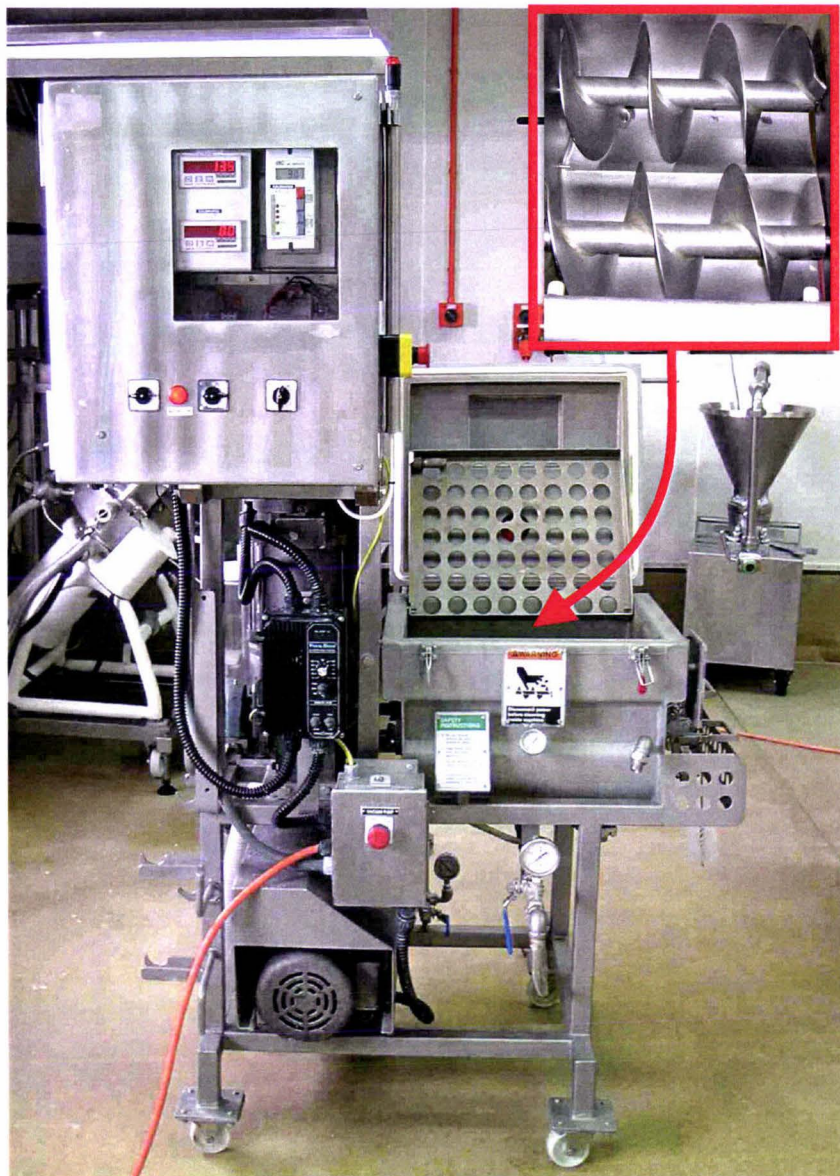


Figure 3.11. Blentech Cooker with augers in the inset.

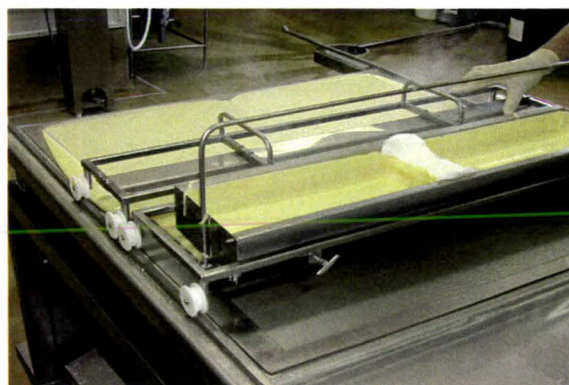
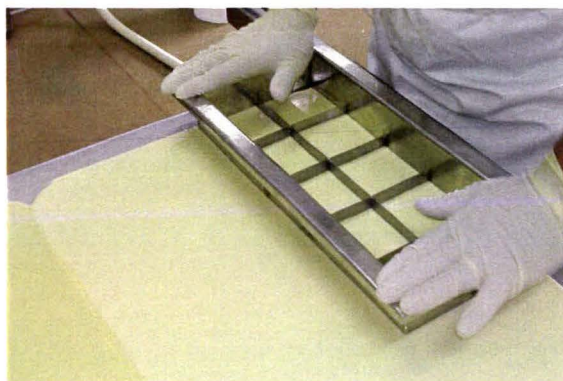


Figure 3.12. Casting the cheese onto the chilled table.



**Figure 3.13.** Cutting slices from the ‘sheared’ mixture.



**Figure 3.14.** Slices being wrapped in polypropylene film.

The slices were removed from the table with the aid of plastic spatulas and wrapped in strips of polypropylene film (Figure 3.14). These slices were stored at 4°C until required for testing. The testing (vane test, cylinder test and melt) of these slices was carried out after 7 days of refrigerated storage (Legg, 2004). Vane tests, cylinder tests and melt tests were carried out.

Penetrometry testing requires a minimum stack of 10 slices to conduct the testing. Ten IWS slices were stacked upon each other. For textural analysis of the processed cheese, vane testing was carried out as described in Section 3.7.6; melt testing as discussed in Section 3.7.5 and penetrometry testing as discussed in Section 3.7.4.

In addition to the method described above, the processed cheese with 15.3% protein and 2% potato starch was prepared on a Stephan cooker (Stephan, Germany, type UMMISK25). The procedure for processed cheese preparation remains the same as in the case of the Blentech cooker. Stirring speed in the Stephan is 700 rpm. Half of the

molten cheese was passed through the shear pump as well. The same test applied for IWS processed cheese prepared on Blentech cooker (Section 3.7) are used to measure the functional properties of the slices prepared on the Stephan cooker.

## **4. Effect of Different Starches on the Rheological Behaviour of Processed Cheese Spreads**

### **4.1 Introduction**

Rennet casein is, in principle a very young cheese, which has the same original calcium content as in young cheese. Like young cheese, it has the ability to form a stable structure. Rennet casein does not suffer from proteolysis and variation of composition compared to cheese. Thus, by using rennet casein based processed cheese formulation uniformity in composition and consistency of attributes such as rheological properties, are more easily achieved (Pereira, 2000).

In this chapter, the effects of different starches on the rheological properties of rennet casein based processed cheese spreads are investigated. Processed cheese spreads were prepared using the Paar-Physica Rheometer starch cell. Viscosity measurement (in-situ), frequency sweep and amplitude sweep measurements were carried out to determine the effect of starches on the processed cheese spreads properties.

The main objectives of the work presented in this chapter were:

- To make model rennet casein based processed cheese spreads at 9.7% protein level and containing ten different starches (cornstarch, waxy cornstarch, high amylose cornstarch, rice starch, waxy rice starch, potato starch, wheat starch, resistant starch, acid converted starch and Perfectamyl) added at six different concentration levels (0, 1, 2, 3, 4 and 5%).
- To measure the rheological properties of the processed cheese spread products.
- To determine the relationship between the rheological behaviour of starch and those of the model processed cheese containing the same starches at different concentrations.

This preliminary work was performed in order to select the starches that give the best rheological properties among all the ten starches and make recommendations for starches to be used for further characterisation such as microstructural measurements.

## 4.2 Experimental approach

A formulation was chosen for rennet casein based processed cheese spreads as mentioned in 3.3.1. Rennet casein was used as a source of protein and provides the structural and functional properties to the processed cheese spreads. To ensure accurate repetition of the samples, the control formulation without starch was prepared and measured on daily basis, and each formulation was prepared in duplicate. The time temperature profile followed to prepare the processed cheese in the starch cell is given in Section 3.3.2. After the processed cheese was made, the rheological measurements were carried out in-situ, with temperature being maintained at 25°C. These rheological measurements consisted of viscosity measurement (during the cheese making) followed by a frequency sweep measurement and an amplitude sweep measurement.

The reasons for selecting the chosen ten starches are reported in Table 4.1 and these include a wide range of varieties. These starches included for example normal rice starch, which is known to have good rheological properties, and resistant starch, which can withstand processing conditions.

The different starches were also characterised by means of particle-size measurements using the Malvern Mastersizer (Section 3.6) and the results are reported in Table 4.2. The results show that the starch granule size varies between 2.9  $\mu\text{m}$  for normal rice starch to 25.4  $\mu\text{m}$  for Perfectamyl starch.

**Table 4.1. Reason for selection of starches for the study**

Starch	Reason for selection
Normal Rice starch	Small granule size, good meltability, good rheological properties <sup>1</sup> .
Waxy rice starch	Waxy form of rice starch that does not form a gel <sup>2</sup> .
Normal Cornstarch	Most abundant of the starches <sup>3</sup> .
Waxy cornstarch	High peak viscosity <sup>4</sup> .
High Amylose Cornstarch (HACS).	Slow gel formation but gives a stable gel on cooling. High pasting temperature <sup>5</sup> .
Normal Potato starch	Reach homogeneous condition more quickly <sup>6</sup> .
Normal Wheat starch	High gelatinisation temperature <sup>7</sup> .
Acid converted starch	Low viscosity during processing, but good gel on cooling <sup>8</sup> .
Perfectamyl (Di-starch phosphate of potato starch)	To compare the effect of a modified starch to the native starches.
Resistant starch	To compare the effect of resistant starch to other starches.

<sup>1</sup> Mounsey and O’Riordan, (2001).

<sup>2</sup> Thomas & Atwell, (1999).

<sup>3</sup> Osman, (1967).

<sup>4</sup> Merkenich et al, (1993).

<sup>5</sup> Bemiller & Whistler, (1996).

<sup>6</sup> Bergthaller, (2004).

<sup>7</sup> Pomeranz, (1985).

<sup>8</sup> Carpenter et al, (1998).

**Table 4.2. Mean Particle size diameter of different starches**

Starch	Mean Particle size ( $\mu\text{m}$ )
Normal Rice starch	2.9
Waxy rice starch	3.1
Normal Cornstarch	8.1
Waxy cornstarch	8.1
High Amylose Cornstarch (HACS).	6.1
Normal Potato starch	20.7
Normal Wheat starch	6.5
Acid converted starch	10.1
Perfectamyl (Di-starch phosphate of Potato Starch)	25.4
Resistant starch	9.2

## **4.3 Results**

### **4.3.1 Starch in water solution**

The pasting behaviour of the ten different starches was performed using a 10 wt% starch solution. This is performed in order to understand how these starches can affect the behaviour of processed cheese spread system. Starch was submitted to the same time-temperature profile as used for processed cheese spreads manufacture (see Section 3.3.2).

For the sake of clarity, the results of the pasting measurements are reported in two different figures. Figure 4.1(A) and Figure 4.1(B) show the viscosity as a function of time for solution containing 10 wt% of different starches. The peak viscosity (the highest viscosity attained approximately after 3.5 minutes) is higher for Perfectamyl starch (Figure 4.1(A)) and lower for HACS (High Amylose Cornstarch) (Figure 4.1(B)). Pasting of 10 wt% cornstarch solution starts slightly later than other starches, which could be seen in Figure 4.1(A). The final viscosity (viscosity attained at the end of 14<sup>th</sup> minute) is highest for Perfectamyl (Figure 4.1(A)) and lowest for HACS (Figure 4.1(B)).

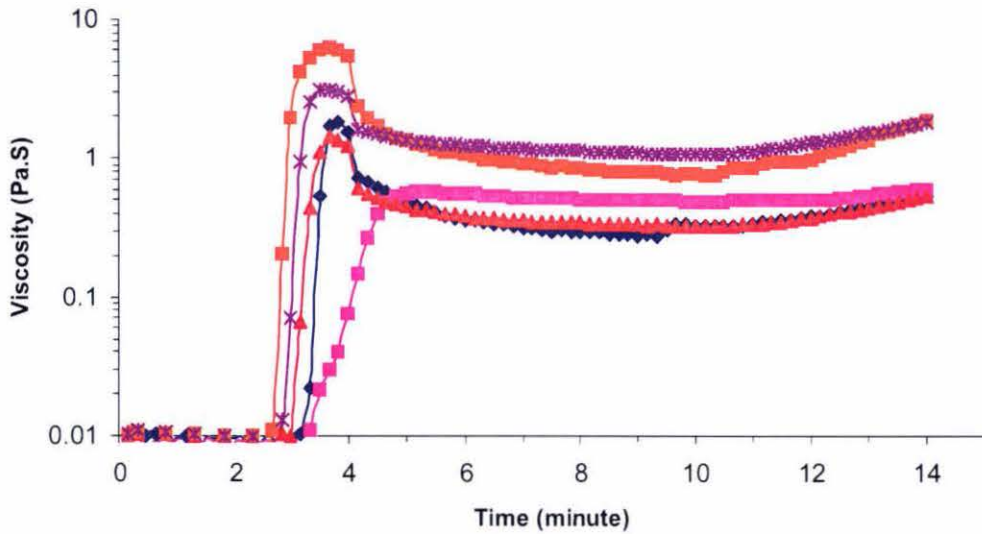


Figure 4.1(A). Viscosity of 10% starch in water solution prepared on Paar-Physica as a function of time. The different starches are waxy cornstarch ( $\blacklozenge$ ), cornstarch ( $\blacksquare$ ), waxy rice starch ( $\blacktriangle$ ), Perfectamyl ( $\blacksquare$ ) and resistant starch (\*).

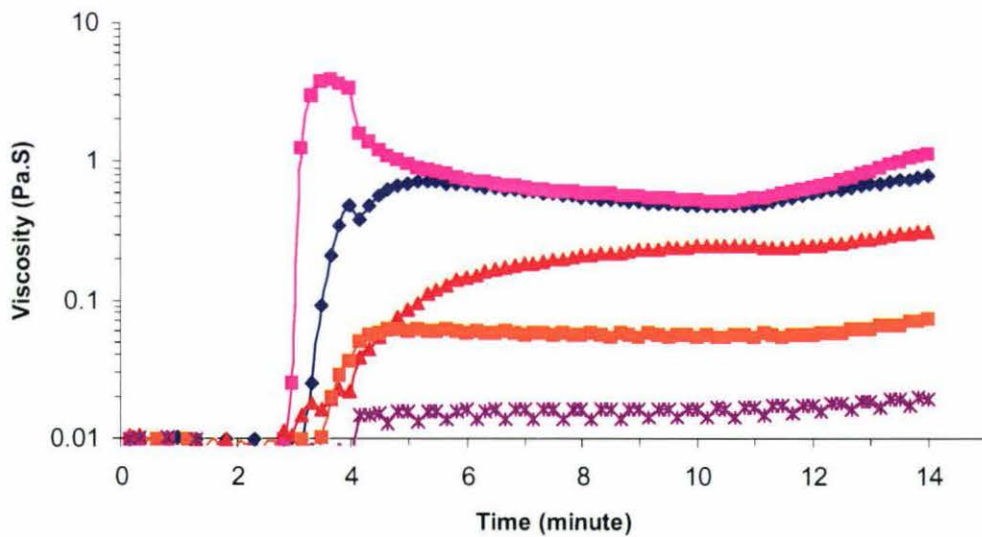
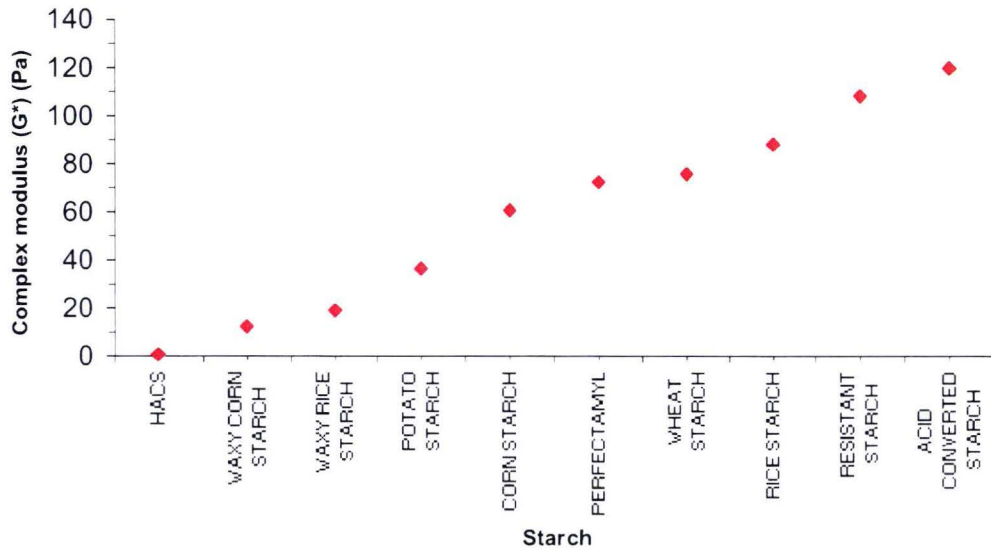


Figure 4.1(B). Viscosity of 10% starch in water solution prepared on Paar-Physica as a function of time. The different starches are rice starch ( $\blacklozenge$ ), potato starch ( $\blacksquare$ ), wheat starch ( $\blacktriangle$ ), acid converted starch ( $\blacksquare$ ) and HACS (\*).



**Figure 4.2. Complex modulus ( $G^*$ ) of 10 wt% solution of different starches at 1Hz.**

To compare the different starches, the complex modulus ( $G^*$ ) values at 1 Hz are reported in Figure 4.2 for 10 wt% solution of different starches. Acid converted starch has the highest  $G^*$  while HACS had the lowest  $G^*$ . The starches had a  $G^*$  in the order acid converted starch > resistant starch > rice starch > wheat starch > Perfectamyl > cornstarch > potato starch > waxy rice starch > waxy cornstarch > HACS.

### 4.3.2 Starch in processed cheese spread

The ten different starches were used for preparing rennet casein based processed cheese spreads. These starches were added at six different concentrations (0, 1, 2, 3, 4, and 5 wt%). The effects of addition of these starches on the viscosity and rheological properties are as discussed below.

Figure 4.3(A) and Figure 4.3(B) shows the viscosity profile of processed cheese spreads containing different starches at 2 wt% addition during the cooking process. Among all the starches added to the processed cheese spread, Perfectamyl (Di-starch phosphate of potato starch) produces the highest increase in the viscosity (Figure 4.3(A)) while HACS produces the least increase in the viscosity (Figure 4.3(B)). The viscosity during processing of the control sample (no starch added) was the smallest. The waxy rice starch and waxy cornstarch do not produce a significant increase in the viscosity of the processed cheese spreads as compared to the normal form of these starches (Figure 4.3(A)).

Figure 4.4(A) and 4.4(B) shows the viscosity of processed cheese spread containing different starches at the 9<sup>th</sup> minute of processing as a function of starch concentration.

Wheat starch has the maximum effect on the viscosity of the processed cheese spreads at 5 wt% starch addition rate (Figure 4.4(B)) while HACS has the least effect at 5 wt% starch addition rate (Figure 4.4(B)). Figure 4.4(A) and Figure 4.4(B) clearly shows that with addition of starch to the processed cheese, there is an increase in the viscosity. All the viscosity values apart from sample containing 1 wt% HACS (Figure 4.4(B)), are higher than the control sample value.

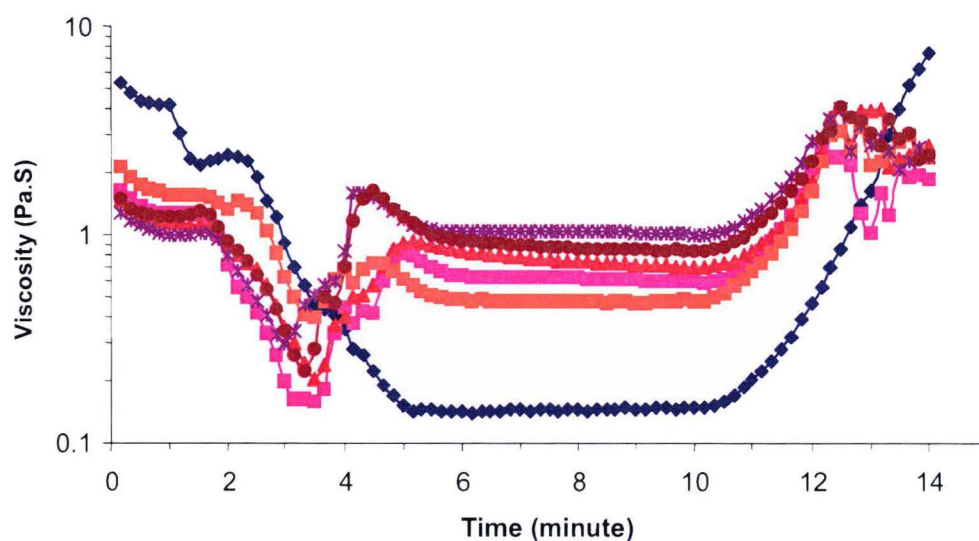


Figure 4.3(A). Viscosity of model processed cheese spread prepared on Paar-Physica as a function of time. The different starches are: 0 wt% control ( $\blacklozenge$ ), 2 wt% waxy cornstarch ( $\blacksquare$ ), 2 wt% cornstarch ( $\blacktriangle$ ), 2 wt% waxy rice starch ( $\blacksquare$ ), 2 wt% Perfectamyl ( $*$ ) and 2 wt% resistant starch ( $\bullet$ ).

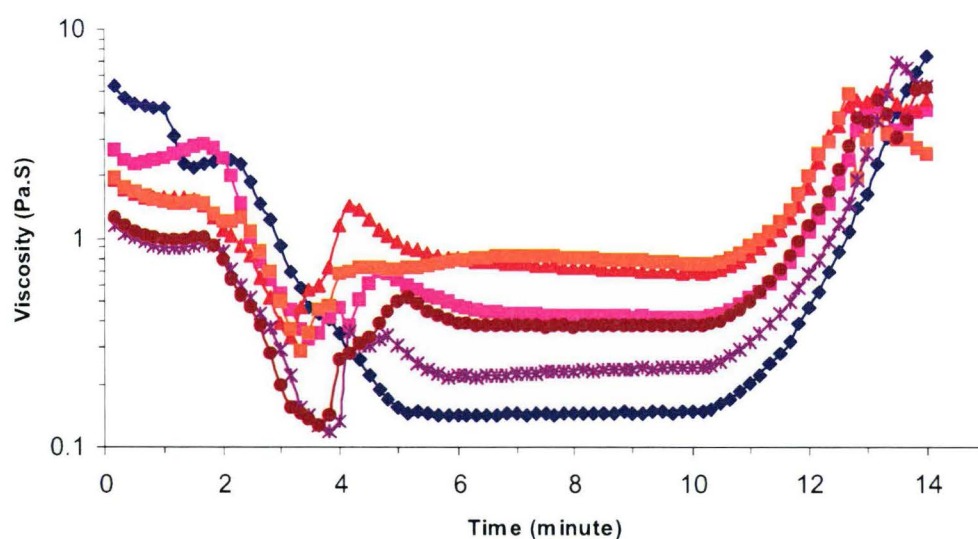


Figure 4.3(B). Viscosity of model processed cheese spread prepared on Paar-Physica as a function of time. The different starches are: 0 wt% ( $\blacklozenge$ ), 2 wt% rice starch ( $\blacksquare$ ), 2 wt% potato starch ( $\blacktriangle$ ), 2 wt% wheat starch ( $\blacksquare$ ), 2 wt% HACS ( $*$ ) and 2 wt% acid converted starch ( $\bullet$ ).

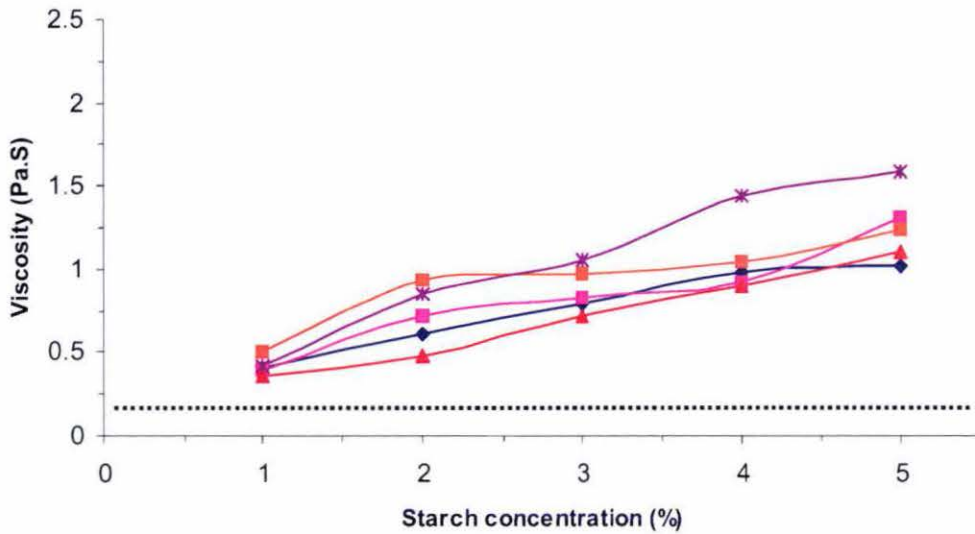


Figure 4.4(A). Viscosity of model processed cheese spread prepared on Paar-Physica as a function of starch concentration at 9<sup>th</sup> minute of processing containing waxy cornstarch (◆), cornstarch (■), waxy rice starch (▲), Perfectamyl (■), resistant starch (\*). Dashed horizontal line is a guide for the value of viscosity of the control sample.

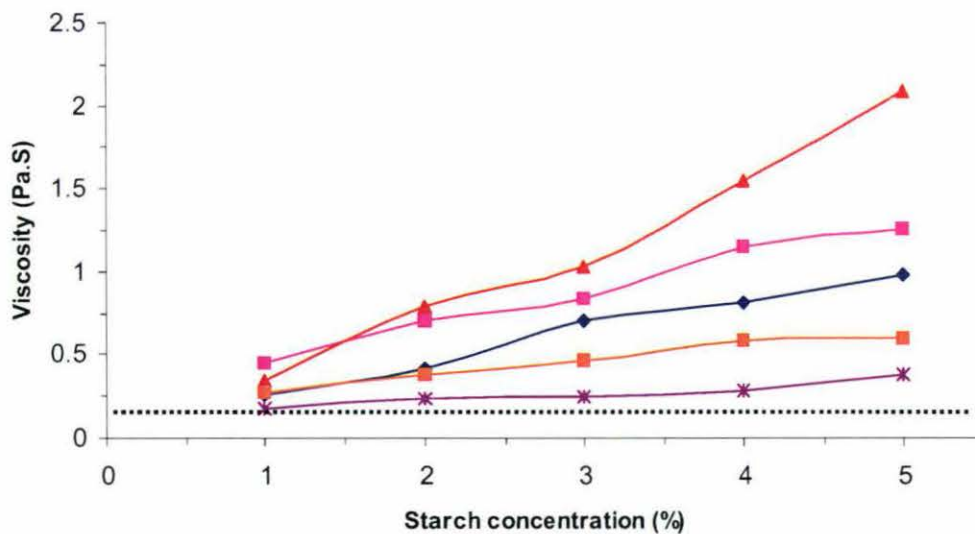


Figure 4.4(B). Viscosity of model processed cheese spread prepared on Paar-Physica as a function of starch concentration at 9<sup>th</sup> minute of processing containing rice starch (◆), potato starch (■), wheat starch (▲), acid converted starch (■) and HACS (\*). Dashed horizontal line is a guide for the value of viscosity of the control sample.

Frequency sweep is one of the most versatile rheological tests to determine the viscoelastic behaviour of materials. A sinusoidal stress or strain of fixed amplitude is imposed and the dynamic moduli are determined over a wide range of frequencies (Gunasekaran & Ak, 2000). Dynamic properties of model processed cheese were measured at a frequency range of 0.01 to 10 Hz at a constant applied strain of 1% (Section 3.3).

Figure 4.5(A) and Figure 4.5(B) shows the elastic modulus ( $G'$ ) and the loss modulus ( $G''$ ) as a function of frequency for processed cheese spread samples containing 2 wt% of different starches. The  $G'$  and  $G''$  values for control sample (no starch added) are also reported for comparison. With increasing frequency (0.01 to 10 Hz),  $G'$  and  $G''$  increased with starch addition. The crossover of  $G'$  and  $G''$  shifts to lower frequencies with starch addition. This shift in crossover frequency is dependent on the type of starch. The crossover frequency is the highest for control and lowest for acid converted starch. This crossover is an indication of the transition in the rheological behaviour from a viscous material ( $G'' > G'$ ) to an elastic material ( $G' > G''$ ). The lower the frequency at which the crossover occurs, the longer the time for the sample to flow.

Complex modulus ( $G^*$ ) ( $G^*=(G'^2 + G''^2)^{1/2}$ ), which includes the contribution of  $G'$  and  $G''$ , gives an idea about the viscoelastic properties. It is a measure of the energy dissipated per cycle of deformation per unit volume (Tunick, 2000). To compare the different samples at different levels of starch addition, complex modulus ( $G^*$ ) values at 1 Hz frequency are reported in Figure 4.6(A) and Figure 4.6(B). The dashed horizontal line is a guide for the value of complex modulus of the control sample. For all the starches there is an increase in the complex modulus value with the increase in the starch addition rate. At 5 wt% starch addition rate, among all the starches HACS (Figure 4.6(B)) has the lowest  $G^*$  value, while acid converted starch (Figure 4.6(B)) has the highest  $G^*$  value.

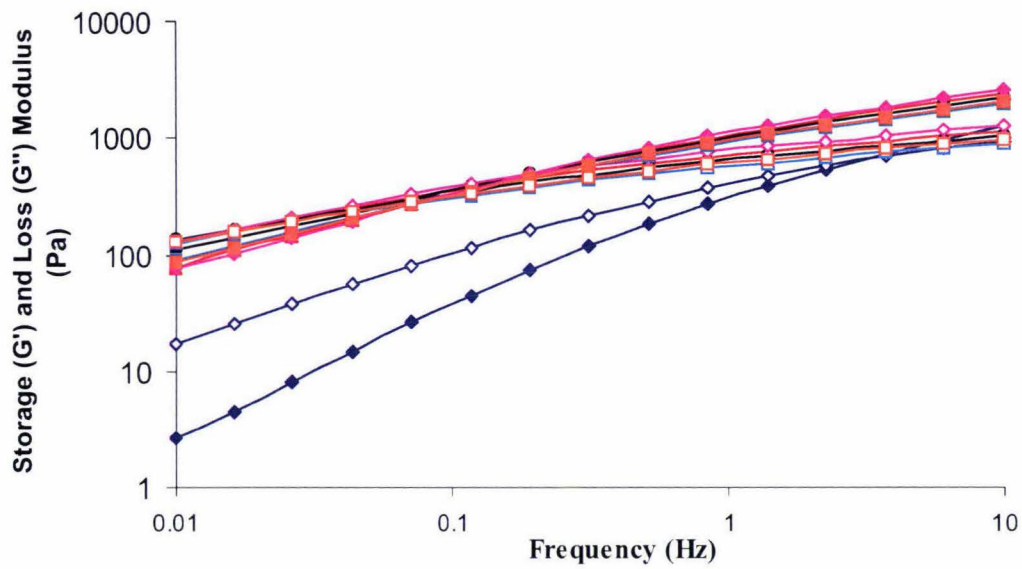


Figure 4.5(A). Storage ( $G'$ ) and Loss ( $G''$ ) modulus of model processed cheese spread prepared on Paar-Physica as a function of frequency at 5°C containing 0 wt% ( $G'$ -♦,  $G''$ -◇), 2 wt% waxy cornstarch ( $G'$ -▲,  $G''$ -△), 2 wt% cornstarch ( $G'$ -●,  $G''$ -○) 2 wt% waxy rice starch ( $G'$ -◆,  $G''$ -◇), 2 wt% Perfectamyl ( $G'$ -■,  $G''$ -□) and 2 wt% resistant starch ( $G'$ -■,  $G''$ -□).

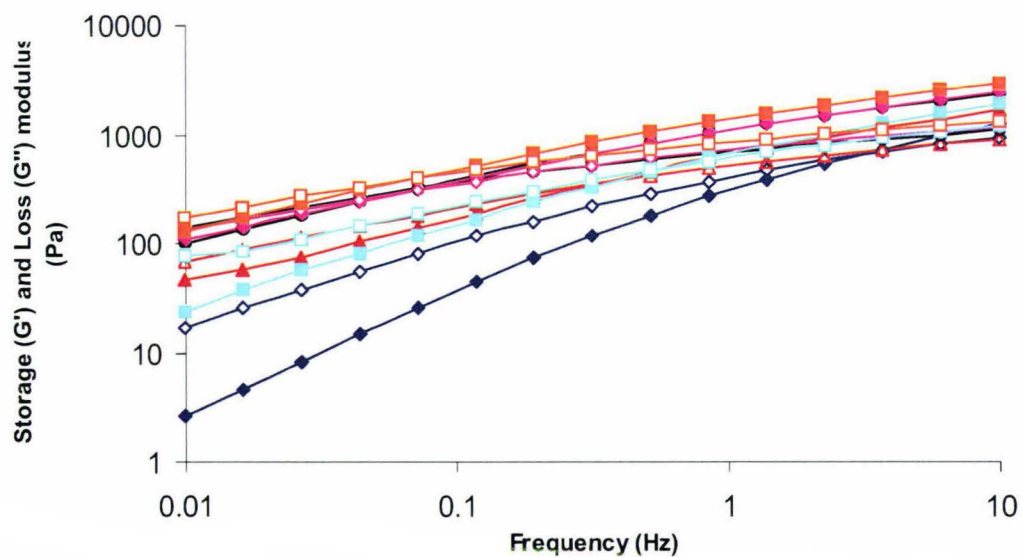
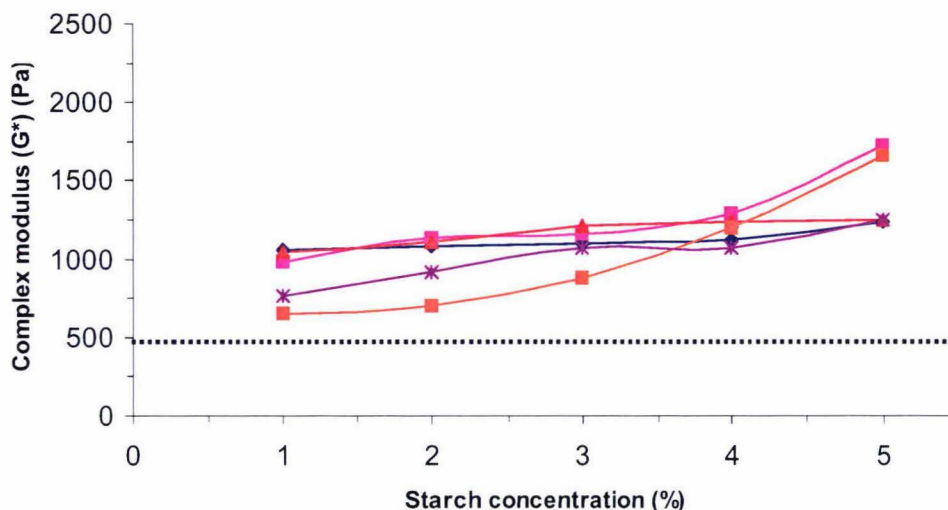
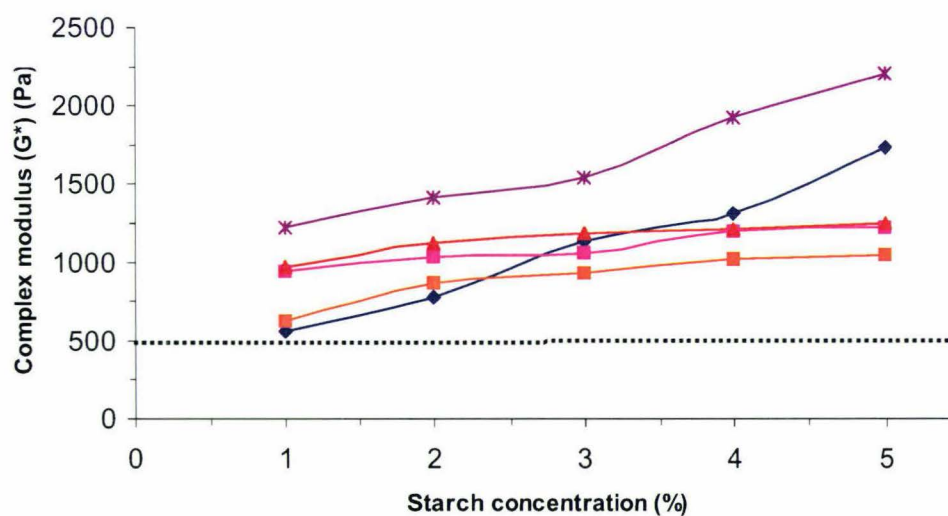


Figure 4.5(B). Storage ( $G'$ ) and Loss ( $G''$ ) modulus of model processed cheese spread prepared on Paar-Physica as a function of frequency at 25°C containing 0 wt % ( $G'$ -♦,  $G''$ -◇), 2wt% rice starch ( $G'$ -▲,  $G''$ -△), 2 wt% potato starch ( $G'$ -●,  $G''$ -○) 2 wt% wheat starch ( $G'$ -◆,  $G''$ -◇), 2 wt% HACS ( $G'$ -■,  $G''$ -□) and 2 wt% acid converted starch ( $G'$ -■,  $G''$ -□).



**Figure 4.6(A).** Complex modulus ( $G^*$ ) of model processed cheese spread prepared on the Paar-Physica as a function of starch concentration at 25°C and at 1 Hz containing waxy cornstarch (♦), cornstarch (■), waxy rice starch (▲), Perfectamyl (■) and resistant starch (\*). Dashed horizontal line is a guide for the value of complex modulus of the control sample.



**Figure 4.6(B).** Complex modulus ( $G^*$ ) of model processed cheese spread prepared on the Paar-Physica as a function of starch concentration at 25°C and at 1 Hz containing rice starch (♦), potato starch (■), wheat starch (▲), HACS (■) and acid converted starch (\*). Dashed horizontal line is a guide for the value of complex modulus of the control sample.

In the strain-sweep experiment, the spread samples were subjected to a strain varying from 0.1% to 1000%, and the dynamic moduli are measured as a function of strain while the frequency is fixed at 1Hz. It is carried out to determine the limits of the linear viscoelasticity and the strain or stress at which the samples “break”. Figure 4.7(A) and 4.7(B) report  $G'$  and  $G''$  as a function of strain for different processed cheese samples containing 2 wt% starch. For all the samples except the control, at low strain (less than 10%),  $G'$  values were higher than  $G''$  values and the samples were independent of strain, this corresponds to the linear viscoelastic region. Above 10% strain,  $G'$  and  $G''$  values decreased with the increase in the strain, as a result of the yielding of the cheese microstructure. This indicates that below 10% deformation, the processed cheese behaved as an elastic material, while at a deformation higher than 10%, the processed cheese behaved as a viscous fluid.

The strain-sweep curves are also used to define the “breakpoint strain”. This strain was chosen to be the strain at which  $G'$  and  $G''$  are equal. Figure 4.8(A) and 4.8(B) reports the “breakpoint strain” for all the measured samples, for different starches at different concentration. For all the samples the value of the “breakpoint strain” increases with increase in the starch concentration. Up to a concentration of 2 wt% except for processed cheese spread with rice starch and HACS, all had comparable “breakpoint strain”. At 5 wt% starch addition rate, Perfectamyl had the highest “breakpoint strain” while HACS has the lowest “breakpoint strain”. The control sample does not show any crossover between  $G'$  and  $G''$  in the strain sweep experiment and hence the values for control are not shown. The “breakpoint strain” of the control was not taken into account as per definition, defined as the cross over between  $G'$  and  $G''$ . Because,  $G''$  was slightly higher than  $G'$  for the control sample, there was no breaking strain. In other words, under large deformation the control sample always flows, instead of breaking.

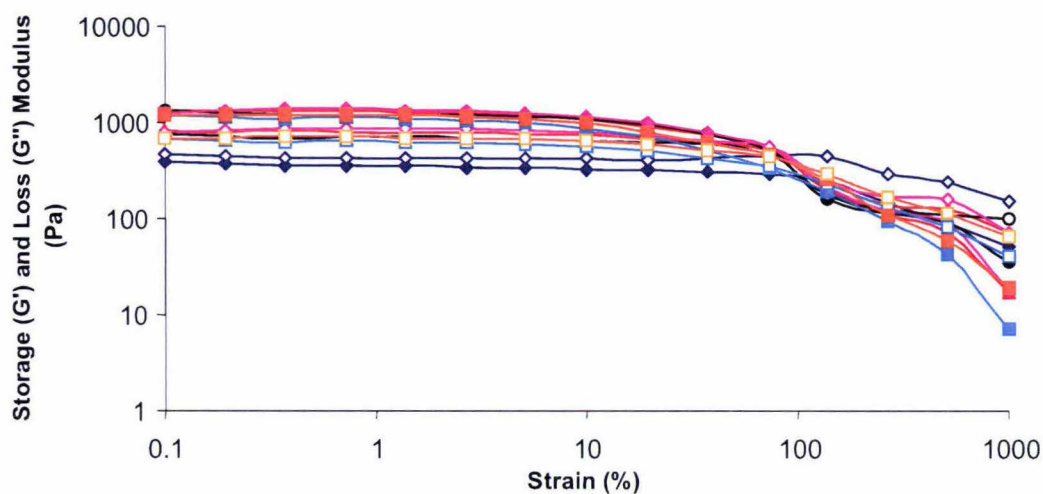


Figure 4.7(A). Storage ( $G'$ ) and Loss ( $G''$ ) modulus of model processed cheese spread prepared on Paar-Physica as a function of strain at 25°C containing 0 wt% ( $G'$ -♦,  $G''$ -◇), 2 wt% waxy cornstarch ( $G'$ -▲,  $G''$ -△), 2 wt% cornstarch ( $G'$ -●,  $G''$ -○) 2 wt% waxy rice starch ( $G'$ -◆,  $G''$ -◇), 2 wt% Perfectamyl ( $G'$ -■,  $G''$ -□) and 2 wt% resistant starch ( $G'$ -■,  $G''$ -□).

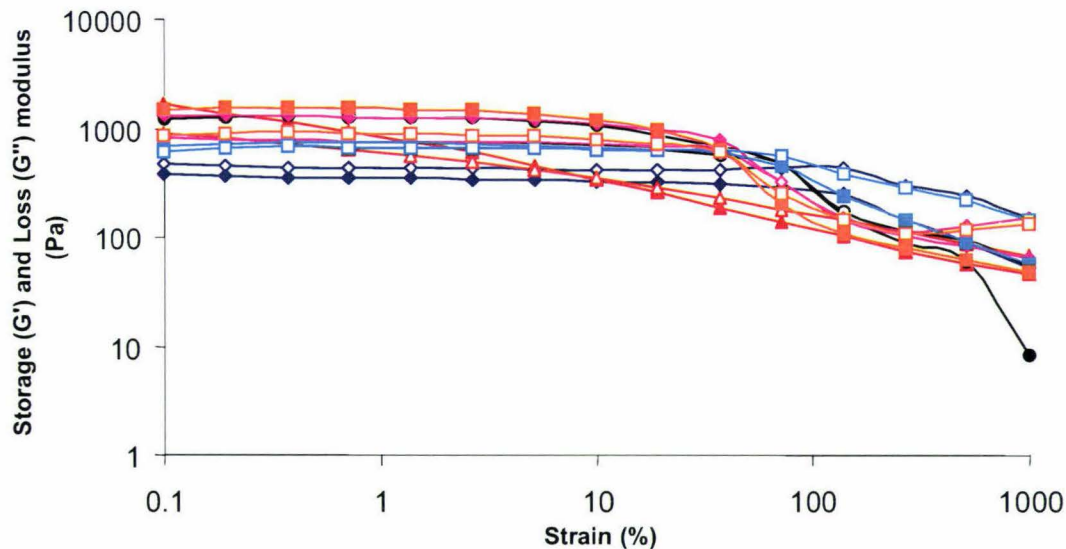


Figure 4.7(B). Storage ( $G'$ ) and Loss ( $G''$ ) modulus of model processed cheese spread prepared on Paar-Physica as a function of strain at 25°C containing 0 wt% ( $G'$ -♦,  $G''$ -◇), 2 wt% rice starch ( $G'$ -▲,  $G''$ -△), 2 wt% potato starch ( $G'$ -●,  $G''$ -○) 2 wt% wheat starch ( $G'$ -◆,  $G''$ -◇), 2 wt% HACS ( $G'$ -■,  $G''$ -□) and 2 wt% acid converted starch ( $G'$ -■,  $G''$ -□).

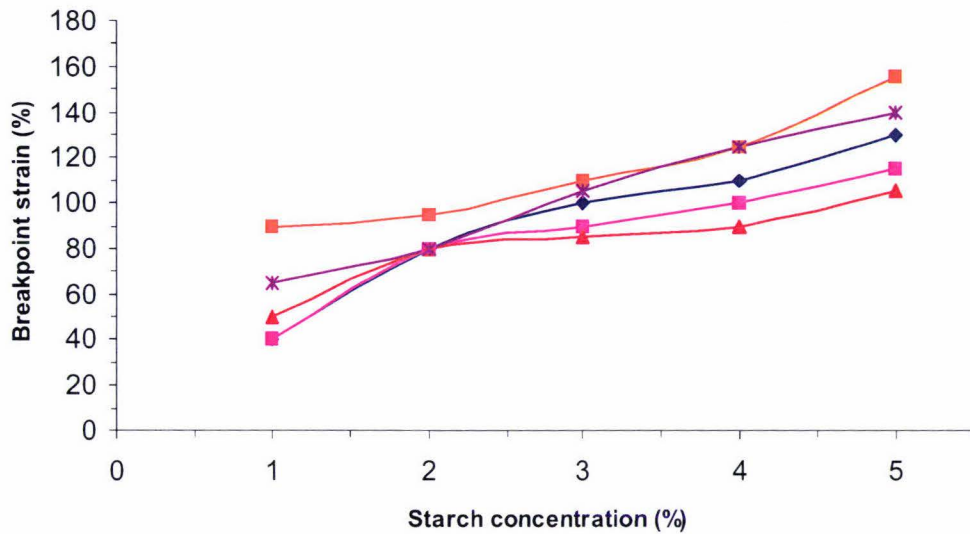


Figure 4.8(A). “Breakpoint strain” of model processed cheese spread prepared on the Paar-Physica as a function of starch concentration at 25°C. Starches are waxy cornstarch (♦), cornstarch (■), waxy rice starch (▲), Perfectamyl (■) and resistant starch (\*).

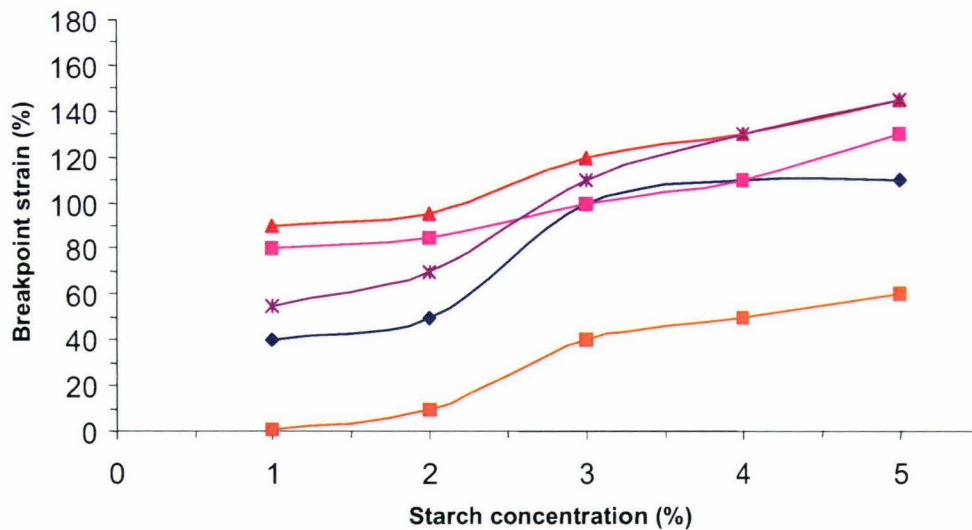


Figure 4.8(B). “Breakpoint strain” of model processed cheese spread prepared on the Paar-Physica as a function of starch concentration at 25°C. Starches are rice starch (♦), potato starch (■), wheat starch (▲), HACS (■) and acid converted starch (\*).

#### 4.4 Discussion

The pasting behaviour of different starch in solution has been extensively studied, but studies on the effect of starches on processed cheese systems are very scarce. Of the ten starches studied in this chapter, it is well known that potato starch has the highest swelling rate among all other native starch (Pomeranz, 1985). Due to this reason and the presence of phosphate monoesters group, more viscosity development in the potato starch containing processed cheese spread is anticipated when it is cooked (Singh et al, 2003). According to Pomeranz (1985), cornstarches gelatinize at a relatively higher temperature and therefore swell rather slowly, giving a delayed pasting of cornstarch solution as compared to other starches. Waxy starches do not form gels and do not produce considerable viscosity increase (Thomas & Atwell, 1999). HACS has a very high gelatinization temperature in the range of about 160-170°C, and essentially does not produce any major increase in viscosity (BeMiller & Whistler, 1996). Acid converted starch does not produce very large viscosity increase during cooking but has a tendency to produce higher viscosity on cooling (Thomas & Atwell, 1999). Rice starch with its small granule size produces soft gels (Bao & Bergman, 2004). Wheat starch with its limited swelling capacity does not produce a significant increase in the viscosity (Figure 4.1(B)) (Mounsey & O’Riordan, 2001).

The pasting behaviour of these starches as reported in the present chapter (Section 4.3.1) is in agreement with what is found in the literature and summarised above. Perfectamyl has the highest viscosity and HACS the lowest viscosity (Figure 4.1(A) and 4.1(B)). Furthermore upon cooling, acid converted starch had the highest elasticity ( $G^*$ ) and HACS the lowest elasticity (Figure 4.2). This could explain partly the effect of the starch in processed cheese. Particularly in the case of HACS, which has a high gelatinisation temperature, and thus does not get gelatinised at temperature of processed cheese cooking temperature (85°C). As a result HACS does not produce any significant effect on the rheological properties of processed cheese spreads as compared to other starches and could be considered as inert filler. In the case of acid converted starch at 2 wt% (Figure 4.2 and Figure 4.6(B)), which exhibited the highest complex modulus ( $G^*$ ) when pasted in water, it did show the highest complex modulus ( $G^*$ ) when added to processed cheese. Though the pasting behaviour corresponds well to the performance in processed cheese for the two extreme cases (HACS and acid converted starch at 2 wt%), this is not true for all the other starches and at the investigated concentrations. This is clearly demonstrated in Figure 4.9 where the viscosity (at 9<sup>th</sup> minute) of

processed cheese containing starch is plotted as function of the viscosity of 10 wt% starch in solution (Figure 4.9(A)) and the  $G^*$  of the processed cheese containing starch is plotted as a function of  $G^*$  of the 10 wt% starch in solution (Figure 4.9(B)). Indeed, Figure 4.9(A) shows that although the viscosity of the processing cheese containing starch during the cheese making is related to the viscosity of the starch, it is not the case when the cheese is cooled down, where  $G^*$  of the processed cheese and the starch solutions do not correlate (Figure 4.9(B)).

This poor correlation in the elasticity of the processed cheese to the elasticity of the added starch could be due to several effects. Processed cheese, although in this chapter simplified to a model processed cheese sample, is a very complex multiphasic system. This system involves protein-protein and protein/fat interactions (Pereira, 2000). The addition of starch increases the number of the different interactions involved by including starch/protein and starch/fat interactions. Furthermore if during cheese manufacture the system is continuously homogenised, phase separation between the starch and the processed cheese network could occur when cooled. This would result in a non-homogeneous microstructure, which exhibit different rheological behaviour from the processed cheese. This phase separation will also depend on several parameters, such as the nature and the concentration of the starch added, and the shear-temperature regime applied during the cheese manufacture. For instance one should expect that at constant shear rates, the higher the viscosity of the system the smaller the resulting fat droplets.

For a better understanding of the effect of starch in processed cheese, in addition to the rheological investigation performed in this chapter, further investigation are required, particularly microstructure observation and fat droplet particle size distribution. These measurements will be performed in the next phase of the study.

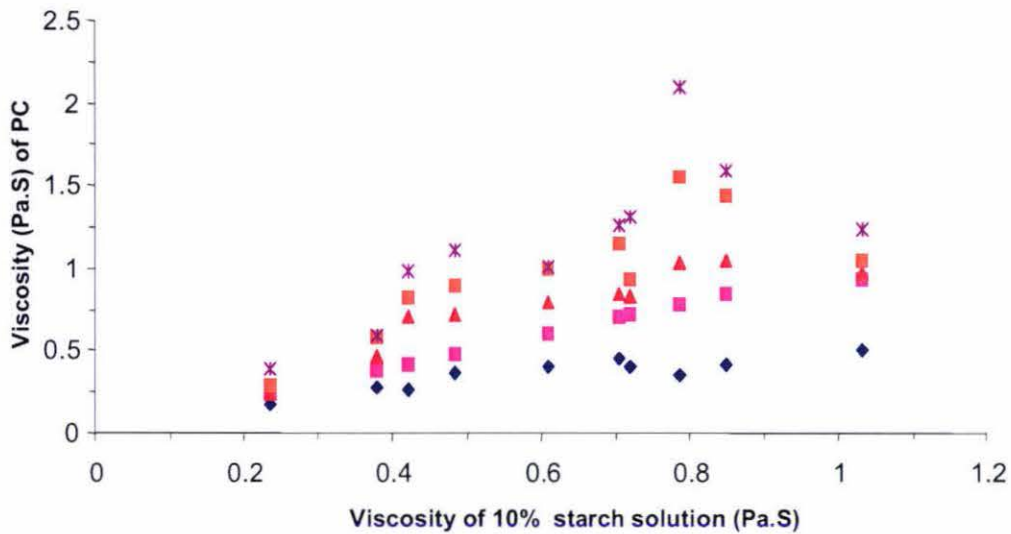


Figure 4.9(A). Correlation between viscosity of the processed cheese (PC) spread samples and the viscosity of the starch in solution with 1 wt% ( $\blacklozenge$ ), 2 wt% ( $\blacksquare$ ), 3 wt%, ( $\blacktriangle$ ), 4 wt% ( $\blacksquare$ ) and 5 wt% ( $\ast$ ) addition of starch. Starch type (from left to right) cornstarch, Perfectamyl, Acid converted starch, wheat starch, potato starch, resistant starch, waxy cornstarch, rice starch, HACS, waxy rice starch.

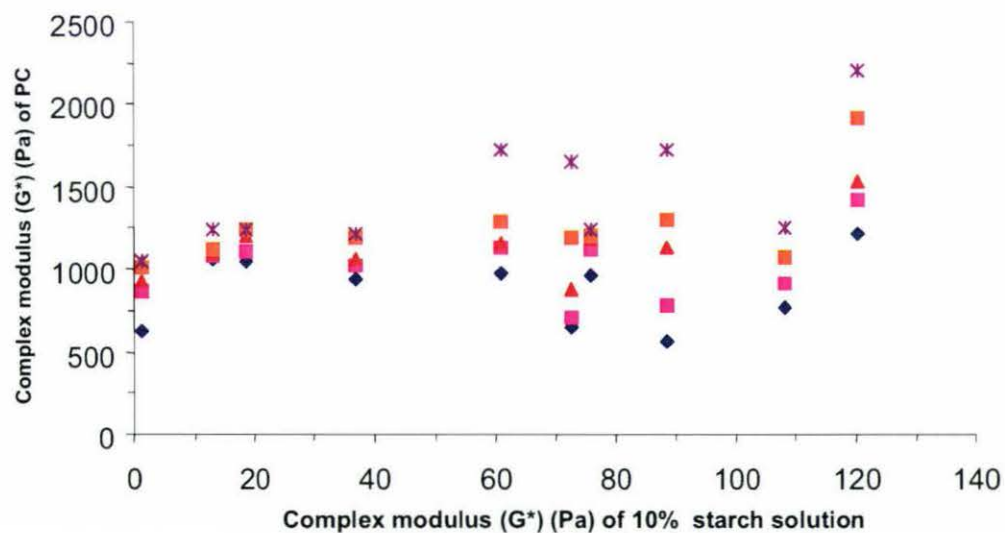


Figure 4.9(B). Correlation between complex modulus ( $G^*$ ) of the processed cheese (PC) spread samples and the complex modulus ( $G^*$ ) of the starch in solution with 1 wt% ( $\blacklozenge$ ), 2 wt% ( $\blacksquare$ ), 3 wt%, ( $\blacktriangle$ ), 4 wt% ( $\blacksquare$ ) and 5 wt% ( $\ast$ ) addition of starch.

#### **4.5 Summary to Chapter**

In this chapter, rennet casein based model processed cheese was prepared using the starch cell of the Paar-Physica rheometer. The effects of ten different starches on the rheological properties were investigated. Acid converted starch had the most significant effect (highest  $G^*$ ) on the processed cheese spread while HACS had the least effect (least  $G^*$ ). Complex modulus, strain and viscosity of the processed cheese spread increase with increase in the starch addition rate. But this increase is totally dependent on the type and physio-chemical properties of the starch. The frequency at which the sample transforms from elastic to viscous is highest for HACS and least for wheat starch. Acid converted starch produces the highest complex modulus ( $G^*$ ) in a processed cheese system, when pasted in water.

## **5. Rheological and Microstructural Properties of Rennet Casein based Processed Cheese Spreads containing Selected Starches**

### **5.1 Introduction**

In the previous chapter (Chapter 4) the effects of ten different starches on the rheological properties of rennet casein-based processed cheese spreads were studied. Different rheological measurements were performed to determine the effect of starches on the rheological properties. It was found that each starch, depending upon its source and its physico-chemical properties, has a different effect on the rheological properties of processed cheese.

In this chapter, in addition to the rheology, the effect of starch on the microstructural properties and the fat particle size is investigated. The effect of starches at different addition rates on the rheological, microstructural and particle size characteristics were determined. However, in this chapter the number of starches investigated is reduced from ten to six. These starches were chosen on the basis of the rheological properties they imparted on the processed cheese spreads.

The Paar Physica and the RVA were used to manufacture processed cheese samples. This allowed the study of the effect of different sample preparation methods and the starch addition on the properties of processed cheese spreads. The time-temperature combination used for sample preparation on Paar-Physica is mentioned in Section 3.3.2, while the samples prepared on RVA are given in Section 3.3.3.

The main objectives of the work presented in this chapter were:

- To make rennet casein based processed cheese spreads using the Paar Physica (starch cell) and the RVA.
- To evaluate six different starches (Potato Starch, Wheat Starch, Rice Starch, HACS, Waxy CornStarch and Acid Converted Starch) added at four different levels (0, 1, 2 and 3 wt%).
- To determine the rheological and the microstructural properties of the processed cheese spreads containing different starches.

- To select the starch which gives the best rheological and microstructural properties.

This work is performed in order to further understand the effect of starch on processed cheese so that recommendation can be made as to which starches could be used for protein reduction.

## 5.2 Experimental approach

From the previous chapter, six starches were selected, namely potato starch, rice starch, wheat starch, waxy cornstarch, acid converted starch and HACS. Based on the preliminary work performed in Chapter 4, acid converted starch was selected because it had maximum effect on the rheological properties (highest complex modulus) of the processed cheese spreads. Potato starch, rice starch and wheat starch were selected mainly on the intermediate functional properties they imparted to processed cheese spreads. HACS has high pasting temperature (160-170°C) compared to other starches, which allows investigating the effect of a non-pasted starch on a processed cheese system. Furthermore, HACS has least effect on the rheological property (complex modulus) of processed cheese spread. To have a wider range of different starches, waxy cornstarch was added, as the five selected starches are mainly normal starches. These six starches were added to a processed cheese spread formulation at 1, 2 and 3 wt% level.

Processed cheese spreads samples can be prepared on the Paar Physica or RVA. Although both equipments rely on the heating and shearing of a sample (Section 3.3.2 and Section 3.3.3), they have different shearing geometry. Due to the difference in the shearing geometry, there would be differences in the rheological and microstructural properties of the prepared samples.

To monitor the effect of storage time, the samples were stored in a refrigerator at 4°C for 30 days. During this period rheological properties were measured for all of the samples at 0, 10, 20 and 30 days after manufacture.

### 5.3 Results

Six different starches were used for preparing rennet casein based processed cheese spreads using the Paar-Physica and the RVA. These starches were added at three different rates (0, 1, 2 and 3 wt%). The effects of addition of these starches on the rheological and microstructural properties are discussed below. Because of the difference in the applied shear due to the difference in the shearing geometries, the results of the Paar-Physica and the RVA will be presented separately.

#### 5.3.1 Samples prepared on Paar-Physica

##### *Rheological Measurement*

Figure 5.1 shows the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) as a function of frequency for processed cheese spread samples containing 2 wt% of various starches. The measurements were performed at 25°C, with an applied strain of 1%. The control sample, which does not contain starch, is reported for comparison. For all the measured frequencies (from 0.01 to 10 Hz), the moduli  $G'$  and  $G''$  increased with increased starch addition. For instance, the cross over frequency is lowest for acid converted starch and highest for the control sample. In addition, the crossover of  $G'$  and  $G''$  shifts to lower frequencies with increased starch addition. This crossover indicates the transition from viscous to elastic behaviour; with the lower the frequency at which the crossover occurs the less the sample flows (Gunasekaran and Ak, 2000).

To compare the different samples at different starch levels, the complex modulus  $G^*$  at 1 Hz is reported in Figure 5.2. The marked horizontal line in Figure 5.2 indicates the complex modulus value of the control processed cheese spread. For all starches there is an increase in  $G^*$  values at all starch addition rates. Among all the starches HACS produces the least increase in the complex modulus values. At 1 wt% starch addition rate, wheat starch gives the maximum value of complex modulus, at 2 wt% starch addition rate acid converted starch gives the maximum complex modulus value and at 3 wt% starch addition rate wheat starch produces the highest  $G^*$  value.

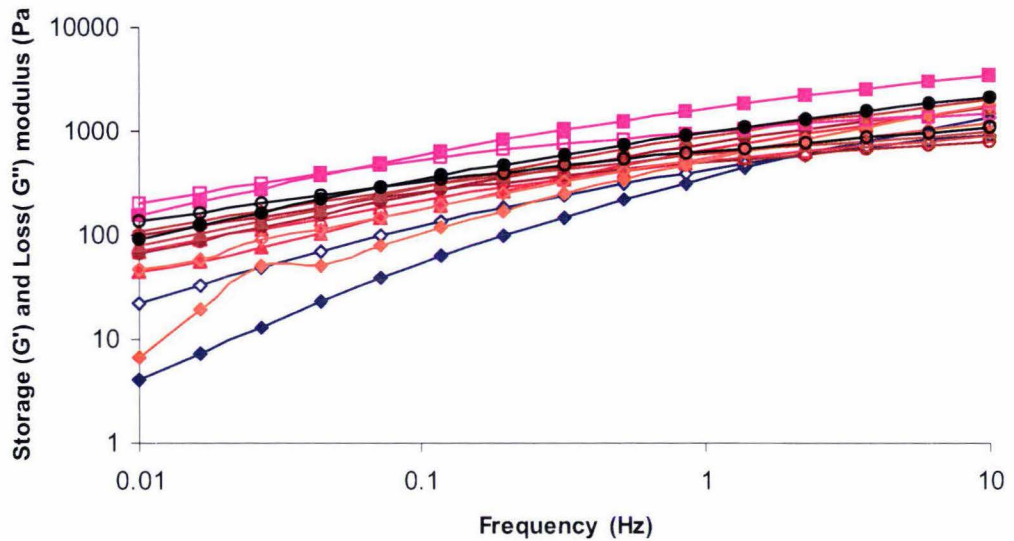


Figure 5.1. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are: 0 wt % ( $G'$ - $\diamond$ ,  $G''$ - $\diamond$ ), 2 wt% rice starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% potato starch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ), 2 wt% wheat starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% HACS ( $G'$ - $\blacklozenge$ ,  $G''$ - $\lozenge$ ), 2 wt % acid converted starch ( $G'$ - $\blacksquare$ ,  $G''$ - $\square$ ) and 2 wt% waxy corn starch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ).

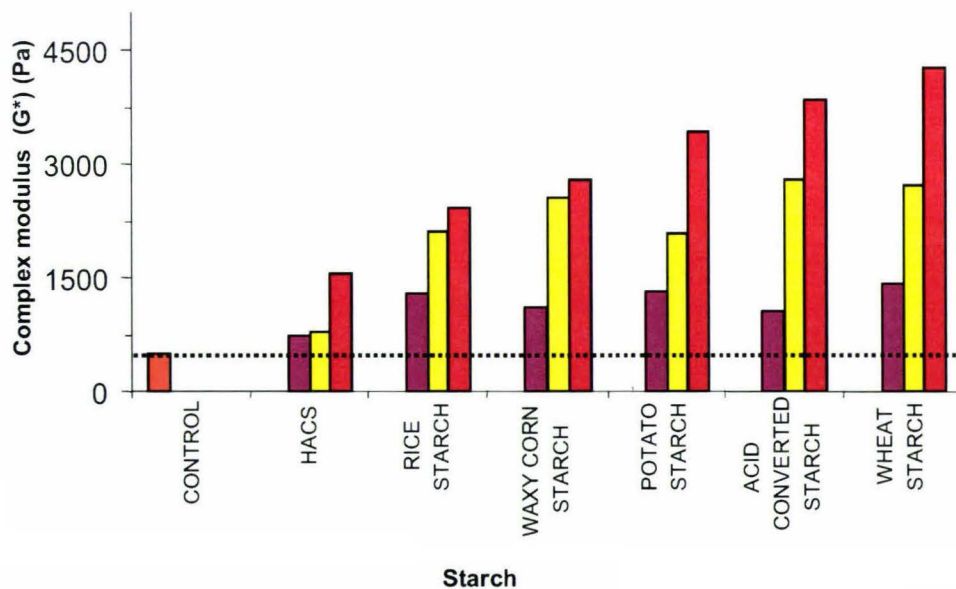


Figure 5.2. Complex modulus ( $G^*$ ) at 1 Hz and measured at 25°C of model processed cheese spread prepared on the Paar-Physica. Starch concentration: 0 wt% ( $\blacksquare$ ), 1 wt % ( $\blacklozenge$ ), 2 wt% ( $\blacktriangle$ ) and 3 wt% ( $\blacklozenge$ ). Dashed horizontal line indicates the value of  $G^*$  of the control sample.

Figure 5.3 reports  $G'$  and  $G''$  as a function of strain for different processed cheese spread samples containing 2 wt% starch. The strain at which a crossover between  $G'$  and  $G''$  occurs, is chosen as the strain at which the sample flows and is here defined as the “breakpoint strain”. Figure 5.4 reports this “breakpoint strain” for all the measured samples, for the different starches and at different starch concentration. For all the samples the value of the “breakpoint strain” increases with the increase in the starch concentration. Up to the concentration of 2 wt%, potato starch, wheat starch and acid converted starch had the highest “breakpoint strain”. At 3 wt% added starch, rice, potato and wheat starches had “breakpoint strain” of comparable values, with HACS being the lowest. Note that the control does not show a crossover between  $G'$  and  $G''$  in the strain-sweep experiment. Thus no “breakpoint strain” comparisons can be made between starch containing samples and the control.

#### ***Particle size measurement***

Figure 5.5, shows the particle size distribution of processed cheese samples containing 2 wt% starch as measured by the Malvern Mastersizer (see Section 3.6). The control sample shows that the particle size distribution is a bimodal exhibiting two main particle size classes having peaks of 0.5  $\mu\text{m}$  and 4  $\mu\text{m}$ . Because the casein protein network making the cheese network is expected to be fully dissolved by EDTA (Section 3.6), the particle size distribution measured is that of the fat droplets. Cheese samples containing waxy cornstarch or HACS showed similar distribution to the control. For the other starches, namely rice starch, wheat starch, potato starch, and acid converted starch, higher particle sizes are measured (see Figure 5.5 at radius higher than 50  $\mu\text{m}$ ). This indicates firstly that the particle sizes observed are much higher than those of the control sample, and secondly that there are other particles sizes which are out of the range of the measuring equipment.

From these particle size distribution, the  $D[4,3]$ , the diameter of equivalent mean volume of the particles, was calculated and reported in Figure 5.6. Although it is very difficult to extract a clear trend from this figure, due to the fact that for some starches the particle size is out of the range of the measurement. Figure 5.6 clearly shows that except for HACS and waxy cornstarch, up to a concentration of 2 wt%, all the samples had mean particle size at least two folds higher than the control sample.

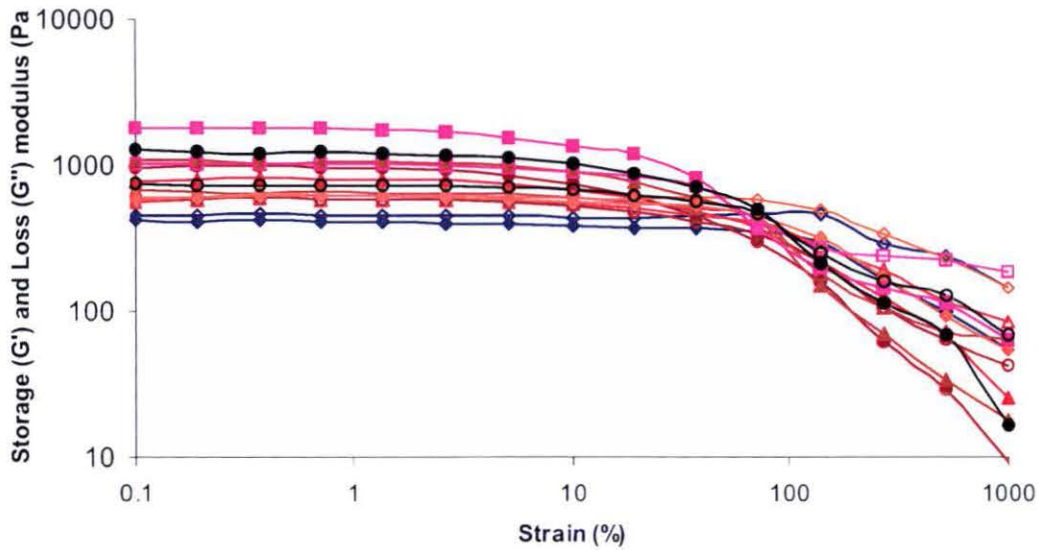


Figure 5.3. Storage ( $G'$ ) and Loss ( $G''$ ) modulus at 1 Hz frequency as a function of strain (%) of model processed cheese spread prepared on Paar-Physica at 25°C. Starches are: 0 wt % ( $G'$ - $\blacklozenge$ ,  $G''$ - $\blacklozenge$ ), 2 wt% rice starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\blacktriangle$ ), 2 wt% potato starch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ), 2 wt% wheat starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% HACS ( $G'$ - $\blacklozenge$ ,  $G''$ - $\blacklozenge$ ), 2 wt % acid converted starch ( $G'$ - $\blacksquare$ ,  $G''$ - $\square$ ) and 2 wt% waxy cornstarch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ).

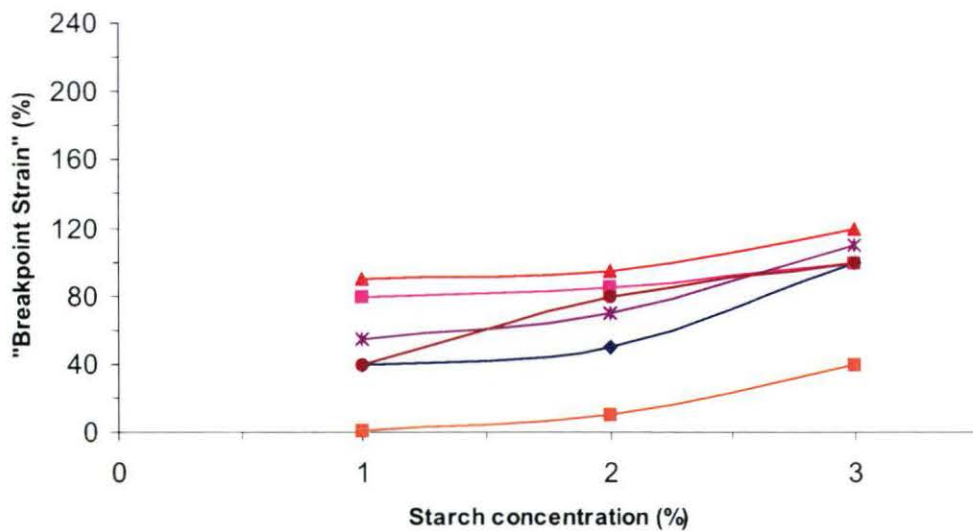


Figure 5.4. "Breakpoint strain" of model processed cheese spread prepared on Paar-Physica as a function of starch concentration at 25°C at 1 Hz frequency containing rice starch ( $\blacklozenge$ ), potato starch ( $\blacksquare$ ), wheat starch ( $\blacktriangle$ ), HACS ( $\blacksquare$ ), acid converted starch ( $*$ ), waxy cornstarch ( $\bullet$ ).

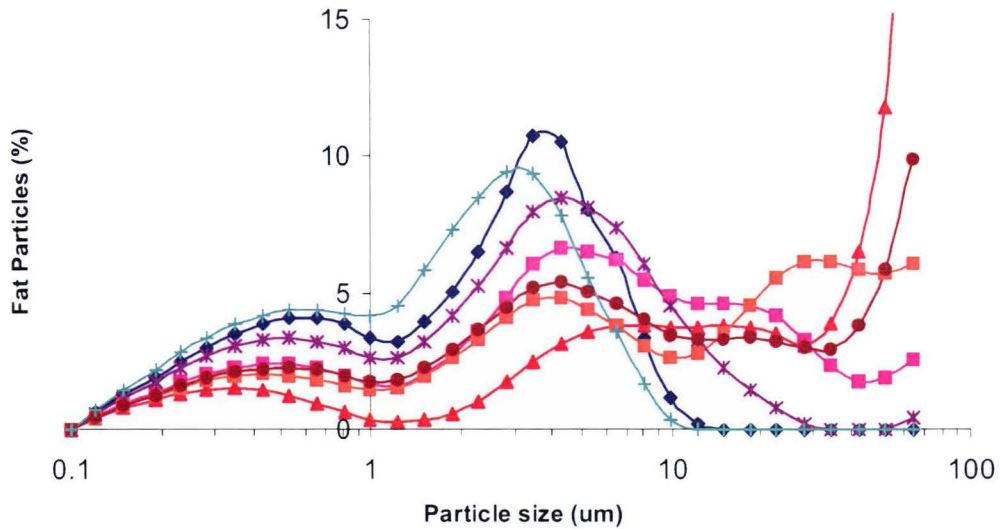


Figure 5.5. Particle size distribution of model processed cheese spreads prepared on Paar-Physica with control ( $\blacklozenge$ ), 2 wt% rice starch ( $\blacksquare$ ), 2 wt% potato starch ( $\blacktriangle$ ), 2 wt% wheat starch ( $\blacksquare$ ), 2 wt% HACS ( $\times$ ), 2 wt% acid converted starch ( $\bullet$ ) and 2 wt% waxy cornstarch ( $+$ ).

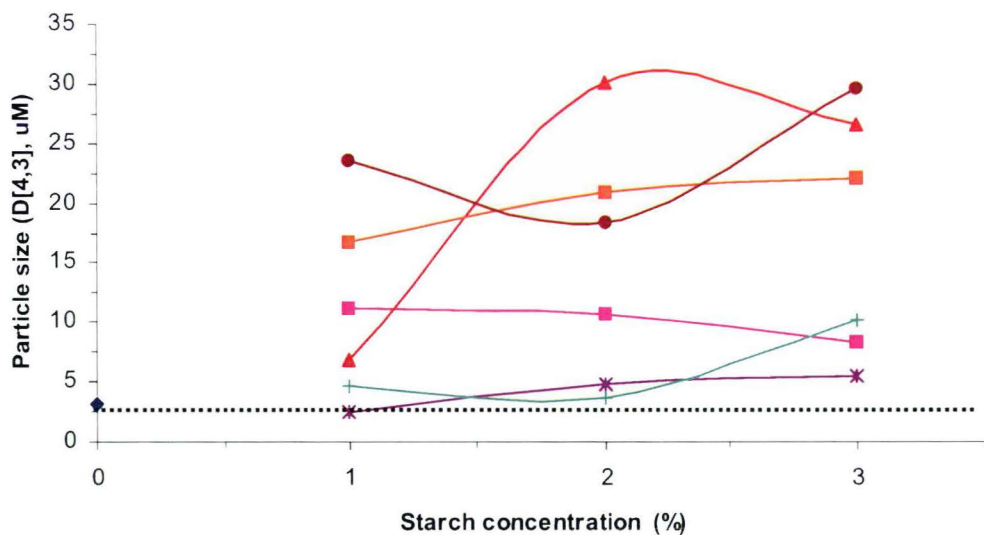
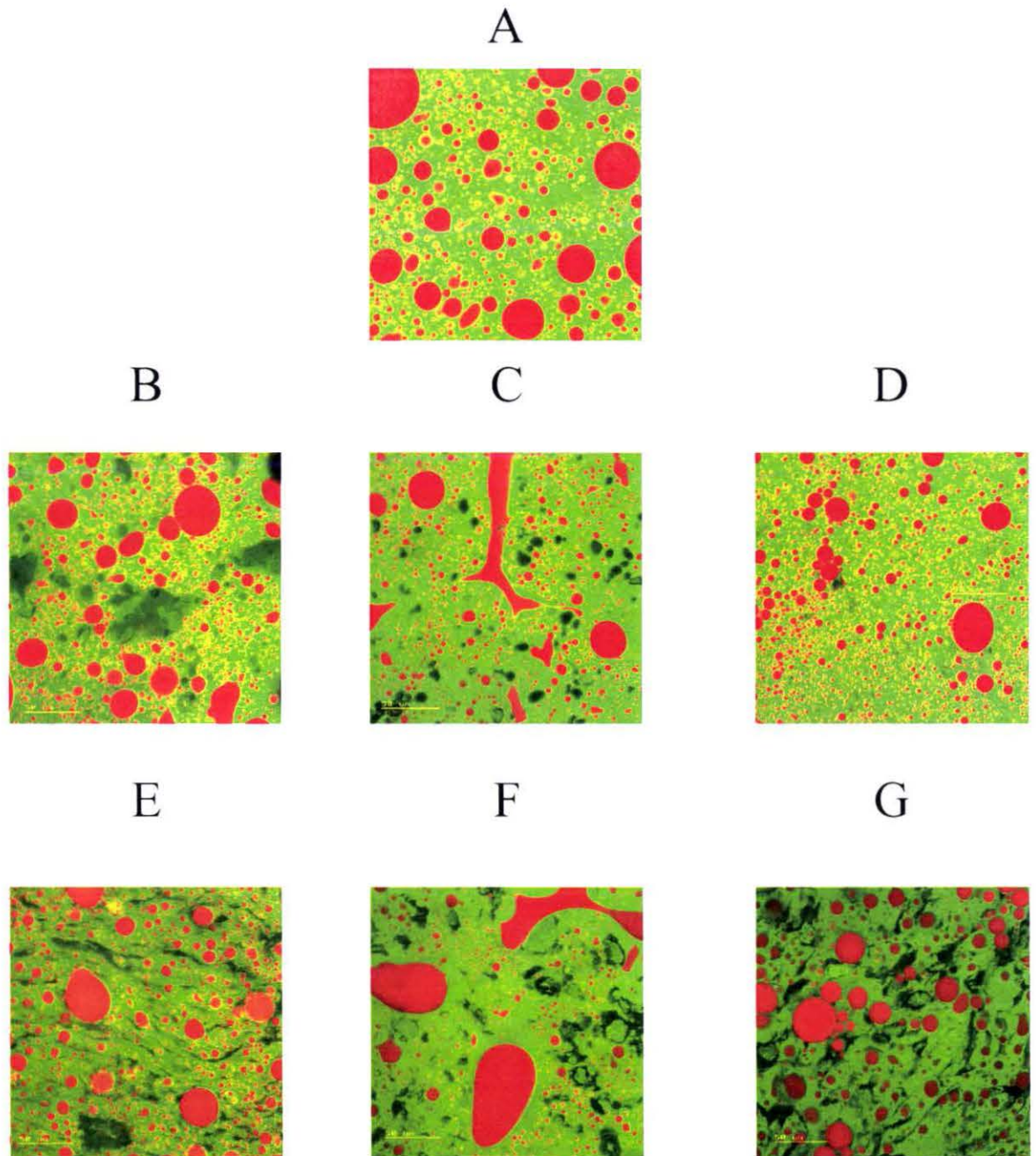


Figure 5.6. Particle size (D [4,3]) of model processed cheese spreads with control ( $\blacklozenge$ ), rice starch ( $\blacksquare$ ), potato starch ( $\blacktriangle$ ), wheat starch ( $\blacksquare$ ), HACS ( $\times$ ), acid converted starch ( $\bullet$ ) and waxy cornstarch ( $+$ ) as a function of starch concentration. Dashed horizontal line is a guide for the D [4,3] value of the control sample.

***Microstructural evaluation***

Figure 5.7 shows the confocal micrographs of processed cheese spreads sample with different starches at 2 wt% addition rate. The micrographs illustrate how fat particles (red area) are entrapped within the protein matrix (green coloured continuous phase). For all observed samples, the fat droplets were uniformly distributed. However, a wide range of particle sizes is observed. Processed cheese spread with wheat starch (Figure 5.7(F)) tends to show some irregular shaped and large fat droplets as compared to spreads with other starches. Processed cheese spread with HACs (Figure 5.7(C)) shows some spherical black spots in the micrograph, which might be the unpasted starch particles. Furthermore, apart from the control sample (Figure 5.7(A)) and the sample containing potato starch (Figure 5.7(D)), dark areas that could be associated with starch-rich areas are observed. In the case of samples containing waxy cornstarch (Figure 5.7(E)) and rice starch (Figure 5.7(G)) these dark areas are much more elongated than in the case of samples containing other starches.



**Figure 5.7. Confocal micrographs of processed cheese spread prepared on Paar-Physica obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACs, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.**

### 5.3.2 Samples prepared on RVA

The samples made using the RVA were submitted to the same measurements as those made on the Paar-Physica.

#### *Rheological Measurement*

Figure 5.8, shows the elastic modulus  $G'$  and the loss modulus  $G''$  as a function of frequency for model processed cheese samples containing 2 wt% starch. As seen in Figure 5.8 there is an increase in the  $G'$  and  $G''$  values with increase in the frequency (0.01 to 10 Hz), except for HACS at 2 wt% addition rate where the values of the  $G'$  and  $G''$  are just lower than the control sample value. Similarly to samples made using the Paar-Physica, the frequency at which  $G'$  and  $G''$  crossovers, decreases with the addition of starch indicating that the samples containing starch flow less than the control. The sample to which acid converted starch was added has the lowest crossover frequency. It is worth mentioning that the values for  $G'$  and  $G''$  for all the processed cheese samples with and without starch addition had higher values for samples prepared on RVA than Paar-Physica.

To report the effect of starch addition at the three levels investigated, the complex modulus at 1 Hz is reported in Figure 5.9. The horizontal line in the graph indicates the complex modulus value for control processed cheese spread. It could be observed that apart from the cheese sample containing 1 and 2 wt% HACS all the samples have a higher  $G^*$  than the control sample (see dashed line on Figure 5.9 for comparison with the control). Acid converted starch and wheat starch had the biggest increase in  $G^*$  followed by rice and potato starches, then waxy cornstarch, and finally HACS had the lowest increase in  $G^*$ .

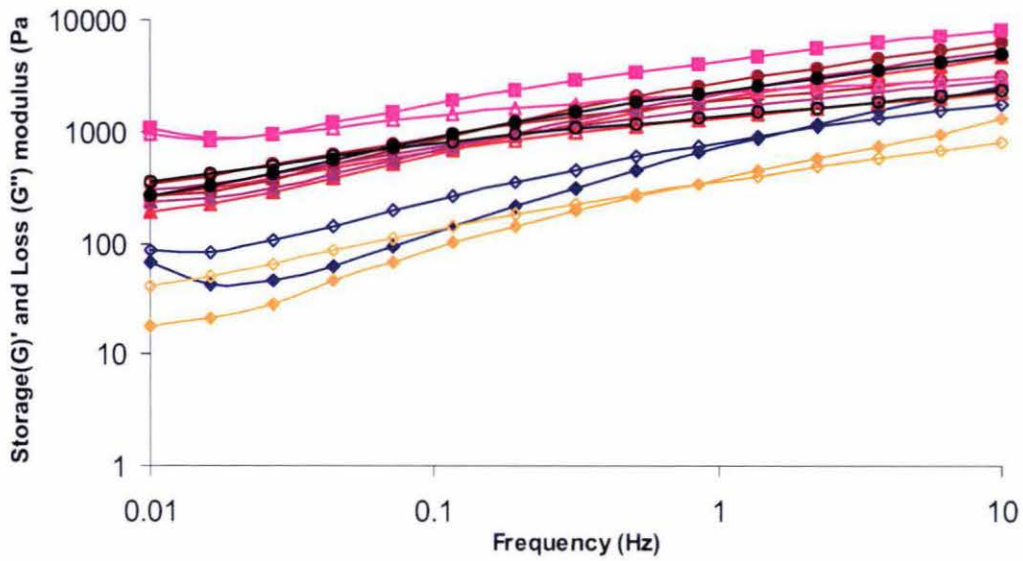


Figure 5.8. Storage ( $G'$ ) and Loss ( $G''$ ) modulus as a function of strain (%) of model processed cheese spread prepared on RVA. Starches are: 0 wt % ( $G'$ - $\diamond$ ,  $G''$ - $\diamond$ ), 2 wt% rice starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% potato starch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ), 2 wt% wheat starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% HACS ( $G'$ - $\blacklozenge$ ,  $G''$ - $\lozenge$ ), 2 wt % acid converted starch ( $G'$ - $\blacksquare$ ,  $G''$ - $\square$ ) and 2 wt% waxy cornstarch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ).

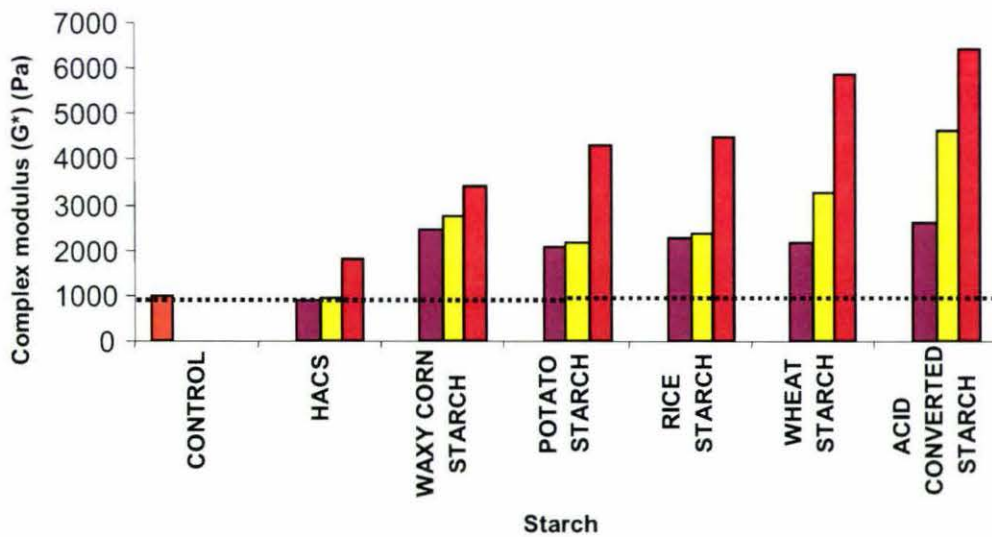


Figure 5.9. Complex modulus ( $G^*$ ) at 1 Hz and measured at 25°C of model processed cheese spread prepared on the RVA. Starch concentration: 0 wt % ( $\blacksquare$ ), 1 wt % ( $\blacksquare$ ), 2 wt% ( $\blacksquare$ ) and 3 wt % ( $\blacksquare$ ). Dashed horizontal line is a guide for the value of  $G^*$  of the control sample.

Figure 5.10 shows  $G'$  and  $G''$  as a function of strain for processed cheese spreads made with 2 wt% starch. At low strain (less than 10%)  $G'$  values are higher than the  $G''$  values for all the samples and both  $G'$  and  $G''$  are independent of the strain. In rheological terms this corresponds to the linear viscoelastic region (Tunick, 2000). These curves are also used to define a “breakpoint strain” corresponding to the strain at which  $G'$  and  $G''$  are equal. This “breakpoint strain” is reported in Figure 5.11 for all the samples made at 1, 2 and 3 wt% starch concentration. HACS has the lowest “breakpoint strain” among all the starches at all the starch concentrations. At 3 wt% starch concentration wheat starch has the highest “breakpoint strain”.

### *Particle size measurement*

Figure 5.12 shows the particle size distribution of the processed cheese spread samples. As with the samples prepared on the Paar-Physica, the fat particle size distribution of the control sample gives a bimodal distribution. However, contrary to the samples made on the RVA, most of the samples containing starch have a smaller particle size distribution than the control. Apart from wheat starch that presented a third peak at 20  $\mu\text{m}$  and potato starch that presented some particle size distribution out of the range of the measurement. To better illustrate the effect of starch addition on fat particle size, the  $D [4,3]$ , is reported on Figure 5.13 for all the samples made. It could be clearly seen that apart for sample made with acid converted starch at 2 and 3 wt%, the potato starch at 1 and 2 wt% and wheat starch at 3 wt% all the samples had a particle size equal or smaller than that of the control. Note also that compared to the samples made on the Paar-Physica the values of  $D [4,3]$  are much smaller.

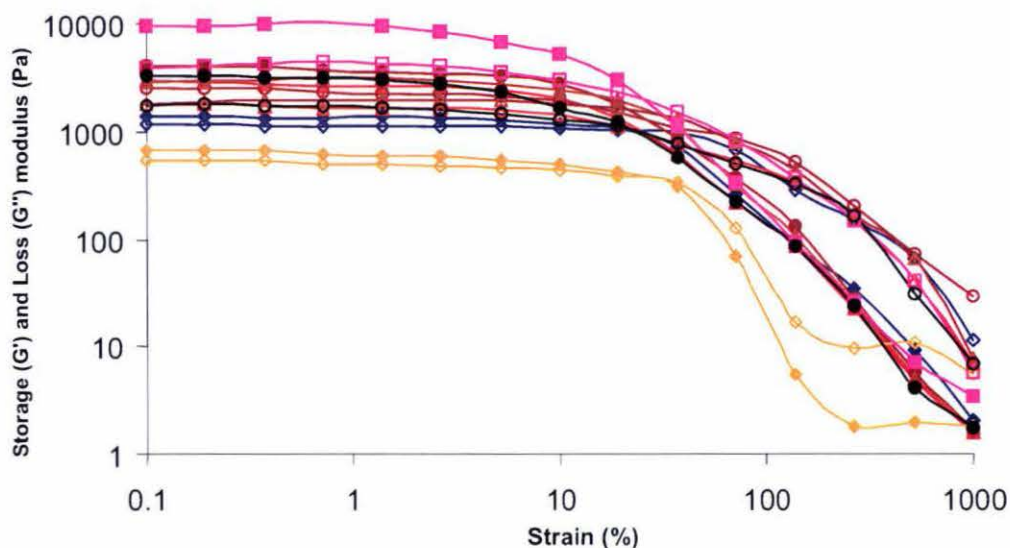


Figure 5.10. Storage ( $G'$ ) and Loss ( $G''$ ) modulus at 1 Hz frequency as a function of strain (%) of model processed cheese spread prepared on RVA at 25°C. Starches are: 0 wt % ( $G'$ - $\diamond$ ,  $G''$ - $\diamond$ ), 2 wt% rice starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% potato starch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ), 2 wt% wheat starch ( $G'$ - $\blacktriangle$ ,  $G''$ - $\triangle$ ), 2 wt% HACS ( $G'$ - $\diamond$ ,  $G''$ - $\diamond$ ) 2 wt % acid converted starch ( $G'$ - $\blacksquare$ ,  $G''$ - $\square$ ) and 2 wt% waxy cornstarch ( $G'$ - $\bullet$ ,  $G''$ - $\circ$ ).

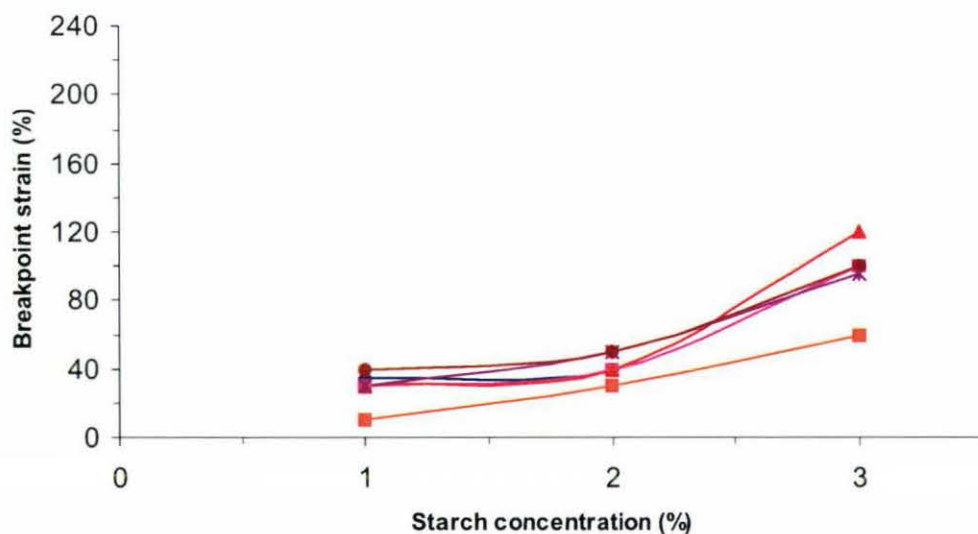


Figure 5.11. "Breakpoint strain" of model processed cheese spread prepared on the RVA as a function of starch concentration at 25°C containing rice starch ( $\diamond$ ), potato starch ( $\blacksquare$ ), wheat starch ( $\blacktriangle$ ), HACS ( $\blacksquare$ ), acid converted starch ( $*$ ) and waxy cornstarch ( $\bullet$ ).

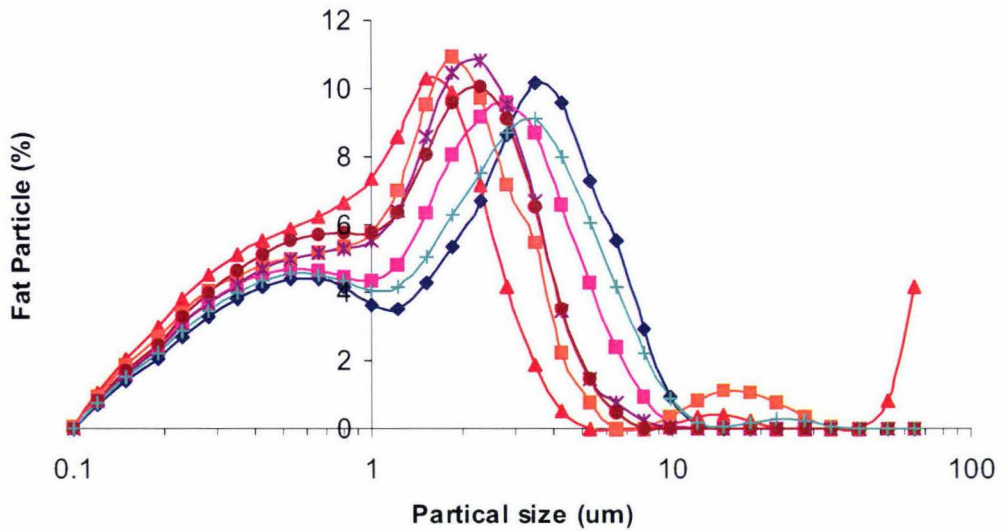


Figure 5.12. Particle size distribution of model processed cheese spreads prepared on RVA as a function of particle size containing control (◆), 2 wt% rice starch (■), 2 wt% potato starch (▲), 2 wt% wheat starch (■), 2 wt% HACS (\*), 2 wt% acid converted starch (●) and 2 wt% waxy cornstarch (+).

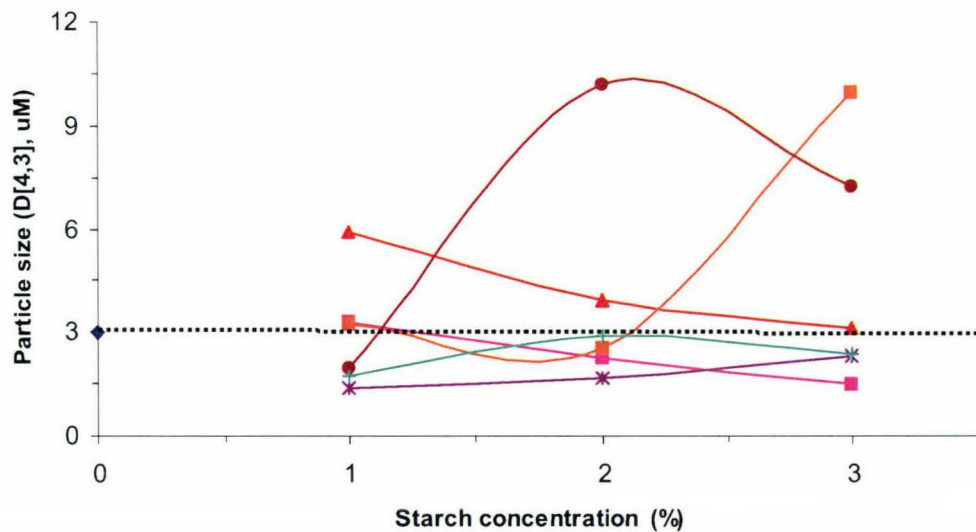
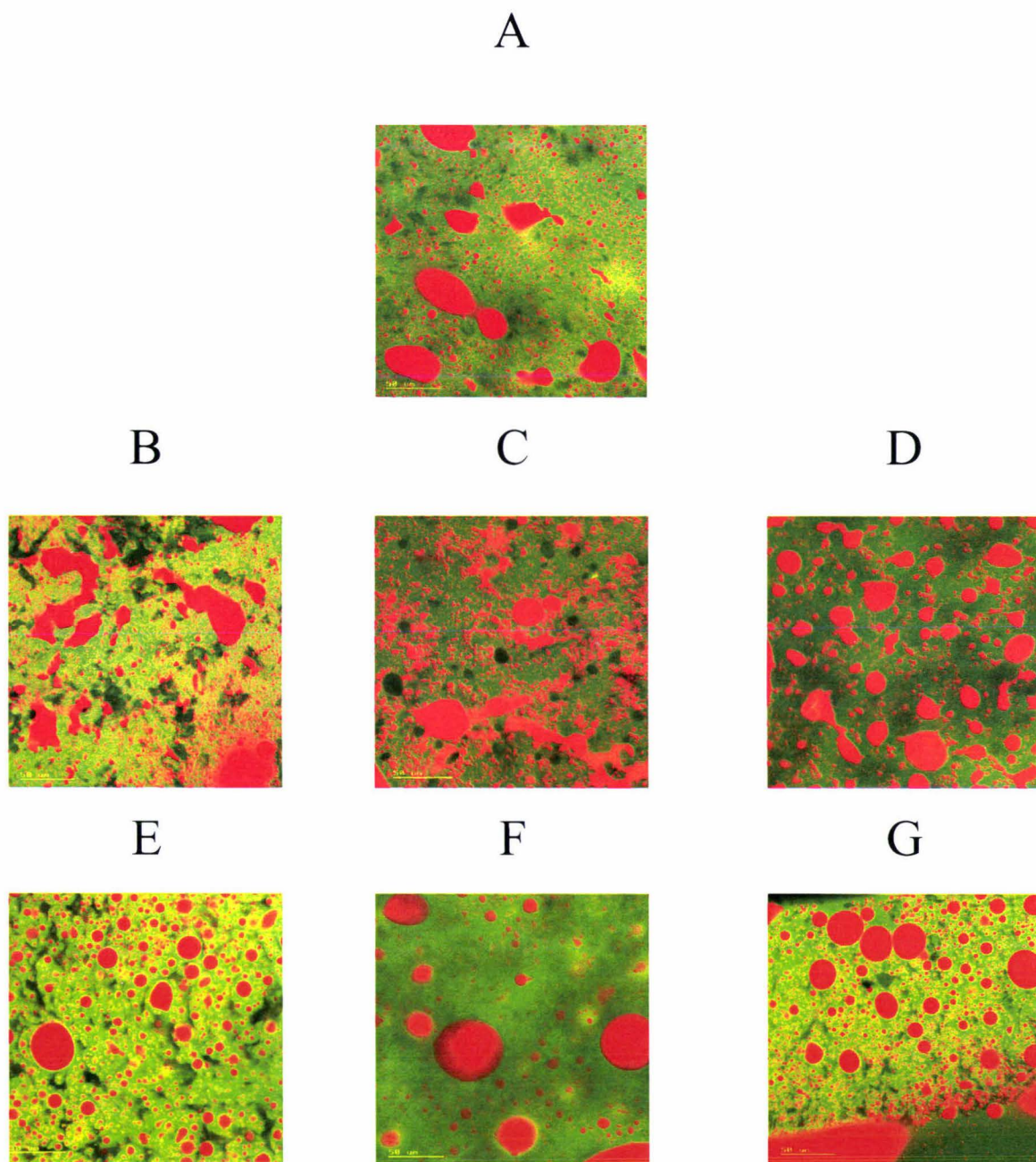


Figure 5.13. Particle size (D [4,3]) of model processed cheese spreads prepared on RVA as a function of starch concentration containing control (◆), rice starch (■), potato starch (▲), wheat starch (■), HACS (×), acid converted starch (●) and waxy cornstarch (+).

***Microstructural evaluation***

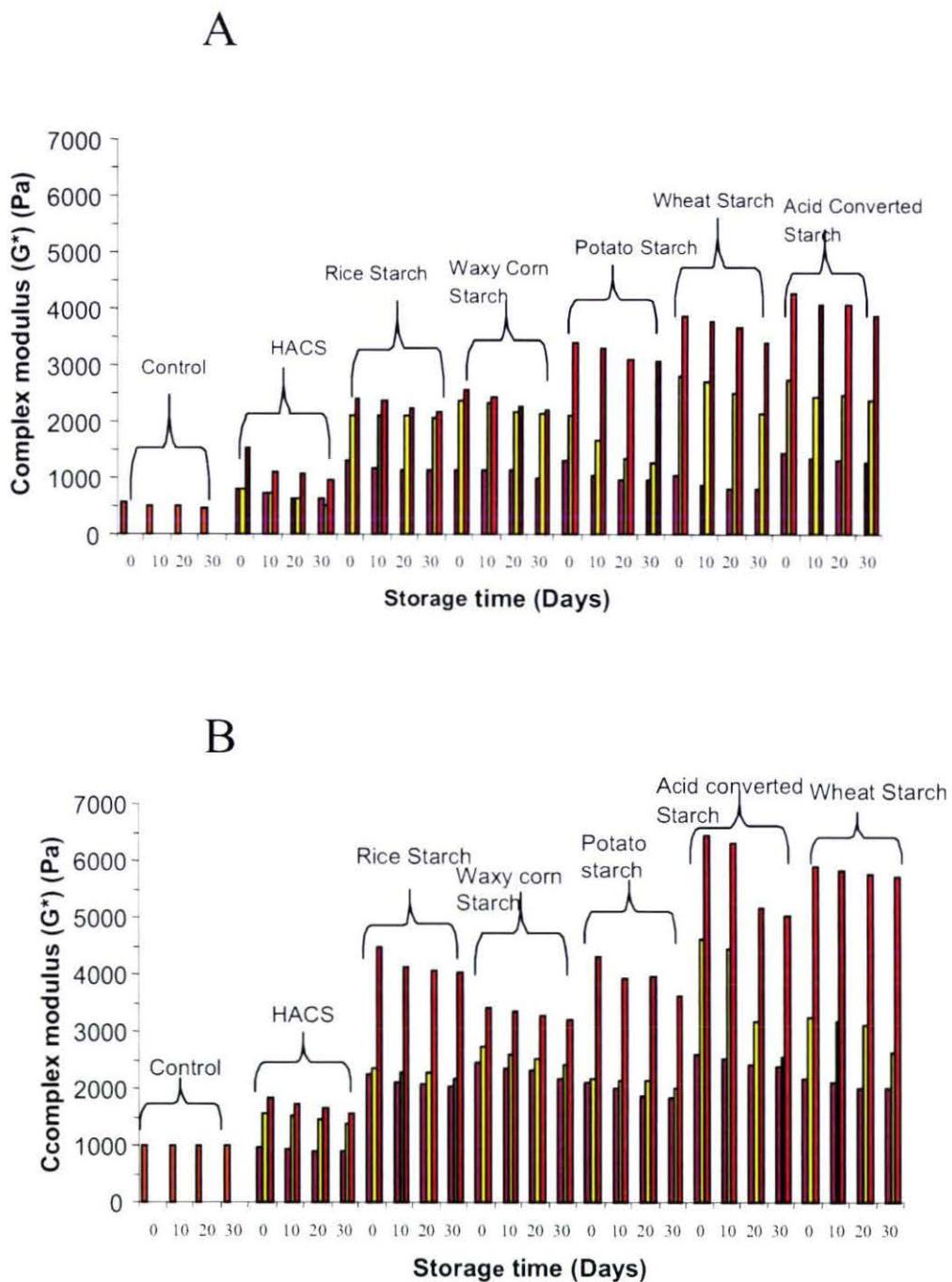
Figure 5.14 shows the confocal micrographs of processed cheese spread samples prepared on RVA with different starches at 2% addition rate. Fat particles (red area) are entrapped within the protein matrix (green coloured continuous phase) as previously seen in the confocal micrographs of the samples made on the Paar-Physica. The micrographs show range of particle of all the sizes. Similarly for the samples made on Paar-Physica, the samples containing HACS (Figure 5.14(C)) shows some black spots in the micrograph, which could be the unpasted starch particles. Fat droplets in waxy corn starch (Figure 5.14(E)), wheat starch (Figure 5.14(F)) and rice starch (Figure 5.14(G)) added processed cheese spreads seems to be more emulsion-like than those seen in the micrographs of other cheese samples. The micrographs of samples made with acid converted starch (Figure 5.14(B)), waxy cornstarch (Figure 5.14(E)) and rice starch (Figure 5.14(G)), shows some dark areas, which could be starch-rich areas.



**Figure 5.14.** Confocal micrographs of processed cheese spread prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are for (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACS, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.

#### 5.4 Effect of storage time

It is important to know if the structure of the product changes over time. The model cheese samples were stored at 4°C for 30 days and their rheological properties tested at 0, 10, 20 and 30 days. Figure 5.15(A) shows the complex modulus values for all the processed cheese samples made on the Paar-Physica as a function of the storage time. For all of the starches and control sample there is a marginal decrease in the complex modulus of the samples on refrigerated storage. Figure 5.15(B) shows the complex modulus values for all the samples made on the RVA and measured at 0, 10, 20 and 30 days. Except for the sample containing acid converted starch where  $G^*$  decreased noticeably after 20 days of storage, there is a slight decrease in the complex modulus of the samples. This clearly indicates that at this storage time and temperature conditions (30 days at 4°C) the rheological properties of the cheese samples containing starch are not drastically affected.



**Figure 5.15.** Complex modulus ( $G^*$ ) of model processed cheese spread as a function of storage time prepared on (A) Paar-Physica and (B) RVA with 0 wt% (■), 1 wt % (■), 2 wt% (■) and 3 wt % (■) containing different starches.

## 5.5 Discussion

For all the measured samples, the moduli ( $G'$ ,  $G''$  and  $G^*$ ) values were higher for processed cheese samples prepared on the RVA as compared to those made on the Paar-Physica (Fig 5.1, 5.2 and Fig 5.8, 5.9). In addition, the particle size distribution for samples prepared on the RVA are smaller than the samples prepared on the Paar-Physica (Figure 5.6 and Figure 5.13). These two results are related, as the decrease in fat droplet size should result in a higher elasticity, or firmness of the processed cheese. In fact, Lee et al (2003) suggested that decreased fat droplets size has the effect of allowing more proteins to be absorbed at the fat-water interface, whereas decrease in the size heterogeneity enhances the inclusion of fat droplets in the protein matrix, and hence the formation of a stronger network. These differences, both in the complex modulus and the particle size distribution, between the samples made on the Paar-Physica and the RVA is due as pointed out earlier to the difference in the shearing geometry of the two equipments. Furthermore, the time-temperature profiles used for sample preparation on both the equipments were different.

When starches are added the rheological parameters ( $G'$  and  $G''$  and  $G^*$ ) increased, for both samples made on the RVA and the Paar-Physica. In the case of Paar-Physica  $G^*$  increased in the order of HACS, rice starch, waxy cornstarch, potato starch, acid converted starch and wheat starch (Figure 5.2). In the case of samples made on RVA,  $G^*$  increased in order, HACS, waxy cornstarch, potato starch, rice starch, wheat starch and acid converted starch (see Figure 5.9). The probable reason for slight difference in the effect of the different starches on the processed cheese ( $G^*$  of wheat starch is higher for the Paar-Physica and acid converted starch is the highest for the RVA) could be due to the amount of shear experienced by the samples and thus how the final microstructure is affected. The confocal micrographs of the samples containing HACS showed that some HACS starch granules were unpasted, this resulted in a low increase in  $G^*$  when compared to the other starches.

Although, as for the previous chapter, one is tempted to relate the rheological behaviour of the processed cheese to the rheological behaviour of the starch (Figure 4.1), this is a very complicated task. In fact HACS has the lowest  $G^*$  and acid converted starch had the highest  $G^*$  when pasted in water (see Chapter 4, Figure 4.2). This could partly support the behaviour of the processed cheeses, however other effects have to be taken

into account. These effects will include the temperature/shear history during the cheese making, the ability of the different starches to compete for water in the presence of fat and protein, the nature of interactions between the starch, protein and fat network and how these affect the cheese microstructure. For instance as observed by confocal microscopy the addition of potato starch appeared to have little effect on the protein/fat network, while waxy cornstarch seemed to disturb the protein/fat network and induce protein-rich and starch-rich phases in the cheese sample. These effects will be discussed in more detail later in the general discussion (Chapter 9).

When considering the strain-sweep experiment (Figure 5.3 and 5.10), the “breakpoint strain” for all of the samples is very similar and not very different from that of the control (~10%). This is an indication that the protein-fat network mainly dominates the rheological behaviour of the sample at high deformation.

It is well known that during storage, the structure of the processed cheese spread changes continuously, typically towards a firmer, shorter texture (Schar & Bosset, 2002). The rheological changes occurring during ageing may be due to changes in pH, moisture and salt content and could also be due to proteolysis of the casein-protein matrix by rennet (Mulvihill & McCarthy, 1994). However, in the present work the measurements have showed that a slight decrease in the complex modulus ( $G^*$ ) is observed during storage. This could be due to the coalescence of fat droplet, thus the reduction in the number of fat droplets reduces the elasticity of the network, although other effects such as starch retrogradation (Thomas & Atwell, 1999) is not to be ruled out.

## 5.6 Summary to Chapter

In this chapter model processed cheese spread samples were made using the RVA and the Paar-Physica and their rheological, particle size and microstructural properties were investigated. The effect of storage time on the rheological behaviour of these samples was also monitored up to 30 days. It was found that RVA produces smaller fat particles as compared to Paar-Physica and as a result gives processed cheese spreads with higher  $G'$  and  $G''$  values. The acid converted starch, in the case of RVA and the wheat starch in the case of Paar-Physica had the highest increase of the elasticity of the processed cheese spread while HACS has the least impact among all the starches. The microstructure of the model processed cheeses was dependent on both the starches and the type of equipment used for their manufacture. This was also observed on the particle size distribution, where RVA tended to produce smaller fat droplets than the Paar-Physica. Furthermore, this study showed that there is no significant change in the rheological properties of the processed cheese spread on refrigerated storage up to 30 days.

## 6. Protein Reduction in Rennet Casein Based Processed Cheese Spreads

### 6.1 Introduction

Chapter 5 discusses the effects of six different starches on the rheological and microstructural properties of processed cheese spreads using different rheological methods. Confocal Laser Scanning Microscopy (CLSM) and particle size measurement were carried out to detect any effects the starches might have on the microstructural properties of processed cheese spreads. It was found that the effects of starch on processed cheese spreads depended both on the source and physio-chemical properties of the particular starch.

This chapter describes and discusses an investigation into the reduction of protein in a processed cheese spread formulation and its replacement by starch. All six starches investigated previously, namely potato starch, wheat starch, rice starch, high amylose cornstarch (HACS), waxy cornstarch and acid converted starch were used. Starches were added at different levels for the different reduced levels of protein considered and the effects of the starches on the rheological and microstructural properties were determined.

Processed cheese spread samples were prepared both on the Paar Physica and the RVA. The time-temperature profile used for the Paar-Physica and the RVA can be found in Section 3.3.2 and Section 3.3.3 respectively.

The main objectives of the work presented in this chapter were:

- To make a model processed cheese spread based on rennet casein using three protein levels (9.7%, 8.5% and 7.5%) in both the RVA and the Paar-Physica.
- To make processed cheese spreads based on rennet casein for each of the protein levels, with each of the six starches being incorporated at four levels (0, 1, 2 and 3 wt%).
- To determine the rheological and microstructural properties of the resulting processed cheese spreads.

- To ascertain the amount of starch required at each protein level to achieve rheological and structural properties that match those of the control as closely as possible.

This work was done in order to recommend the type and level of starch to be used for protein reduction in Individually Wrapped Slice (IWS) processed cheese.

## **6.2 Experimental approach**

The six starches were used to replace protein in processed cheese spreads. The protein levels in the processed cheese spread formulations were reduced to either 8.5% or 7.5% from the 9.7% used in the control. The control formulation (54.8% moisture, 9.7% protein) is discussed in Section 3.3.1. All other materials and methods were outlined in Chapter 3.

To obtain the two protein reductions, an amount of rennet casein was removed from the formulation and replaced with water. The ingredients and target compositions are shown in Tables 6.1 and 6.2.

To monitor the effect of storage time, the samples prepared were kept in a refrigerator at 4°C for 30 days. During this period, rheological properties were measured for all the samples at 0, 15 and 30 days after manufacture.

**Table 6.1. Ingredients for rennet casein based processed cheese spread with different protein levels**

	<b>9.7%</b>	<b>8.5%</b>	<b>7.5%</b>
	<b>Protein</b>	<b>Protein</b>	<b>Protein</b>
	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>
Rennet casein	3.52	3.06	2.70
Soya oil	7.885	7.885	7.885
Lactose	1.26	1.26	1.26
Citric acid	0.215	0.215	0.215
Trisodium citrate	0.84	0.84	0.84
Sodium chloride	0.30	0.30	0.30
Added Water	15.98	16.44	16.80
<b>Total Weight</b>	<b>30.0</b>	<b>30.0</b>	<b>30.0</b>

**Table 6.2. Target composition for rennet casein based processed cheese spreads with different protein levels**

	<b>9.7% Protein</b>	<b>8.5% Protein</b>	<b>7.5% Protein.</b>
	<b>Control</b>		
Moisture (%)	54.74	56.13	57.21
Fat (%)	26.33	26.32	26.32
Protein (%)	9.79	8.51	7.51
Intact casein (%)	7.55	6.51	5.79
Salt (%)	1.00	1.00	1.00
Lactose (%)	4.19	4.19	4.19
Target pH	5.70	5.70	5.70

### 6.3 Results

Six different starches were added at four different rates (0, 1, 2 and 3 wt%) at each different protein level (9.7%, 8.5% and 7.5%) and the processed cheese were made using the Paar-Physica and the RVA. The effects of addition of these starches at each different protein level on the rheological and microstructural properties are discussed below. The results from the Paar-Physica and the RVA experiments are presented separately. The control experiments (9.7% protein) were reported in Chapter 5. This work was not repeated in this chapter but reference to these results will be made when required.

#### 6.3.1 Samples prepared on the Paar-Physica

##### *Rheological measurements*

The complex modulus ( $G^*$ ) at 1 Hz was used to compare different samples at different protein levels and different starch addition rates. Figure 6.1(A) and figure 6.1(B) shows the values of  $G^*$  for processed cheese containing 8.5% protein and 7.5% protein respectively. Among the starches, HACS had the smallest effect on the complex modulus values at both protein levels. For a processed cheese spread with 8.5% protein, potato starch produced maximum effect on the complex modulus at 1 wt% starch addition rate, acid converted starch produced a maximum effect at 2 wt% starch addition and wheat starch produced maximum effect at 3 wt% starch addition rate. Apart from HACS at the two addition rate in a processed cheese spread with 7.5% protein (Figure 6.1(B)), all the starches increased the  $G^*$  value, no matter what the level of incorporation. At all starch addition rates, wheat starch gave the maximum increase on the  $G^*$  values (Figure 6.1(B)). Compared with processed cheese containing 9.7% protein there was a decrease of the  $G^*$  values as the protein level was reduced.

As defined previously (Chapter 5) “Breakpoint strain” is the strain at which a sample starts to flow and the elastic modulus  $G'$  becomes smaller than the viscous modulus  $G''$ . Figures 6.2(A) and 6.2(B) show the breakpoint strain values at 8.5 and 7.5% protein levels respectively. For all the samples there was an increase in the strain value as the starch level increased and a decrease in the strain value as the protein level decreased. Incorporating wheat starch into the processed cheese spread formulation resulted in the highest “breakpoint strain” value for both 8.5 and 7.5% protein for all starch concentrations. Neither the control sample nor the HACS containing sample at all protein level showed a crossover of  $G'$  and  $G''$  in the strain sweep experiments.

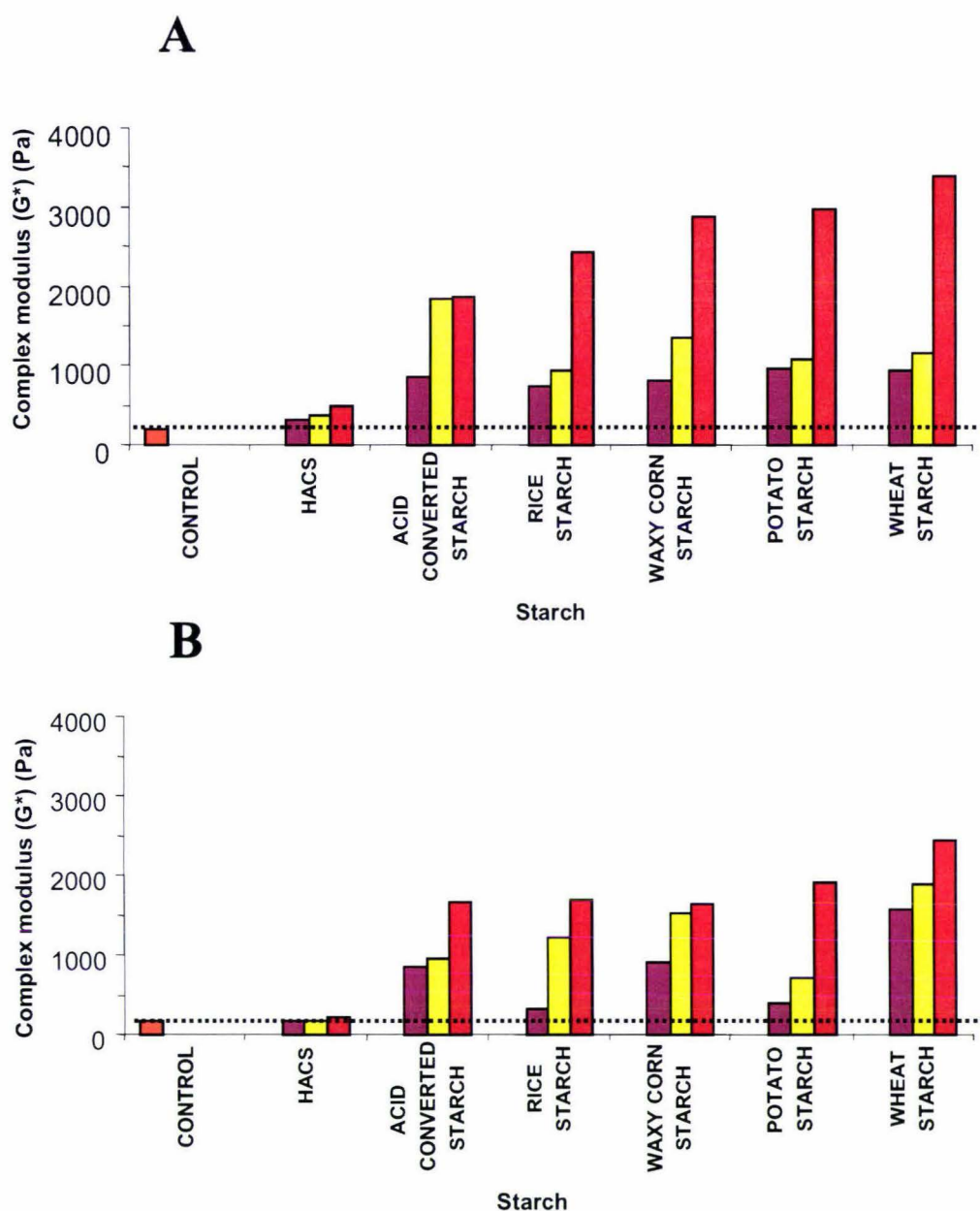
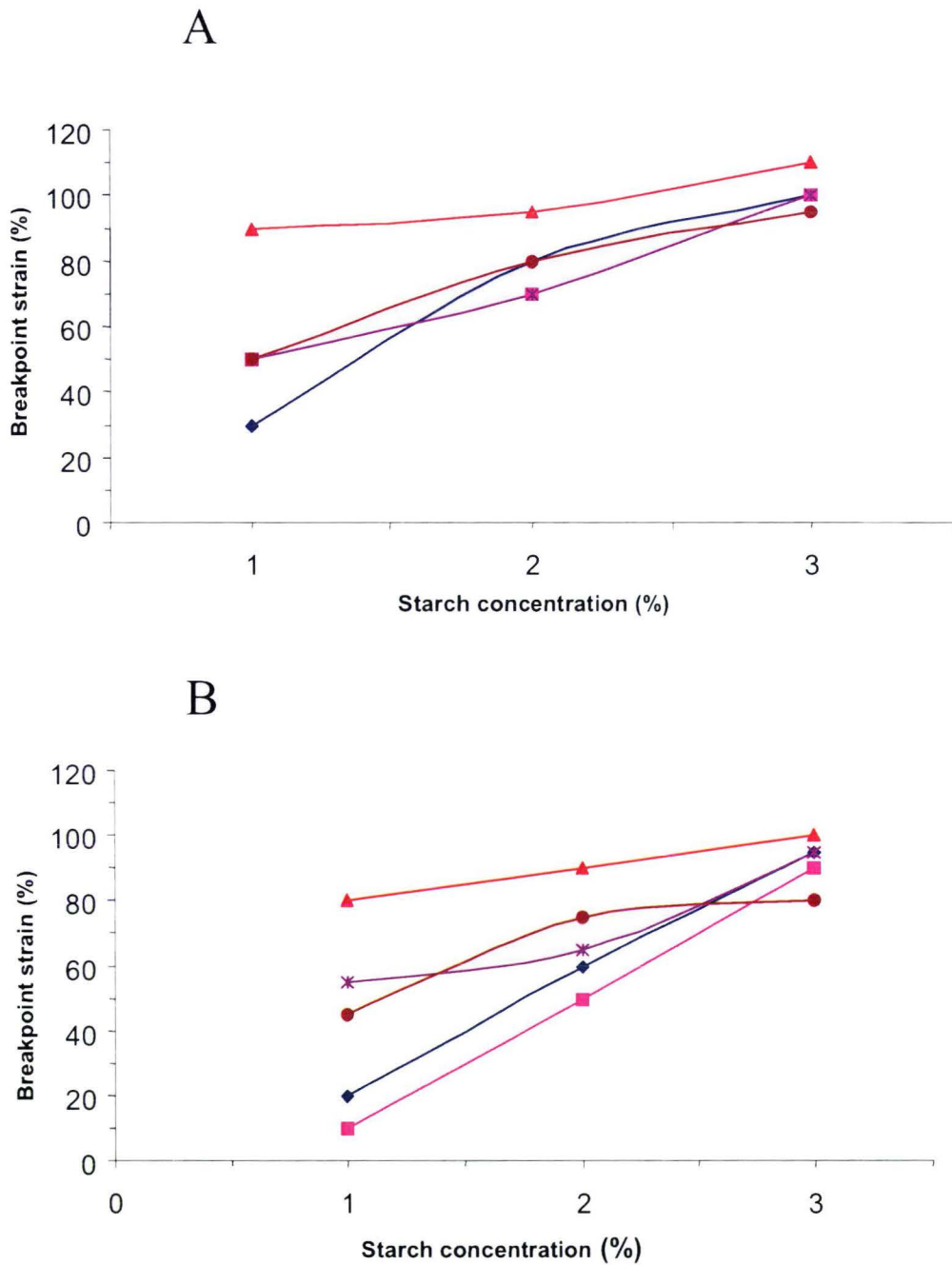


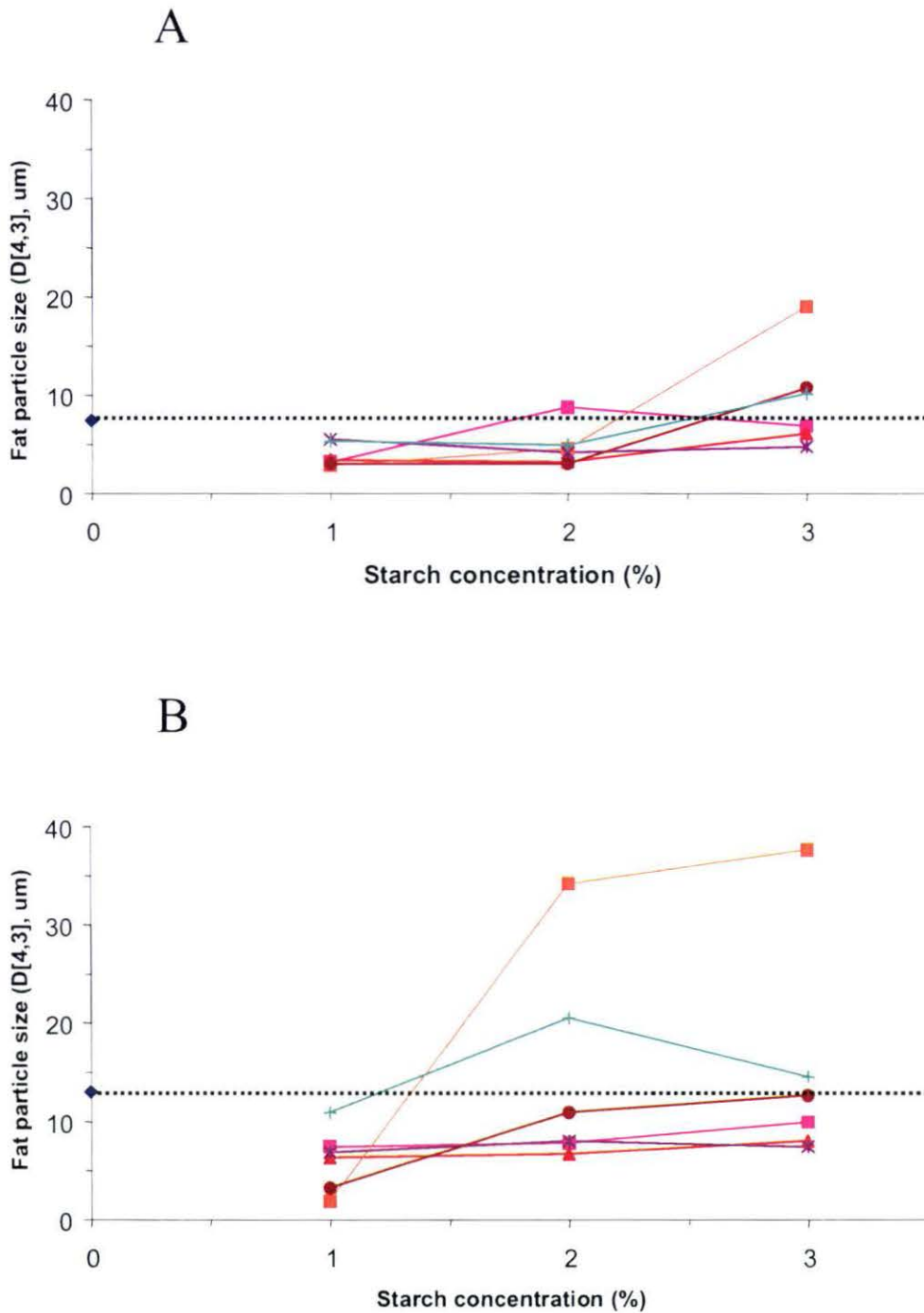
Figure 6.1. Complex modulus ( $G^*$ ) at 1 Hz and measured at 25°C of model processed cheese spread prepared on Paar-Physica at (A) 8.5% protein level and (B) 7.5% protein level. Starch concentration: 0 wt% (■), 1 wt% (■), 2 wt% (■) and 3 wt% (■). Dashed horizontal line is a guide for the value of  $G^*$  of the control sample.



**Figure 6.2. “Breakpoint strain” of model processed cheese spread as a function of starch concentration at 25°C prepared on Paar-Physica at (A) 8.5% protein and (B) 7.5% protein, containing rice starch (♦), potato starch (■), wheat starch (▲), HACS (■), acid converted starch (\*) and waxy cornstarch (●).**

***Particle size measurement***

Figure 6.3 shows, the  $D [4,3]$ , the diameter of equivalent mean volume of the particles obtained from the particle size distribution measurement (Section 3.6). Figure 6.3 (A) and Figure 6.3(B) show the particle size distribution of processed cheese spreads with the six starches at 8.5% protein level and 7.5% protein level respectively. The dotted horizontal line indicates the  $D [4,3]$  value for the control processed cheese spread. The value for the control sample with 7.5% protein was higher than that of the sample containing 8.5% protein. As discussed in Chapter 5, it is difficult to identify a clear trend, partly because of the limitations of the technique. However, apart from the samples with 3 wt% of acid converted starch, wheat starch and potato starch, all the processed cheese spreads had mean particle sizes less than that of the control sample. Except for acid converted starch at 2 and 3 wt% and potato starch at 2 and 3 wt% processed cheese spreads containing 7.5% protein, all had particle size values less than that of the control sample. There was an increase in the particle size of the control sample as the protein level was decreased.

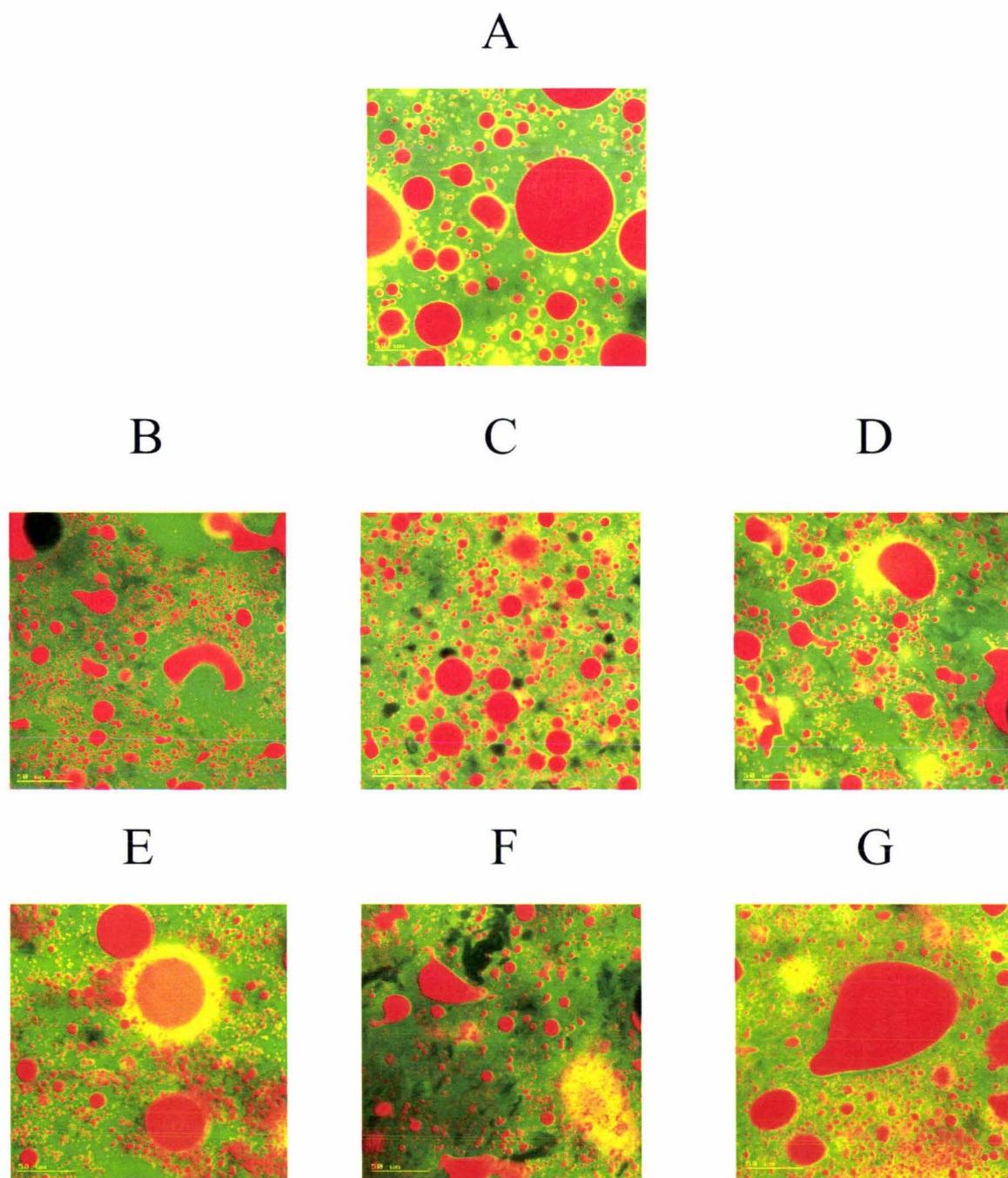


**Figure 6.3.** Particle size (D [4,3]) as a function of starch concentration of model processed cheese spreads prepared on the Paar-Physica with (A) 8.5% protein and (B) 7.5% protein containing control ( $\diamond$ ), rice starch ( $\blacksquare$ ), waxy cornstarch ( $\blacktriangle$ ), acid converted starch ( $\blacksquare$ ), HACs ( $*$ ), wheat starch ( $\bullet$ ) and potato starch ( $+$ ). Dashed horizontal line is a guide for the value of particle size of the control sample.

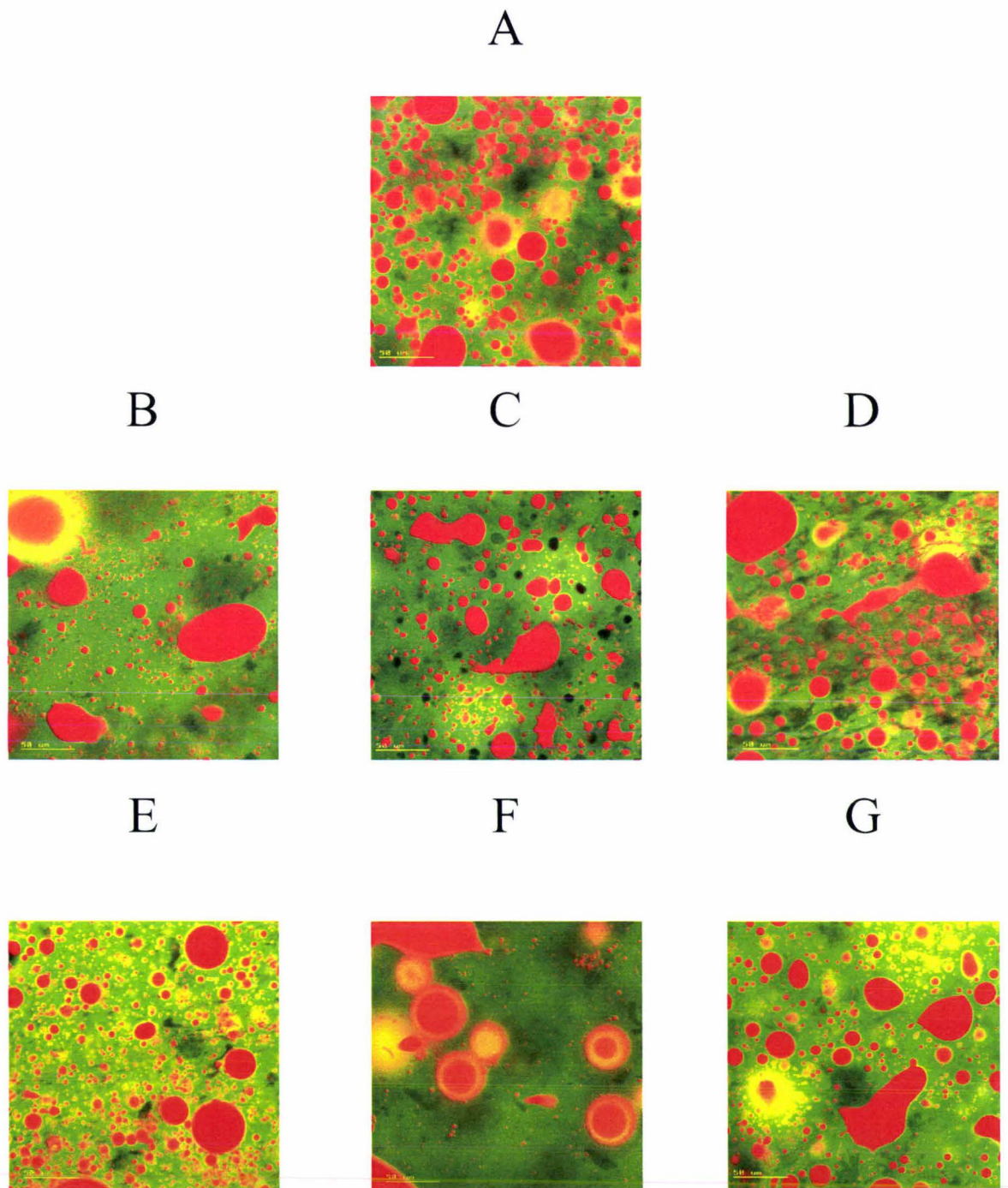
***Microstructural evaluation***

Figure 6.4 shows the confocal micrographs of processed cheese spreads samples containing different starches at the 2 wt% addition rate with 8.5% protein. The micrographs illustrate how fat droplets (red area) are entrapped within the protein matrix (green coloured continuous phase). A wide range of fat droplets sizes was observed. Processed cheese spread containing HACS (Figure 6.4(C)) showed some black spots in the micrograph similar to the ones seen in the 9.7% protein processed cheese spread control. These could be non-pasted starch granules. The black spots seen in the micrograph of processed cheese spread containing acid converted starch (Figure 6.4(B)) are probably air bubbles. As they are too large to be unpasted starch granules. There are dark yellow regions seen around the fat droplets, which were not present in the 9.7% protein processed cheese micrographs. These yellow spots might be regions with excess protein (in this case the protein concentration is higher at the fat interface than in the continuous phase) or they might be computer-generated artefacts. Apart from the control sample (Figure 6.4(A)), waxy cornstarch (Figure 6.4(E)) and HACS (Figure 6.4(C)), all the micrographs showed some large irregular shaped fat droplets.

Figure 6.5 shows the confocal micrographs of processed cheese spread samples with 7.5% protein and with different starches added at the 2 wt% level. Again a wide range of particle sizes was observed. A processed cheese spread containing HACS (Figure 6.5(C)) showed some black spots in the micrograph similar to the ones seen in the 9.7% protein processed cheese spread micrographs. Again, there were dark yellow regions around the fat droplets, which were not present in the 9.7% protein processed cheese micrographs. All the processed cheese micrographs, apart from that for the one containing waxy cornstarch (Figure 6.5(E)), had large irregular shaped fat droplets.



**Figure 6.4.** Confocal micrographs of processed cheese spread containing 8.5% protein prepared on Paar-Physica obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACs, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.



**Figure 6.5.** Confocal micrographs of processed cheese spread containing 7.5% protein prepared on Paar-Physica obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACCS, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.

### 6.3.2 Samples prepared on the RVA

#### *Rheological measurement*

The effect of starch addition on the rheological properties of processed cheese spreads at different protein levels is shown in Figure 6.6. Figures 6.6(A) and 6.6(B) shows the effect of different starches on the complex modulus at both 8.5% and 7.5% protein. At the 8.5% protein level, the  $G^*$  values for all the samples were higher than that of the control sample (dashed line on Figure 6.6(A)). The processed cheese spreads containing acid converted starch had the highest  $G^*$  values for both 1 and 3 wt% starch addition rate. At 2 wt% starch addition, wheat starch had the highest impact on the  $G^*$  values compared with the control samples. At 7.5% protein level, the  $G^*$  values for the processed cheese spreads containing HACS were similar to those of the control sample (Figure 6.6(B)). For all the other starches the  $G^*$  values were higher than that of the control sample. Acid converted starch gave the highest  $G^*$  value at 1 wt% addition rate while wheat starch had the highest impact at both 2 and 3 wt% addition rate. Processed cheese containing 9.7% protein had higher  $G^*$  values than those with 8.5% protein with those containing 7.5% protein having lowest  $G^*$  values.

Figure 6.7 shows the “breakpoint strain” at the both 8.5% protein (Figure 6.7(A)) and 7.5% protein (Figure 6.7(B)) for samples prepared on the RVA. For all the samples there was an increase in the strain value, the starch concentration increased and there was a corresponding decrease in the strain value as the protein level decreased. The addition of 3 wt% wheat starch produced the highest “breakpoint strain” value at both 8.5 and 7.5% protein levels. At 7.5% protein the values for all the starch containing samples were similar. Neither the control sample nor the processed cheese with 7.5% protein containing HACS showed a crossover of  $G'$  and  $G''$  in the strain sweep experiments.

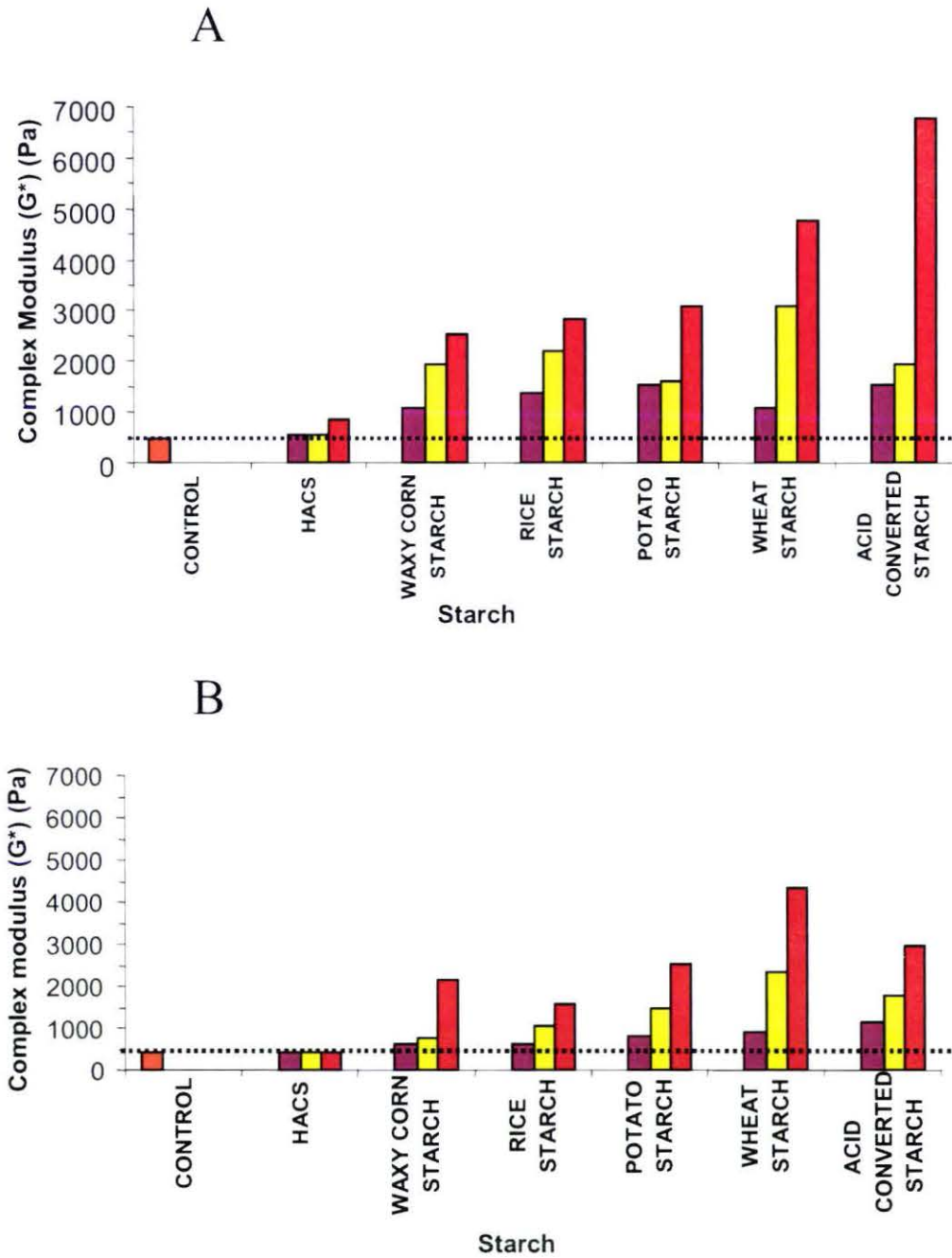
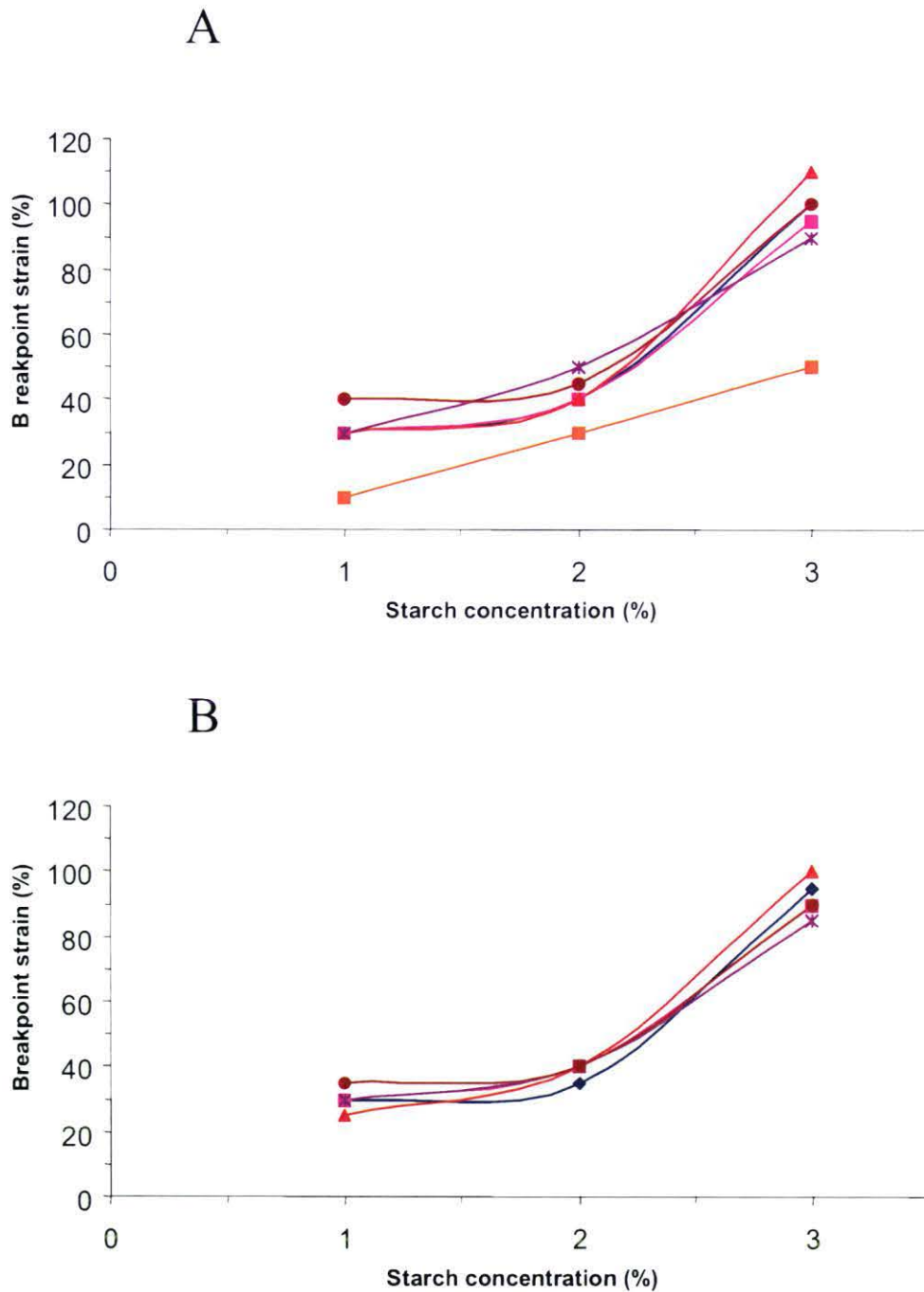


Figure 6.6. Complex modulus ( $G^*$ ) at 1 Hz and measured at 25°C of model processed cheese spread prepared on the RVA at (A) 8.5% protein level and (B) 7.5% protein level. Starch concentration: 0 wt% (red), 1 wt% (purple), 2 wt% (yellow) and 3 wt% (dark red). Dashed horizontal line is a guide for the value of  $G^*$  of the control sample.



**Figure 6.7.** “Breakpoint strain” of model processed cheese spread prepared on the RVA as a function of starch concentration at 25°C at (A) 8.5% protein and (B) 7.5% protein, containing rice starch (◆), potato starch (■), wheat starch (▲), HACS (■), acid converted starch (\*) and waxy cornstarch (●).

### ***Particle size measurement***

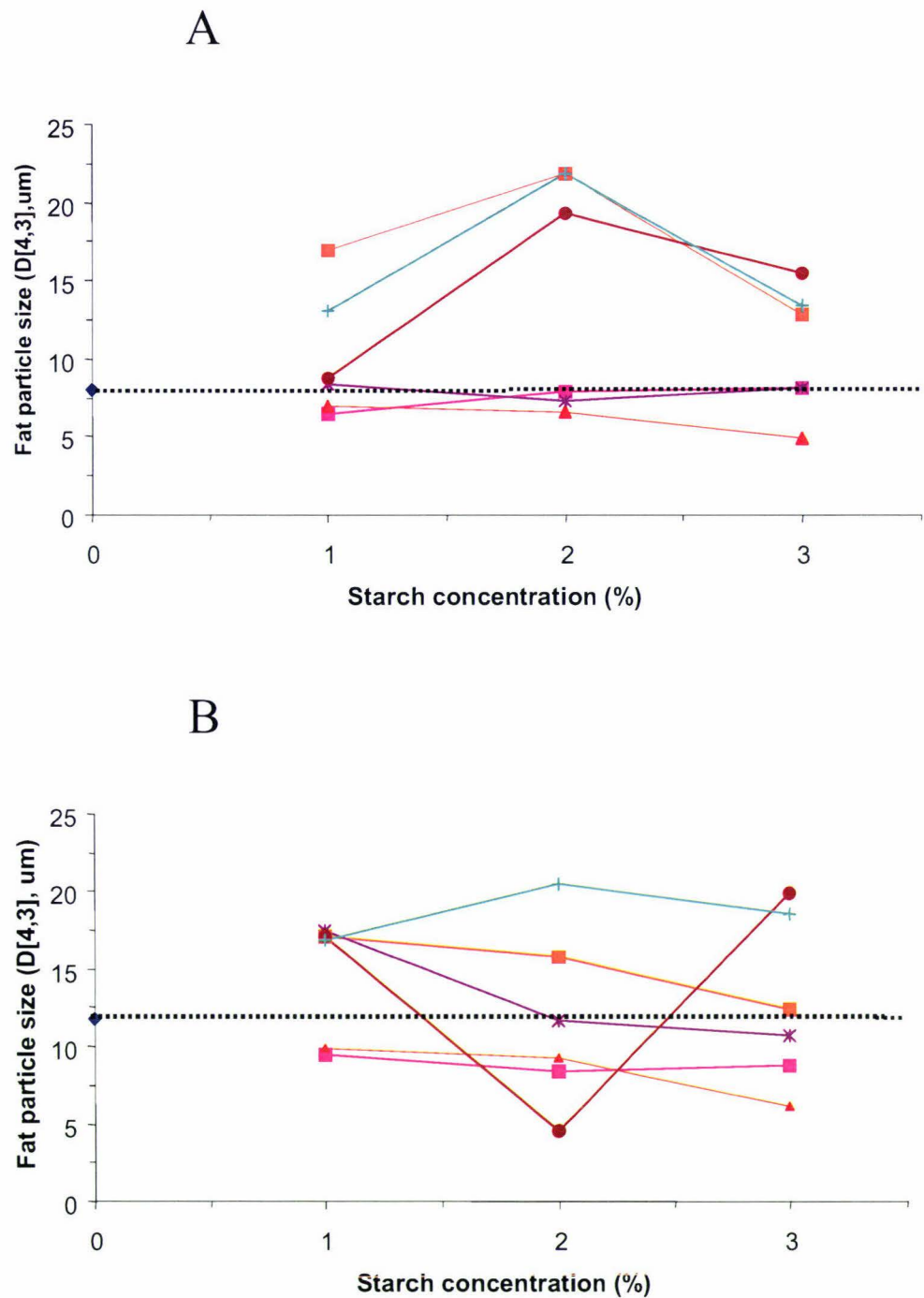
Figure 6.8 shows the particle size distribution  $D [4,3]$  of processed cheese spreads containing starch. Figures 6.8(A) and 6.8(B) illustrate the  $D [4,3]$  for processed cheese containing 8.5% protein and 7.5% protein respectively. It could be seen from the Figure 6.8(A) that acid converted starch, potato starch and wheat starch gave particle size distributions that were greater than that of the control, whereas those for the processed cheeses with HACS, waxy cornstarch and rice starch were similar to that of the control.

As was found for samples made on the Paar-Physica, it was difficult to see a clear trend in the results as shown in Figure 6.8(B). Both potato starch and acid converted starch gave values higher than that of the control sample. Wheat starch at both 1 and 3 wt% addition rates gave higher values than did the control sample. HACS at 1 wt% addition rate gave a particle size distribution that was greater than that of the control sample.

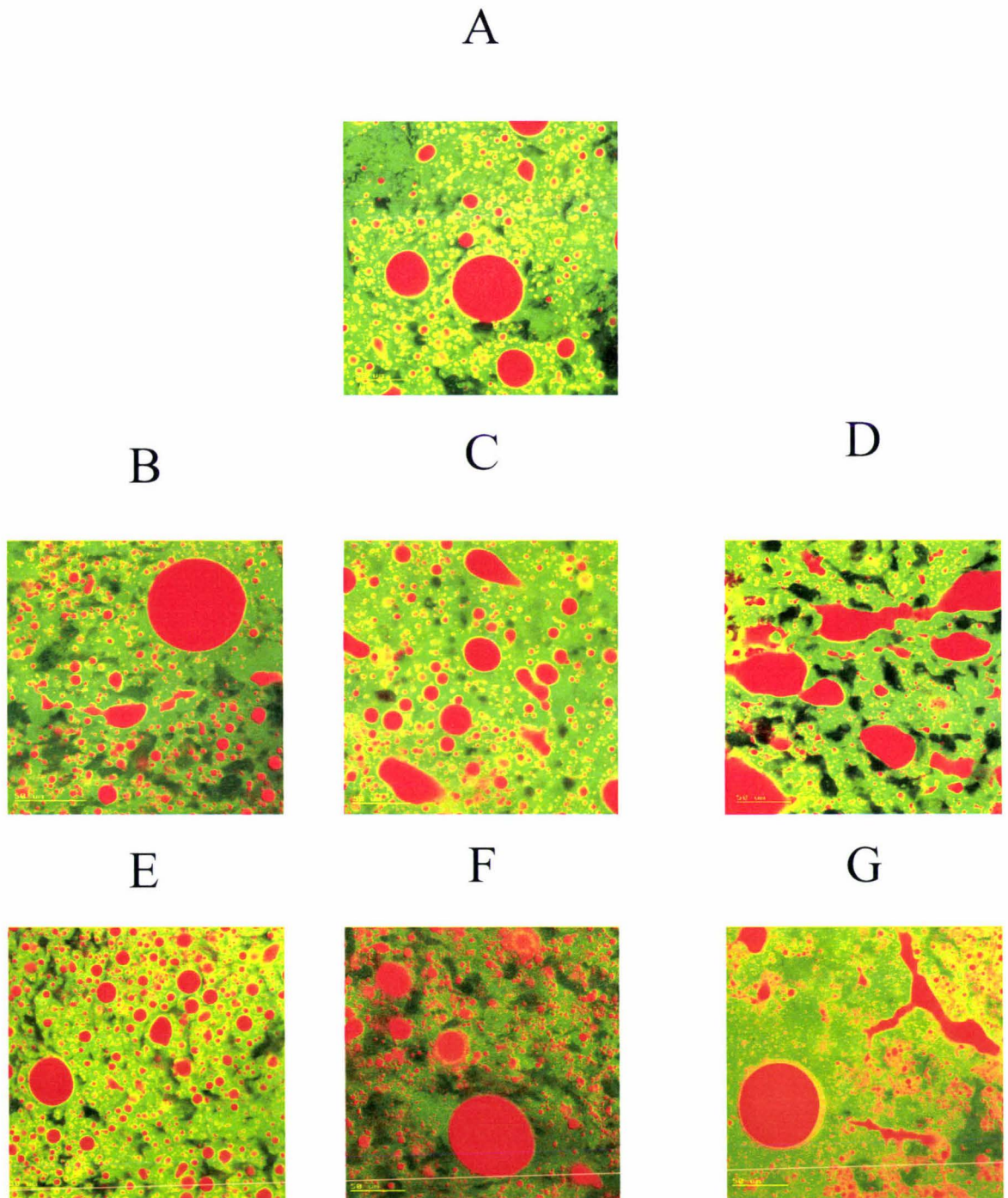
### ***Microstructural evaluation***

Figure 6.9 shows the confocal micrographs of processed cheese spreads samples with 8.5% protein and different starches added at 2 wt% addition rate, and prepared on the RVA. Like samples prepared on the Paar-Physica, the micrographs of samples containing HACS (Figure 6.9(C)) showed black spots, which could represent unpasted starch granules. A yellow colouration could also be seen around the fat droplets. The micrographs showed a range of fat globule sizes. The micrographs of samples with added starch showed irregularly shaped fat droplets compared with the control sample.

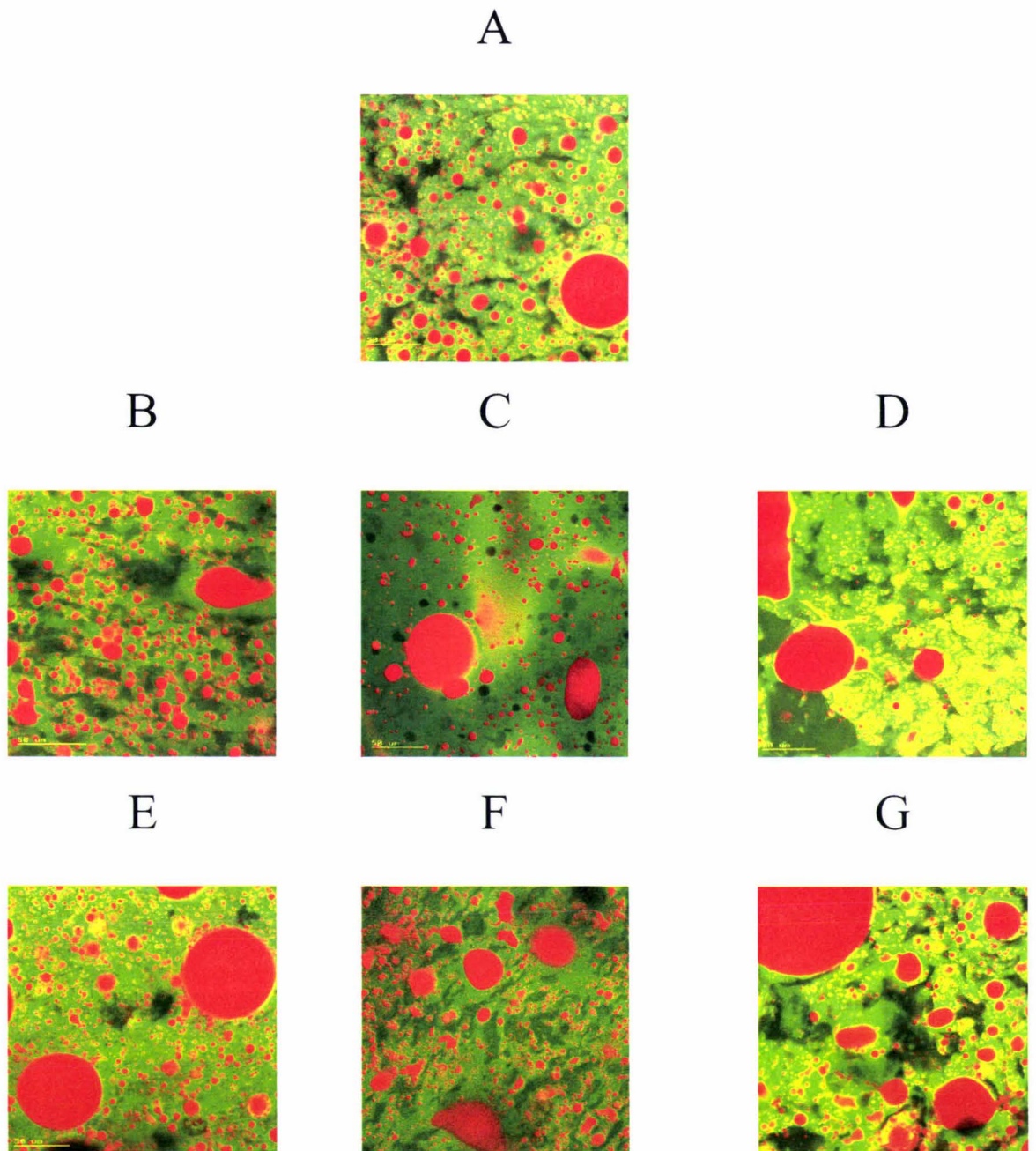
Figure 6.10 shows the micrographs of 7.5% protein processed cheese spreads containing different starches added at the 2 wt% addition rate. Black spots are clearly visible in those samples containing HACS (Figure 6.10(C)). Again there was a range of fat globule sizes observed for all the starches. The micrographs of samples made with acid converted starch (Figure 6.10(B)), potato starch (Figure 6.10(D)), wheat starch (Figure 6.10(F)) and waxy cornstarch (Figure 6.10(E)) clearly showed starch rich areas as dark areas. Yellow colouration, suggesting either regions excess protein or computer generated artefacts, was visible in all the micrographs of processed cheeses containing starch as well as in the micrograph of the control sample.



**Figure 6.8.** Particle size ( $D [4,3]$ ) as a function of starch concentration of model processed cheese spreads with (A) 8.5% protein and (B) 7.5% protein containing control (◆), rice starch (■), waxy cornstarch (▲), acid converted starch (■), HACs (\*), wheat starch (●) and potato starch (+). Dashed horizontal line is a guide for the value of particle size of the control sample.



**Figure 6.9.** Confocal micrographs of processed cheese spread prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are for 8.5% protein level with (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACs, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.



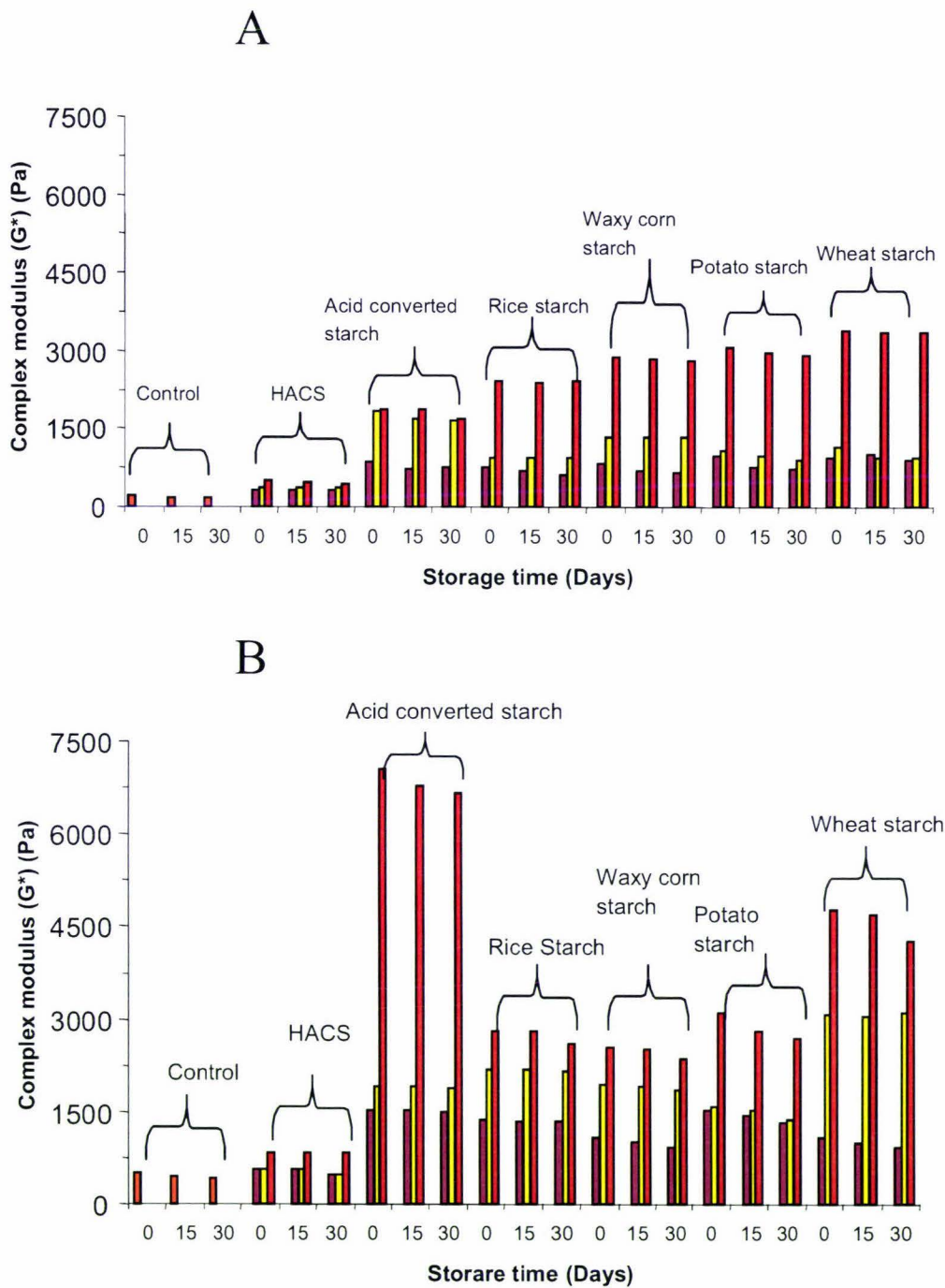
**Figure 6.10.** Confocal micrographs of processed cheese spread at 7.5% protein prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control, (B) 2 wt% acid converted starch, (C) 2 wt% HACs, (D) 2 wt% potato starch, (E) 2 wt% waxy cornstarch, (F) 2 wt% wheat starch and (G) 2 wt% rice starch addition.

#### 6.4 Effect of storage time

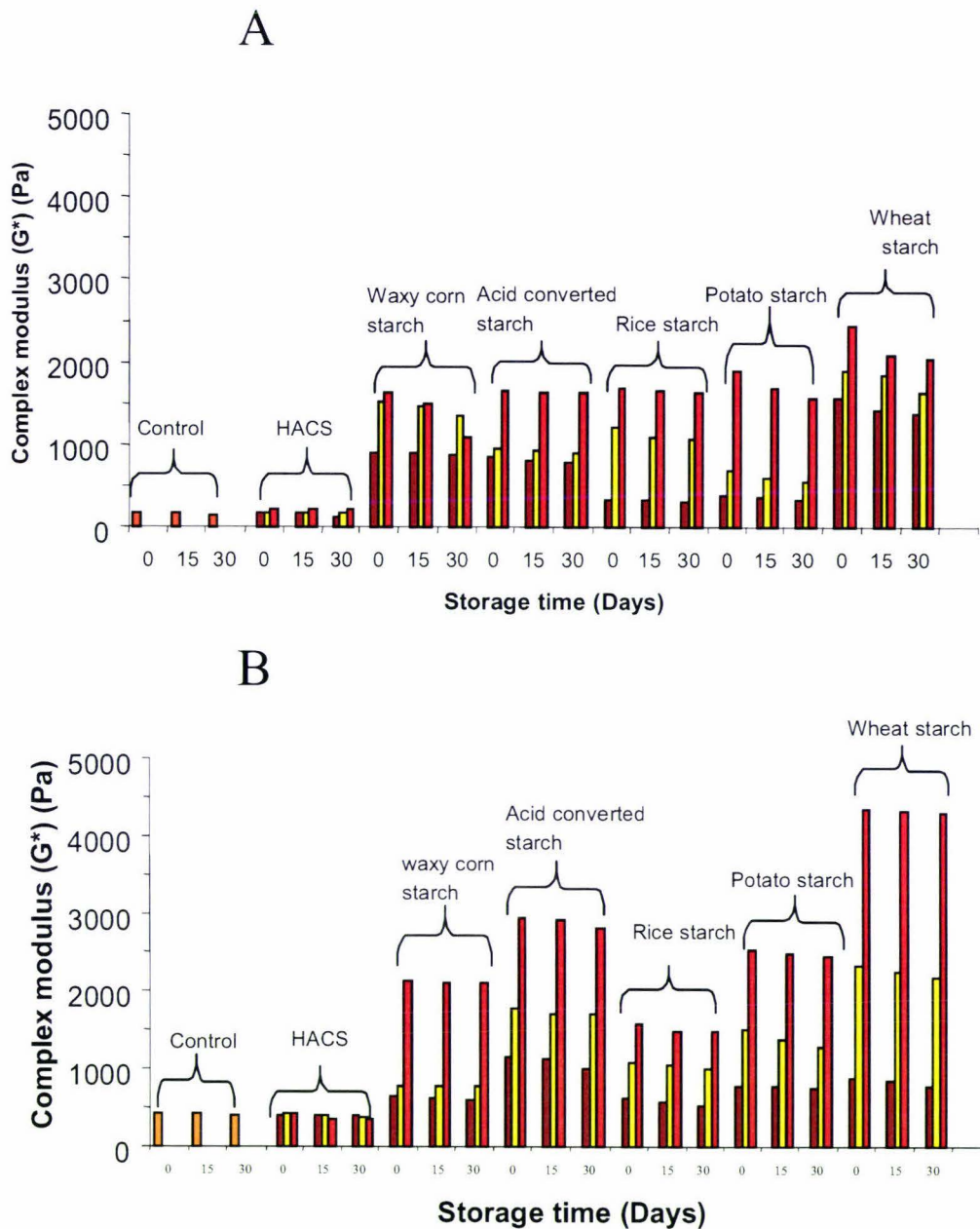
Figure 6.11 shows the complex modulus values for all processed cheese samples made on Paar-Physica and the RVA at 8.5% protein as a function of storage time. Figure 6.12 shows the complex modulus values for all processed cheese spreads samples prepared on Paar-Physica and RVA at 7.5% protein as a function of storage time.

The rennet casein based processed cheese spreads were stored at 4°C for 30 days and their rheological properties were determined at 0, 15 and 30 days. Figure 6.11(A) shows the  $G^*$  values for processed cheese spreads with 8.5% protein prepared on the Paar-Physica. For all the processed cheeses containing starch and the control sample there is a slight decrease (~ 10-12%) in the  $G^*$  values during refrigerated storage. Figure 6.11(B) shows the complex modulus ( $G^*$ ) value at 8.5% protein processed cheese spread prepared on RVA and measured at 0,15 and 30 days of storage. There seems to be a slight decrease in the  $G^*$  values of the samples. It suggests that at reduced protein (reduction by 1 wt%), this storage time and temperature have marginal effects on the rheological properties of processed cheese spreads.

Figure 6.12 shows the complex modulus values for all processed cheese samples made on Paar-Physica and the RVA at 7.5% protein as a function of storage time. Figure 6.12(A) shows the  $G^*$  values of processed cheese spreads prepared on the Paar-Physica at 7.5% protein level and stored at refrigerated temperature (4°C). Apart from the processed cheese samples containing potato and wheat starches, where  $G^*$  decreased noticeably after 15 days of storage, the decrease in the  $G^*$  values of the samples was small. Figure 6.12(B) shows the complex modulus values for samples prepared on RVA at 7.5% protein level. For all the samples there is a small decrease (<10%) in the  $G^*$  values indicating that at this storage conditions, the rheological properties of cheese samples containing starches are not drastically affected.



**Figure 6.11.** Complex modulus ( $G^*$ ) of model processed cheese spread as a function of storage time at 8.5% protein prepared on (A) Paar-Physica and (B) RVA with 0 wt% (■), 1 wt % (■), 2 wt% (■) and 3 wt % (■) containing different starches.



**Figure 6.12.** Complex modulus ( $G^*$ ) of model processed cheese spread as a function of storage time at 7.5% protein prepared on (A) Paar-Physica and (B) RVA with 0 wt% (■), 1 wt % (■), 2 wt% (■) and 3 wt % (■) containing different starches.

## 6.4 Discussion

The complex modulus ( $G^*$ ) values, for all the processed cheese spreads samples prepared on the RVA were higher than those prepared on the Paar-Physica confirming the findings discussed in Chapter 5. In addition, the particle size for samples prepared on the RVA was smaller than their equivalents prepared on the Paar-Physica. As stated in Chapter 5 (Section 5.5), a decrease in particle size results in product with higher elasticity.

The decrease in protein concentration will have two consequences. Firstly the protein network is weakened, thus the elasticity of the continuous protein phase is lower, resulting in a lower  $G^*$ . Secondly lowering the level of protein will result in a decrease in emulsifying power, since there is less protein to interact effectively to stabilise the fat and form a protein network (Shimp, 1985). This results in processed cheese products that have a lower elasticity and large fat globule size. This was confirmed by the confocal micrographs for processed cheese spreads containing both 8.5% protein and 7.5% protein where larger fat globules were observed.

When starches were added to the processed cheese spreads, complex modulus values increased at all protein levels both for samples prepared on the Paar-Physica and for those prepared on the RVA. Processed cheese spreads containing different starches showed different rheological behaviour when the starches were added at different protein levels and prepared on different equipments. The reason for differences in the behaviour of the different processed cheese spreads is because of the different shearing geometry of the two pieces of equipments (RVA and Paar-Physica) used to prepare the samples and the difference in the physio-chemical properties of the starches. These differences in the starch physio-chemical properties dictated the final microstructure of the cheese as observed by microscopy. For example cheeses made with waxy cornstarch showed areas that might be starch rich areas, while cheeses made with HACS showed unpasted starch granules.

Generally on refrigerated storage (4°C for 30 days), a slight decrease in the  $G^*$  values was observed. The  $G^*$  values decreased with a decrease in protein level in the processed cheese spreads. This was true for samples prepared on both the Paar-Physica

and the RVA. This slight decrease in the  $G^*$  value was thought to be mainly due to fat coalescing on storage.

To illustrate whether the aim of this work - to produce a processed cheese similar to the control by replacing protein with starch has been achieved, Table 6.3 shows the starch containing processed cheese spreads that have comparable complex moduli ( $G^*$ ) and particle size to that of the control sample made with 9.7% protein. It is clear from Table 6.3, that for different processes and different protein contents, different starches perform differently. However, for further studies, wheat starch, potato starch, rice starch and HACS will be considered for an IWS processed cheese. The reasons are:

- Wheat starch gives the highest elasticity.
- Potato starch is the cheapest.
- Rice starch gives intermediate properties to the processed cheese spread.
- HACS, selected mainly to see the effect of unpasted starch on the functionality of IWS processed cheese.

Waxy cornstarch gives a corny flavour to the final product and therefore was not selected. Acid converted starch, a modified starch, is costlier than commercially available native starches and also is out of the scope of this research work which was confined to a study of native starches.

**Table 6.3. Comparison of elasticity ( $G^*$ ) and particle size of processed cheese spreads containing starches with control (9.7% protein)**

Protein level	Equipment	Elasticity	Particle size.
8.5%	Paar-Physica	HACS (1%, 2% and 3%)	1% Rice starch. Acid converted starch (1%). Wheat starch (1% and 2%) Waxy cornstarch (1% and 2%)
	RVA	HACS (1%)	None
7.5%	Paar-Physica	Potato starch (1%). Rice starch (1%).	Wheat starch (1%).
	RVA	Potato starch (1%). Wheat starch (1%) Acid converted starch (1%) Waxy cornstarch (2%). Rice starch (2%).	None

## 6.5 Summary to Chapter

Model processed cheese spreads were made using both the Paar-Physica and the RVA. Six different starches replaced protein, protein levels were reduced to 8.5% and 7.5% from the control value of 9.7% protein. Rheological, particle size and microstructural properties of all the samples prepared were investigated, both as made and after refrigerated storage. It was found that reducing the level of protein in the processed cheese influenced the rheological properties, the mean particle size and the microstructural properties of model processed cheese spreads. The processed cheeses made in the RVA had smaller fat droplets compared with those made in the Paar-Physica and as a result the complex modulus values were higher. Using HACS in the formulation had the least impact on the properties of all the starches investigated. Microstructure was dependent on the starch type and the equipment used to manufacture the processed cheese. Refrigerated storage had no significant effect on the structure of the processed cheese spread.

## **7. Effect of Different Starches on the Functional Properties of IWS Processed Cheese**

### **7.1 Introduction**

This chapter describes the validation of the effects of starch on rennet casein-based processed cheese spreads, discussed in previous chapters in an Individually Wrapped Slice (IWS) processed cheese system. The formulation was changed from a rennet casein-based processed cheese spread formulation to an IWS processed cheese formulation containing natural cheese. Most of the intact casein is obtained from the young cheese while mature cheese contributes to the flavour properties of the IWS processed cheese. Four different starches were used in this investigation (potato starch, wheat starch, rice starch and high amylose cornstarch (HACS)).

Earlier the effect of reducing the protein level in a processed cheese spread made from rennet casein was studied, by investigating the rheological, microstructural properties and particle size (Chapters 6). In this chapter, each formulation was prepared in the RVA and the different starches were added on top of the formulation. After preparation, three functionality tests, vane test, cylinder test and melt test, were carried out to determine the effects of the starches on the properties of the processed cheese.

The main objectives of the work presented in this chapter were:

- To make IWS processed cheese with a target protein level of approximately 17.3% in the RVA.
- To evaluate the effects of four different starches (potato starch, wheat starch, rice starch and HACS) added at four different levels (0, 1, 2 and 3%).
- To measure the textural and melt properties of the IWS processed cheese products.
- To determine which starch (es) gave the best IWS processed cheese textural properties. The selected starch or starches will then be used for protein reduction in IWS processed cheese.

## 7.2 Experimental approach

The IWS formulations are shown in Table 7.1 with their respective overall compositions shown in Table 7.2. Young, high-solids cheese was used as a source of intact casein to provide most of the functional properties of the product. The mature cheese, used only at low levels, is used to provide the required flavour characteristics in the processed cheese.

Intact casein is the non-proteolysed protein present in the cheese, and consists mainly of  $\alpha$ -casein and  $\beta$ -casein (Berger et al, 1998). The level of intact casein largely determines the texture and melting properties of the product, and in the present sets of experiments the target intact casein level was approximately 8.0%. This was achieved by mixing appropriate quantities of young and mature cheddar cheeses to reach a target of ~17.3% total protein.

Based on the results described in Chapter 6, four different starches, potato, rice, wheat and HACS, were selected for this phase of work. Potato starch, rice starch and wheat starch were selected mainly on the functional properties (firmness, melt characteristics, elasticity) they imparted to processed cheese. HACS has high pasting temperature compared to other starches (160-170°C). This enabled us to look at the effect of the presence of non-pasted starch in a processed cheese system. Potato starch is the cheapest of the starches available. Wheat starch generally gives the highest firmness among all the starches.

To further establish the effects, from a solids perspective, which the added starch could have on the product, the experiments were repeated using lactose in place of the starch. Lactose is considered as inert filler and should give an incremental increase in the viscosity of the continuous phase in proportion to the amount of lactose added. Allowance is made in the formulation for differences in moisture content of starch and lactose.

**Table 7.1. Ingredients for IWS processed cheese containing starch at different addition levels**

	<b>Control</b>	<b>1% starch</b>	<b>2% starch</b>	<b>3% starch</b>
	<b>(g)</b>	<b>addition</b>	<b>addition</b>	<b>addition</b>
	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>
High solids cheese (young)	18.55	18.55	18.55	18.55
Cheddar (mature)	2.00	2.00	2.00	2.00
Lactose	0.60	0.60	0.60	0.60
Trisodium citrate	0.93	0.93	0.93	0.93
Citric acid	0.06	0.06	0.06	0.06
Sodium chloride	0.18	0.17	0.17	0.17
Potassium sorbate	0.06	0.06	0.06	0.06
Added water	7.62	7.63	7.63	7.63
Starch	0.00	0.30	0.60	0.90
<b>Total weight</b>	<b>30.00</b>	<b>30.30</b>	<b>30.60</b>	<b>30.90</b>

**Table 7.2. Target composition of IWS processed cheese containing different starch addition levels**

	<b>Typical</b>	<b>1% starch</b>	<b>2% starch</b>	<b>3% starch</b>
	<b>composition</b>	<b>addition</b>	<b>addition</b>	<b>addition</b>
	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>
Moisture	47.0	46.7	46.3	46.0
Fat	25.9	25.6	25.4	25.1
Protein	17.3	17.1	16.9	16.8
Intact casein	8.0	7.9	7.9	7.8
Salt	1.7	1.7	1.7	1.6
Lactose	1.9	1.9	1.9	1.9
<b>Target pH</b>	<b>5.7</b>	<b>5.7</b>	<b>5.7</b>	<b>5.7</b>

All of the formulations were prepared in triplicate except for those formulations containing HACS and extra lactose. The trials were carried out on separate days where on a given day, a starch was selected and five IWS slices for each different addition rate along with five control (no starch addition) slices were prepared. The five control samples were prepared everyday, to monitor day-to-day variation in the processed cheese. Functional tests (vane test, cylinder test and melt test) were carried out after seven days of refrigerated storage of the IWS slices.

The formulations for the experiments carried out with extra-added lactose are shown in Table 7.3.

**Table 7.3. Formulation for processed cheese containing extra lactose**

	<b>Control</b>	<b>1% extra lactose</b>	<b>2% extra lactose</b>	<b>3% extra lactose</b>
	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>
High solids cheese- Young	18.55	18.55	18.55	18.55
Cheddar - mature	2.00	2.00	2.00	2.00
<b>Lactose</b>	<b>0.60</b>	<b>0.87</b>	<b>0.93</b>	<b>1.39</b>
Trisodium citrate	0.93	0.93	0.93	0.93
Citric acid	0.06	0.06	0.06	0.06
Sodium chloride	0.18	0.17	0.17	0.17
Potassium sorbate	0.06	0.06	0.06	0.06
Added water	7.62	7.66	7.70	7.74
<b>Total Weight</b>	<b>30.0</b>	<b>30.30</b>	<b>30.40</b>	<b>30.90</b>

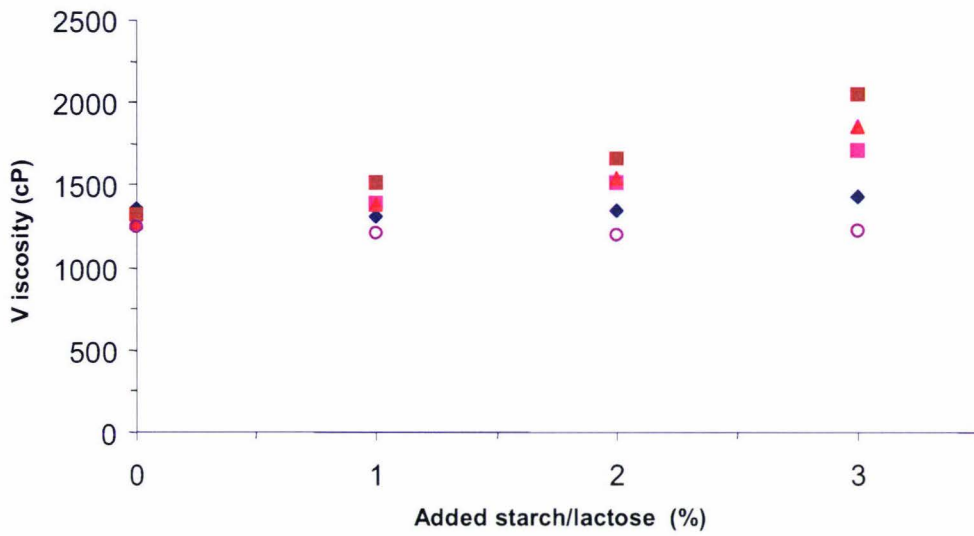
## **7.3 Results**

### **7.3.1 Viscosity measurement**

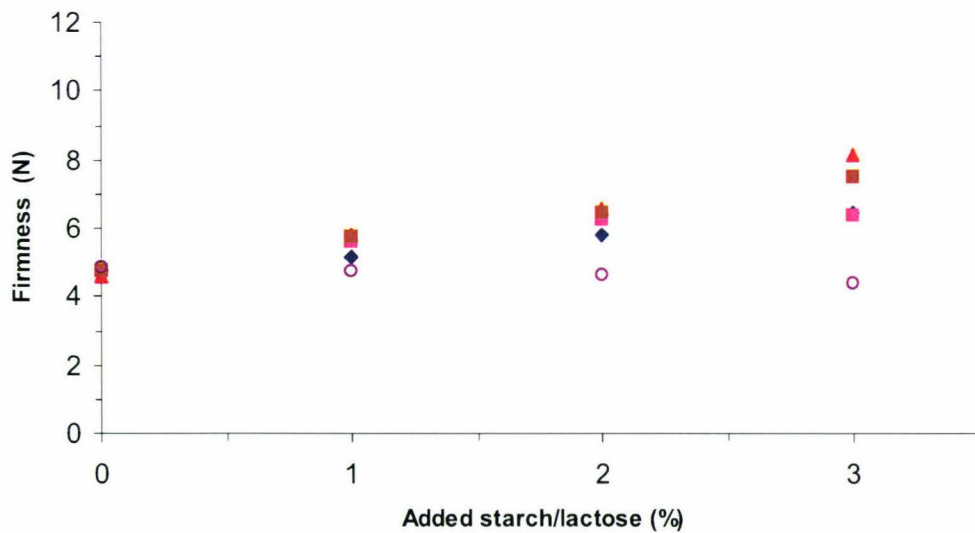
Apparent viscosity is measured in-situ by the RVA. The plastic stirrer stirs the contents of the canister and the measured torque on the stirrer is used to derive an apparent viscosity. The final viscosity only is considered here. Figure 7.1 shows the viscosity values for IWS processed cheeses with the four starches and with the extra lactose added. The viscosity was found to increase with starch concentration for all the starches. However, there was no significant change in the viscosity of processed cheese when lactose was added. IWS processed cheese containing wheat starch gave the highest viscosity followed by potato, rice and HACS. Processed cheese with lactose addition had the lowest viscosity. It is possible that because potato starch and wheat starch have larger granules (see Table 4.2, Chapter 4) and more swelling ability than rice starch they could impart a higher viscosity rise compared with other starches. Rice starch with small granules increases the viscosity but less than both potato and wheat starches. As confocal micrographs in previous chapters showed, HACS was not fully pasted during the cooking and this could result in a small increase in viscosity, compared with the other starches.

### **7.3.2 Firmness**

Figure 7.2 shows the firmness data for all of the IWS processed cheese, both with the starches and with lactose addition. The firmness of the processed cheese was observed to increase as the starch level increased. In contrast, addition of extra lactose had little effect on firmness. The firmness of IWS processed cheese was found to be higher for potato starch followed by wheat starch, rice starch and HACS. The IWS processed cheese with extra lactose was the softest of all. The increase in firmness is possibly due to an increase in hydrogen bonding of the amylose fractions, leached out during the cooking process and the shape of the starch granules (Mounsey & O’Riordan, (2001)).



**Figure 7.1.** Final viscosity of IWS processed cheese as a function of starch/lactose concentration prepared on the RVA containing HACCS (◆), rice starch (■), potato starch (▲), wheat starch (■) and extra lactose (○).



**Figure 7.2.** Firmness of IWS processed cheese as a function of starch/lactose concentration prepared on the RVA containing HACCS (◆), rice starch (■), potato starch (▲), wheat starch (■) and extra lactose (○).

### 7.3.3 Stress and Strain

Figure 7.3 shows the Vane test stress values for IWS processed cheeses made with the starches and with extra lactose. The vane test uses a four bladed vane, inserted into a sample, and the vane is rotated until the sample yields. The resulting data is converted to stress and strain values (Section 3.7.6). There was an increase in the stress values for all of the processed cheese that contained starches, but there was a decrease in the stress value for processed cheeses with extra lactose. At 3% starch addition, potato starch gave the highest stress value followed by wheat starch, rice starch and HACS. This was in agreement with the results obtained from the firmness testing.

Figure 7.4 shows the strain values for IWS processed cheese made with the starches and extra lactose. It can be seen that the addition of starch did not seem to affect the strain value of the cheese. Although the addition of starch did affect the elasticity (Figure 7.3), this could be achieved without affecting its deformation, as the two rheological attributes are different.

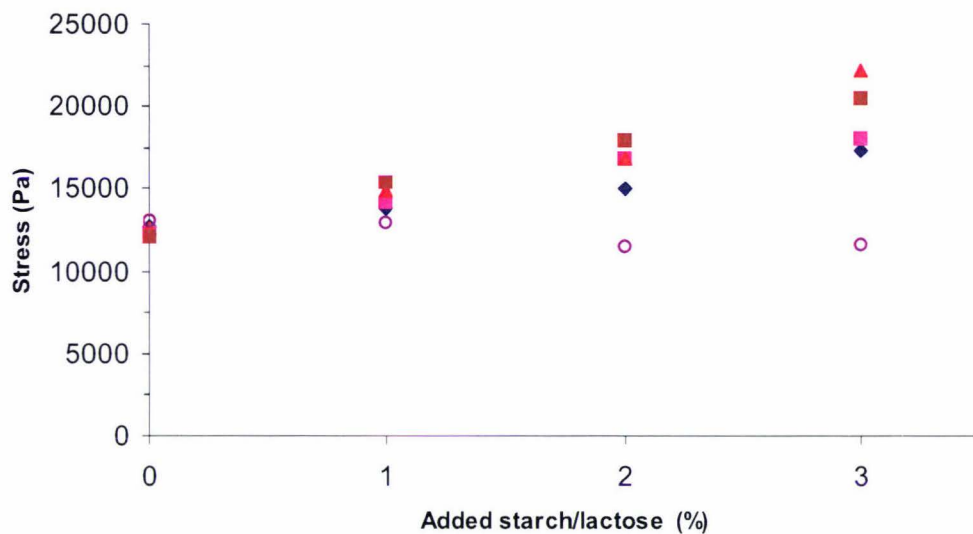


Figure 7.3. Stress of IWS processed cheese as a function of starch/lactose concentration prepared on RVA containing HACs (◆), rice starch (■), potato starch (▲), wheat starch (■) and extra lactose (○).

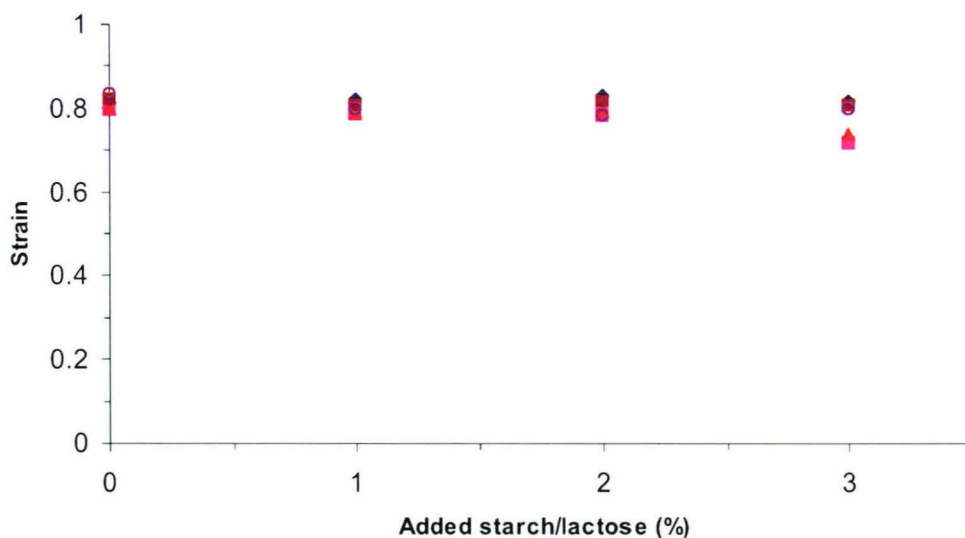


Figure 7.4. Strain of IWS processed cheese as a function of starch/lactose concentration prepared on RVA containing HACs (◆), rice starch (■), potato starch (▲), wheat starch (■) and extra lactose (○).

### 7.3.4 Melt index

Figure 7.5 shows the Melt Index values for IWS processed cheese with the added starches and extra lactose.

The Melt Index of the processed cheeses decreased when more starch was added. Lactose appeared to have little impact on the melt. This confirms the observations reported by Mounsey & O’Riordan, (1999) who suggested that the incorporation of starches to processed cheese during its manufacture, leads to the dehydration of the protein matrix resulting in a decrease in the meltability.

### 7.3.5 Microstructural evaluation

Figure 7.6 shows confocal micrographs of IWS processed cheese samples containing the different starches at a 2% addition rate. The micrographs illustrate how fat droplets (dark red area) are entrapped within the protein matrix (green coloured continuous phase). Fat droplets, although of a wide range of sizes, were observed to be uniformly distributed. Processed cheese slices with wheat and potato starch tended to show larger fat droplets (Figure 7.6(D)), compared with those with the other starches. When HACS is added to a processed cheese, black spots were observed in the micrograph as seen previously (Chapter 5, Figure 5.14(C)). These are likely to be the unpasted starch granules. The processed cheese with rice starch showed the smaller fat droplets than those containing either wheat (Figure 7.6(D)) or potato starches (Figure 7.6(B)).

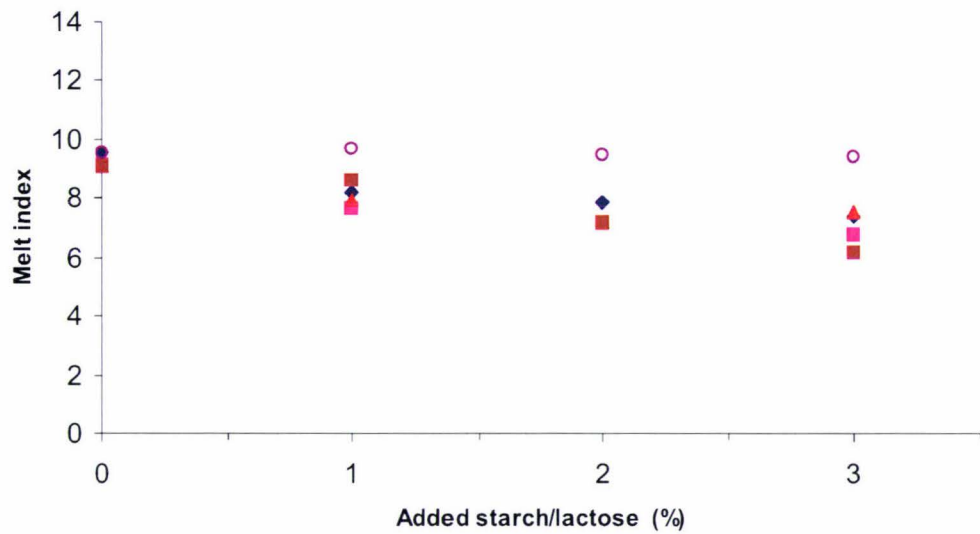
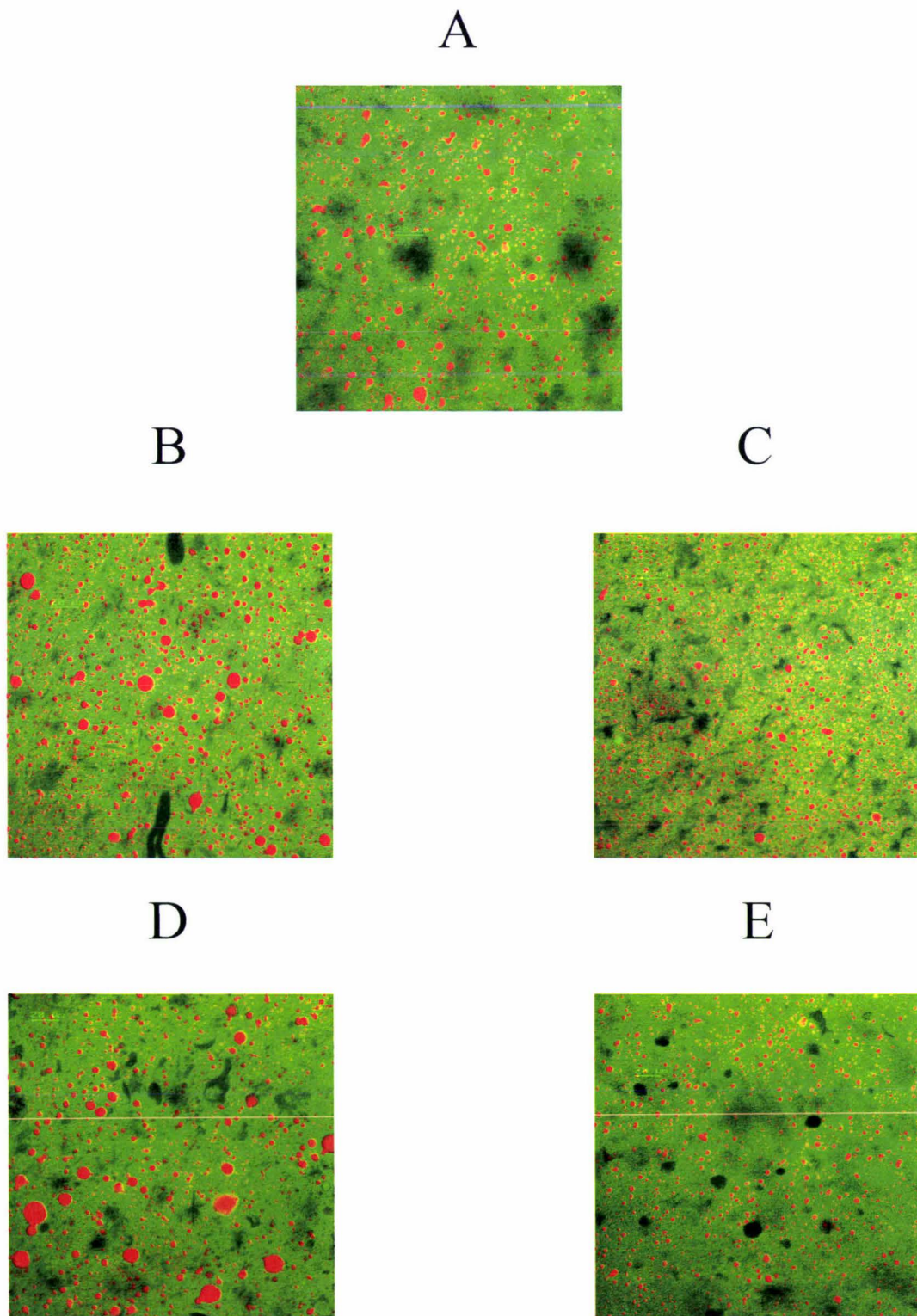


Figure 7.5. Melt Index of IWS processed cheese as a function of starch/lactose concentration prepared on the RVA containing HACs (◆), rice starch (■), potato starch (▲), wheat starch (■) and extra lactose (○).



**Figure 7.6.** Confocal micrographs of processed cheese spread prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control, (B) 2 wt% potato starch, (C) 2 wt% rice starch addition, (D) 2 wt% wheat starch and (E) 2 wt% HACS.

## 7.4 Discussion

The addition of the starches to IWS processed cheese led to increased viscosity, firmness and stress. The strain was largely unaffected and the melt index decreased. Adding lactose instead of starch had little impact on the texture of processed cheese.

The increase in viscosity and elasticity (stress in Fig. 7.3) of the IWS cheese, as seen for the model cheeses (Chapter 4) is due to the presence of starch molecules (amylose and amylopectin) and their water binding ability which resulted in an increase in the viscosity of the cheese continuous phase. Furthermore, the leaching out of amylose during the cooking of processed cheese forms hydrogen bonds and gelifies, resulting in the increase in the firmness. Because potato, wheat, and rice starches have high swelling power and high amylose contents their addition resulted in an increase of both viscosity and firmness of the IWS cheeses. HACS contributed slightly to the firmness and viscosity of the IWS processed cheese, as it was not fully pasted during the cooking process.

Strain values were largely unaffected by starch addition. This suggests that the key cheese structure with respect to strain, is a continuous protein network. As all the cheeses described in this chapter contained the same amount of protein, the strain measured by the vane test is constant. Watkinson (2005, personal communication) has observed previously, that strain measurement in some types of cheeses is remarkably insensitive to changes in the moisture content.

The meltability of the cheeses all decreased with the addition of starches. Although cheese melting is highly complicated and not yet fully understood (Gunasakeran & Mehmet Ak, 2002) it is possible that, the lack of thermoplasticity of cross-linked, leached amylose, adversely affects the meltability of the processed cheese as suggested by Mounsey & O'Riordan (2001). They also suggested that swollen starch granules or gelatinized starch might immobilize water resulting in dehydration of the protein matrix and an increase in hydrophobic protein-protein interactions, ultimately causing poor meltability. This could explain why HACS had the least effect on the melt property of the cheese, as it was not being pasted (gelatinised) to the same extent as wheat, rice or potato starches.

The results of the added lactose experiments were interesting. Lactose is considered an inert filler (Guinee, 2002) and hence it was thought that substituting starch for lactose would allow the effect of the starch solids addition to be measured. However, in this work, lactose had a softening effect on the processed cheese, in contrast to observation made by Berger et al (1998) who concluded that an increase in the amount of lactose in processed cheese should increase the firmness. What was observed in this work might be a dilution effect of lactose on the protein, i.e. a weaker structure is formed (Lee et al, 1999).

## **7.5 Summary to Chapter**

The effect of different starches on the meltability and textural properties of IWS processed cheese were dependent on the type of starch. Potato starch, wheat starch and rice starch reinforced the processed cheese structure, increasing the firmness and viscosity. However, as the firmness increased the melt decreased. The decrease in the meltability could be due to dehydration of protein matrix and the hydrogen bonding of the leached amylose from the starch granules. Among all the starches, potato starch gave the best results in terms of the textural properties of the processed cheese, particularly firmness.

## 8. Protein Reduction in IWS Processed Cheese

### 8.1 Introduction

One of the objectives of this research programme was formulation cost reduction for IWS processed cheese. It was envisioned that reducing the level of young cheese used in a formulation and compensating for the loss of structure by adding starch would achieve significant cost savings. In Chapter 7 it was shown that potato starch gave the largest gain in structural properties of processed cheese such as firmness and it is also the cheapest among all the starches. Thus, it was decided to examine three target protein levels (17.3%, 16.3% and 15.3%) and four potato starch levels (0, 1, 2 and 3 wt%) in processed cheese to find out the optimum level of starch required to replace protein from young cheese without significantly affecting the properties of processed cheese.

The main objectives of the work presented in this chapter were:

- To make IWS processed cheese with three target protein levels (17.3%, 16.3% and 15.3%) in the RVA.
- For each protein level, make IWS containing four levels of potato starch (0, 1, 2 and 3 wt%) and measure the impact on firmness, melt and viscosity.
- Ascertain the amount of starch required to restore the texture of a product to that of the control at each reduced protein level.
- To make recommendations for a scale-up trial in the Fonterra Innovation pilot plant.

### 8.2 Experimental approach

The same control formulation (~47% moisture, ~17.3% protein) was used as in Chapter 7. However, a different block of raw material cheese was used for this piece of work. All other materials and methods are as outlined in Chapter 3.

To achieve the two reduced protein levels, an amount of young cheese was removed from the formulation. The cheese protein was replaced with lactose, fat with butter and moisture with added water. The ingredients and target compositions are shown in Tables 8.1 and 8.2.

**Table 8.1. Ingredients for IWS processed cheese at different protein levels**

	<b>17.3% protein (g)</b>	<b>16.3% protein (g)</b>	<b>15.3% protein (g)</b>
High solids cheese- (young)	18.55	17.40	16.22
Cheddar (mature)	2.00	2.00	2.00
Butter	0.00	0.57	1.16
Lactose	0.60	0.90	1.20
Trisodium citrate (TSC)	0.95	0.95	0.95
Citric acid	0.04	0.04	0.04
Sodium chloride	0.18	0.2	0.22
Potassium sorbate	0.06	0.06	0.06
Added water	7.62	7.88	8.15
<b>Total Weight</b>	<b>30.0</b>	<b>30.0</b>	<b>30.0</b>

**Table 8.2. Composition for IWS processed cheese at different protein levels**

	<b>17.3% protein (Control)</b>	<b>16.3% protein</b>	<b>15.3% protein</b>
Moisture (%)	47.0	47.0	47.0
Fat (%)	25.9	26.0	26.1
Protein (%)	17.3	16.3	15.3
Intact casein (%)	8.1	7.6	7.1
Salt (%)	1.7	1.0	1.8
Lactose (%)	1.9	3.0	4.0
Target pH	5.70	5.70	5.70

Three protein levels and four starch addition rates gave a total of 12 formulations. As described in Chapter 7, each formulation required the manufacture of 5 slices for testing purposes (see Table 8.3). As it was necessary to repeat in triplicate i.e. a set of five slices were made on each of three different days.

**Table 8.3. Preparation of IWS slices with different starch and protein levels-repeated in triplicate**

	0% starch (Control)	1% starch	2% starch	3% starch
17.3% protein	5	5	5	5
16.3% protein	5	5	5	5
15.3% protein	5	5	5	5

To monitor day-to-day variability a set (five slices) of controls processed cheese was made everyday. Each day, one protein level was selected and a set of five slices made at each starch addition rate. Texture testing was carried out seven days after manufacture.

### 8.3 Day-to-day variation

The RVA makes processed cheese on a very small scale. Thus it is particularly sensitive to weighing error or variation in starting materials. Each day, a set of controls was made so that properties such as viscosity and firmness could be monitored. If any significant day-to-day variability was observed, then data could be standardised against daily control values.

#### 8.3.1 Viscosity Measurement

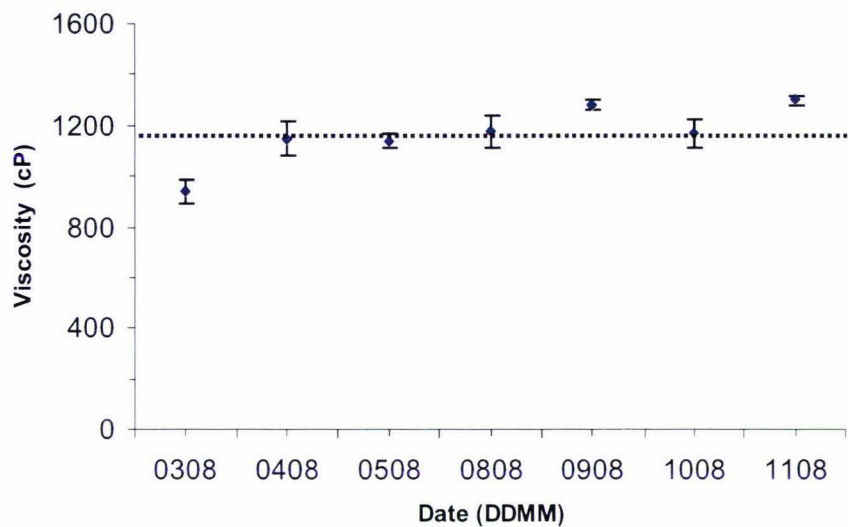
Figure 8.1 displays the viscosity (end point viscosity) data from the RVA for the 17.3% protein controls from each day. Some variation in viscosity within and between days was evident. This is partly a result of the measurement technique because only a single viscosity measurement is recorded for a food system where the creaming process results in a constantly changing viscosity (Dimitreli & Thomareis, 2004). Furthermore if the variation is  $\pm 10\%$  this is likely to be within the precision of the RVA.

### 8.3.2 Firmness

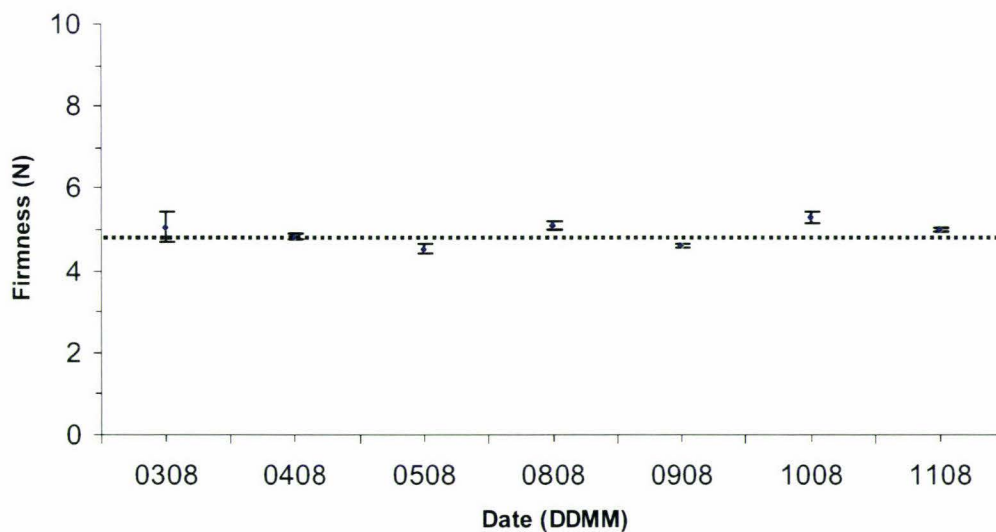
Figure 8.2 displays the firmness data from the RVA for the 17.3% protein controls made each day. Again, some variation within and between days was evident. Some textural variation is to be expected in food systems of this nature. Initially all data were standardised against the relevant daily controls. Although this helped to reduce the apparent day-to-day variation, it complicated the comparison of the different protein levels. Thus only raw data were plotted.

Variation in the firmness might have arisen because of:

- Weighing/transfer: weighing small quantities of ingredients increases the potential weighing errors. Small losses during transfer of ingredients can have significant effects on the final composition resulting in errors in the textural measurement.
- Cheese structure: although cheese raw materials were stored at a low temperature ( $-2^{\circ}\text{C}$ ), proteolysis can still occur and this will affect the intact casein levels in the cheese. Thus, during storage, the body of cheese can weaken. Compositional variation within a block of cheese can also be an issue.
- The rheological tools (RVA and texture analyser) used in this study will also introduce some errors in the measurement, due to the sensitivity of the instruments to accurately measure the torque or forces applied during the measurements.



**Figure 8.1. Viscosity on daily basis for processed cheese containing 17.3% protein. The dotted line is the overall average control viscosity.**



**Figure 8.2. Firmness on daily basis for processed cheese containing 17.3% protein. The dotted line is the overall average control firmness.**

## 8.4 Results

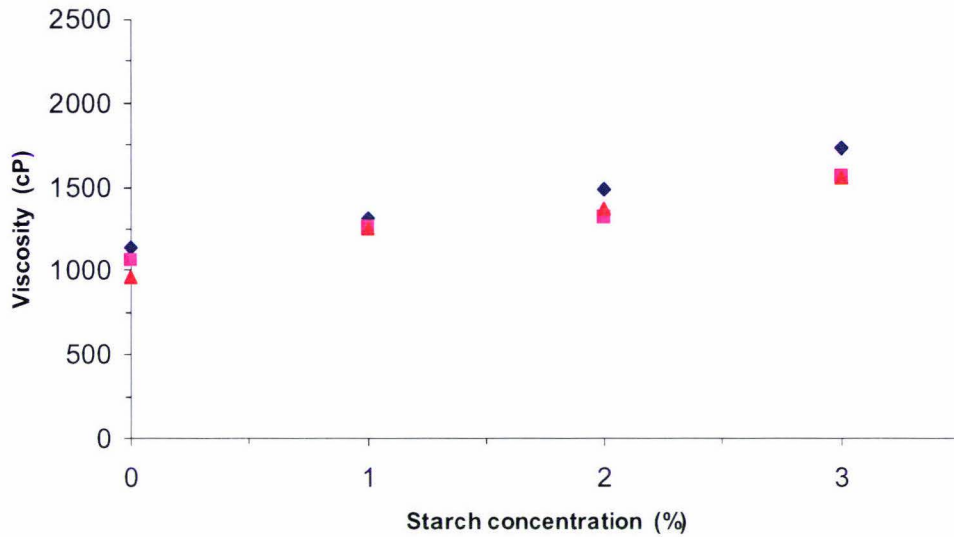
From the standard formulation containing ~17.3% protein, either 1% or 2% protein was removed by reducing the proportion of young cheese. To compensate for the fat and moisture loss, butter and water were added. Protein was compensated by adding lactose. Potato starch was then added to the formulation at three different levels (1, 2 and 3%). As the protein levels were reduced, the IWS products became stickier, that is they failed to peel cleanly from the wrapper. Addition of starch did not wholly fix the problem.

### 8.4.1 Viscosity Measurement

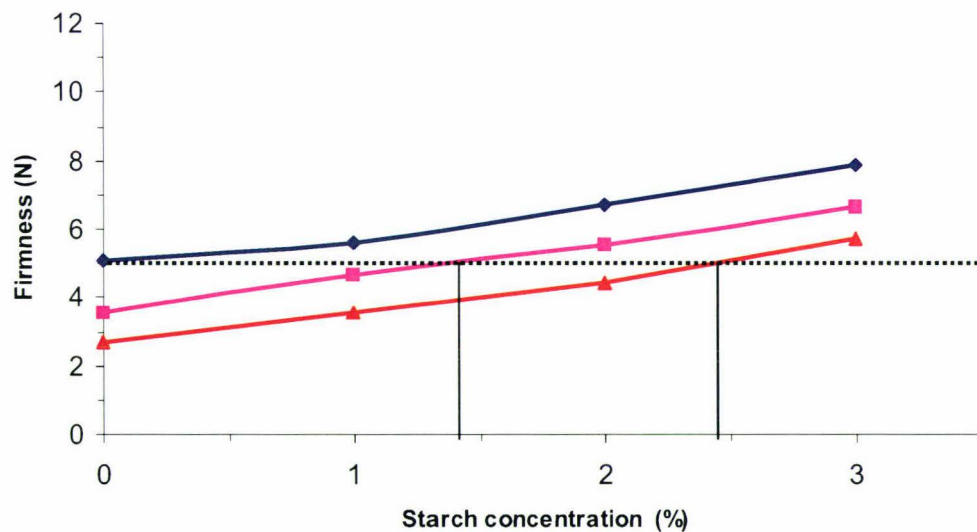
The RVA viscosity data for the ~17.3%, 16.3% and 15.3% protein IWS slices is plotted in Figure 8.3. Each data point is the average of three measurements. Figure 8.3 shows the viscosity results for all of the protein levels (17.3%, 16.3% and 15.3%). As expected, processed cheese containing 17.3% protein generally had the highest viscosity followed by that of the processed cheese containing 16.3% and that of the processed cheese made with 15.3% protein. Higher levels of protein assist structure formation leading to higher viscosity. Viscosity also increases as the starch addition rate increases.

### 8.4.2 Firmness

The firmness data from the 17.3%, 16.3% and 15.3% protein IWS slices is plotted in Figure 8.4. The firmness of processed cheese containing 17.3% protein was the highest of all the processed cheeses, as expected. As the starch addition rate increased there was also an increase in the firmness of the cheese at all protein levels. Note also that as for the viscosity, the firmness increases proportionally to the starch added. The marked horizontal line in the graph indicates the average firmness value of 17.3% control processed cheese. At 1 wt% starch addition rate and 16.3% protein level, similar firmness was observed to that found for 17.3% protein with no starch addition. At 15.3% protein and 2 wt% starch addition, a firmness value comparable with that of the 17.3% protein control was observed.



**Figure 8.3.** Viscosity of IWS processed cheese as a function of potato starch concentration prepared on RVA containing 17.3% protein (◆), 16.3% protein (■) and 15.3% protein (▲).



**Figure 8.4.** Firmness of IWS processed cheese as a function of potato starch concentration prepared on RVA containing 17.3% protein (◆), 16.3% protein (■) and 15.3% protein (▲). The dotted line is the control firmness.

### 8.4.3 Stress and Strain

The stress data from the Vane Test for 17.3%, 16.3% and 15.3% protein IWS slices is plotted in the Figure 8.5. The horizontal line is the 17.3% protein control stress value for reference. The shear stress for 17.3% protein IWS was higher than those of the 16.3% and 15.3% equivalents. At 1 wt% starch addition rate and 16.3% protein, the same shear stress values were observed as for the 17.3% protein control. At 15.3% protein and 2 wt% starch addition, the stress was comparable to that of the control. This is similar to the firmness data.

The strain data from the Vane Test for all protein levels are plotted in Figure 8.6. For the control slices, strain decreased slightly as starch levels increased. The reduction of the strain upon increase in starch addition is also observed for samples made with 16.3% and 15.3% proteins. This is another indication that although the starch addition significantly affected the firmness and stress measured, only a slight decrease in strain is observed. As proposed in the previous chapter (Chapter 7), this could be an indication that the cheese is made of a protein continuous network that has the biggest influence on the strain measurement.

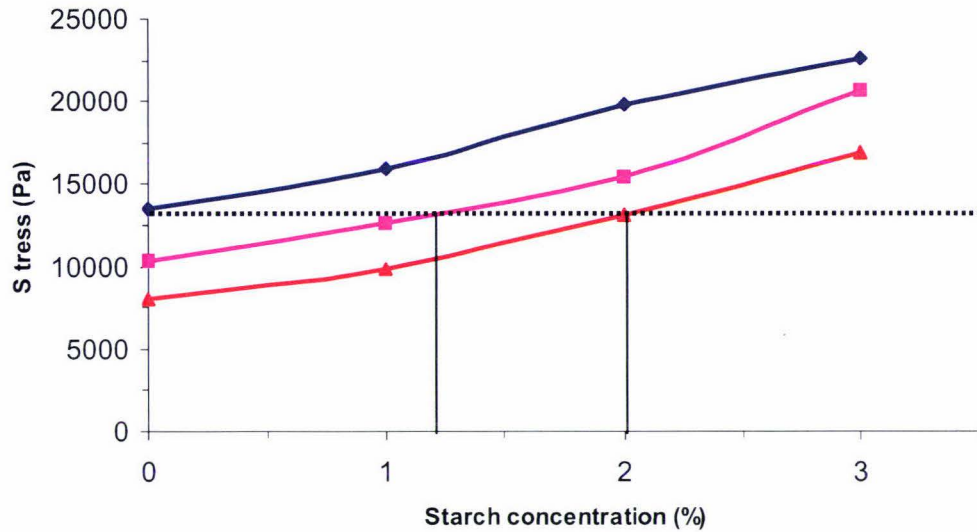


Figure 8.5. Stress of IWS processed cheese as a function of potato starch concentration prepared on RVA containing 17.3% protein (◆), 16.3% protein (■) and 15.3% protein (▲). The dotted line is the control stress.

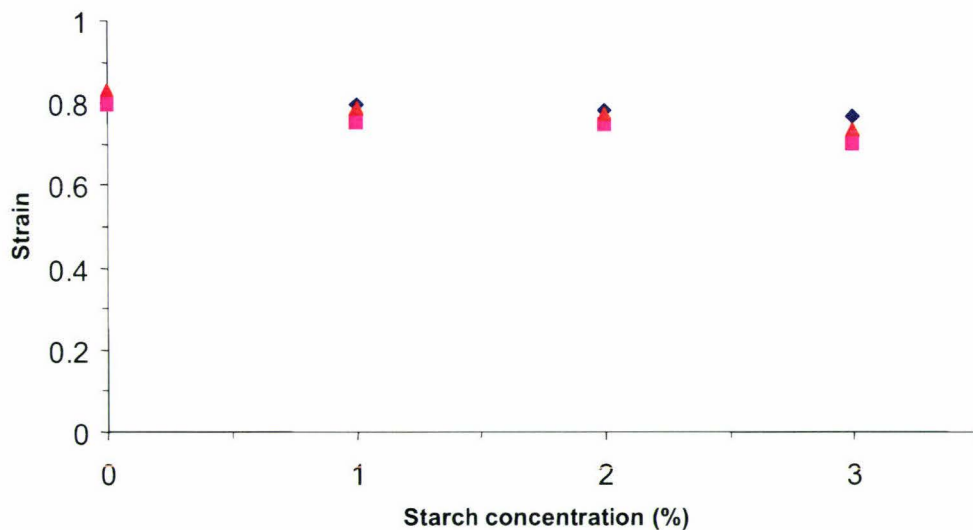


Figure 8.6. Strain of IWS processed cheese as a function of potato starch concentration prepared on RVA containing 17.3% protein (◆), 16.3% protein (■) and 15.3% protein (▲).

#### **8.4.4 Melt index**

The melt index data from the 17.3%, 16.3% and 15.3% protein IWS slices are plotted in the Figure 8.7.

There was a decrease in the melting index of the processed cheese as the starch level increased, and this was true for all the proteins level. There was no significant difference in the melting behaviour of processed cheeses with different protein levels when starch was added.

#### **8.4.5 Microstructural evaluation**

Figures 8.8 and 8.9 show the confocal micrographs of IWS processed cheese samples with potato starch at different levels of protein and starch addition. The micrographs illustrate how fat particles (red areas) are entrapped within the protein matrix (green coloured continuous phase). The micrographs show a range of particle sizes. Processed cheese slices with both 17.3% protein (Figure 8.8 (B), (C) and (D)) and 15.3% protein (Figure 8.9 (A) and (B)) showed a decrease in the fat globule size with increase in starch addition rate. The fat in the cheese samples seems to be more aggregated with larger gaps between the aggregates. The dark black region seen in confocal micrographs may be water droplets air voids or starch rich regions. As protein and fat has been labelled with specific dyes, it should be something other than fat and protein. Fat seems to be well distributed across the protein network and has formed a good emulsion.

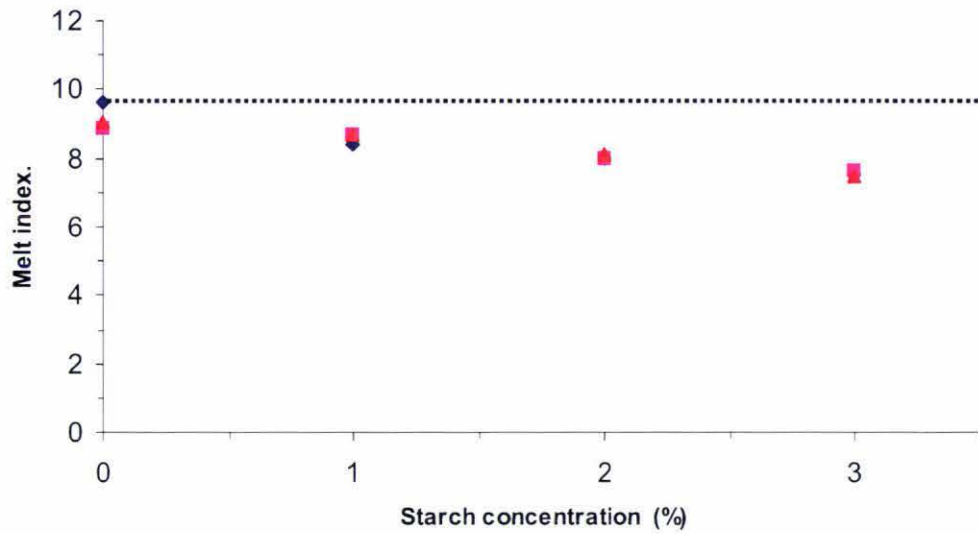
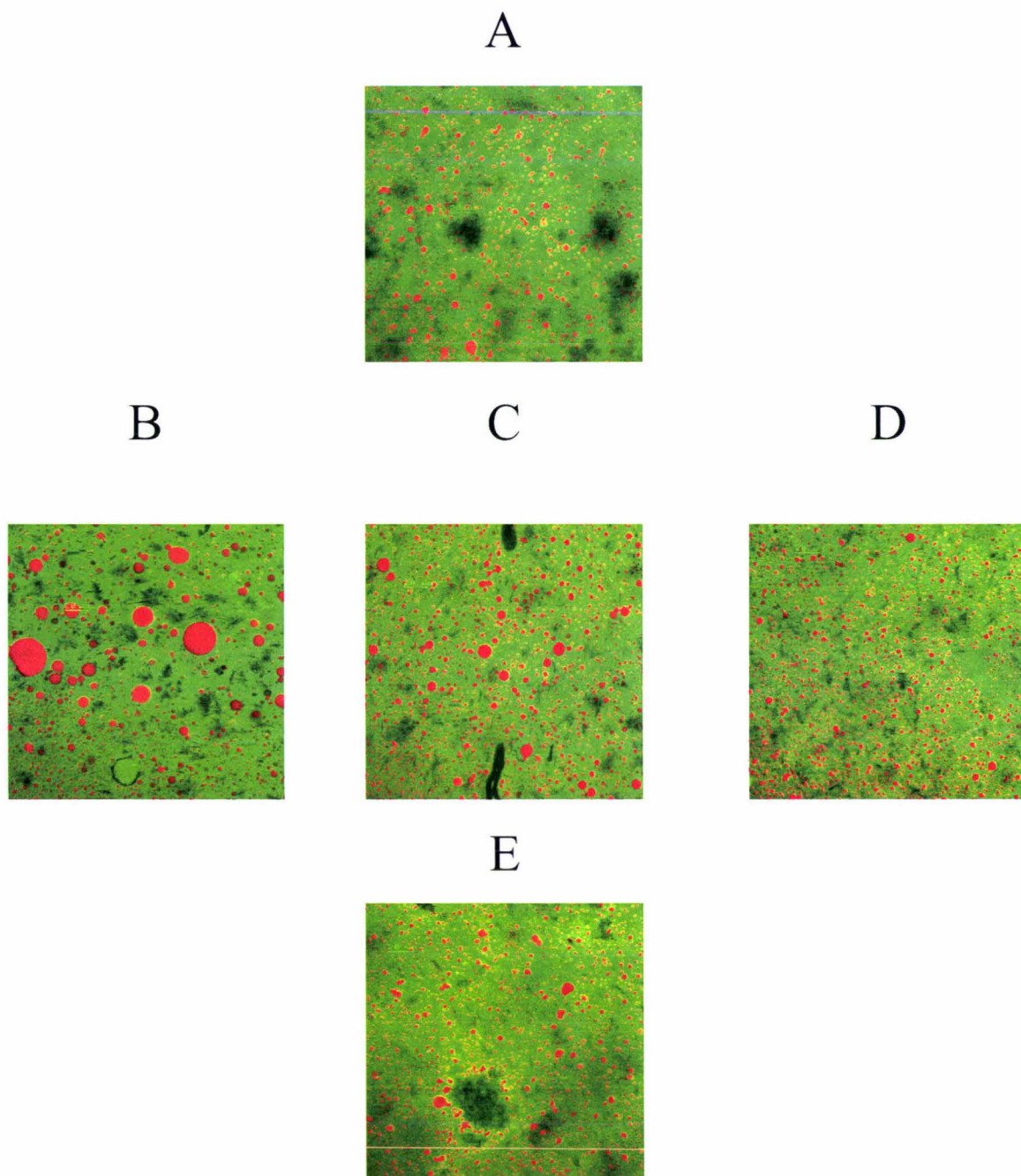
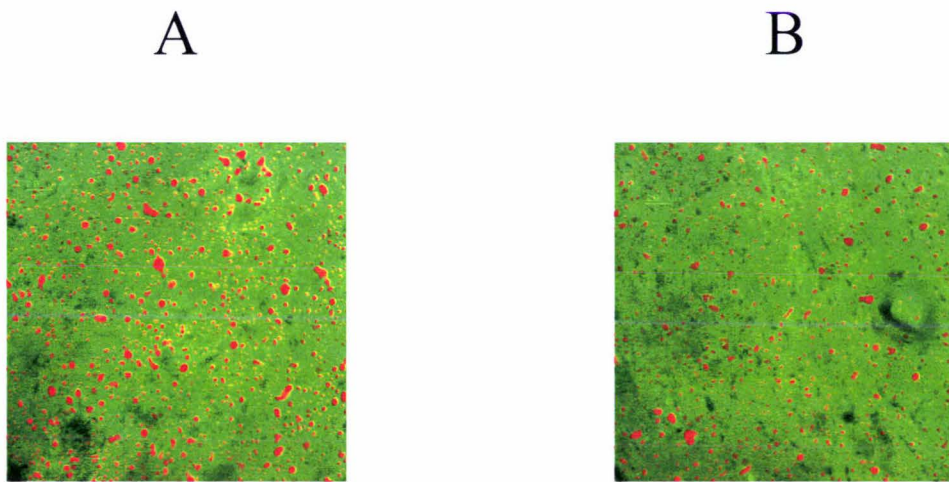


Figure 8.7. Melt Index of IWS processed cheese as a function of potato starch concentration prepared on RVA containing 17.3% protein (◆), 16.3% protein (■) and 15.3% protein (▲). The dotted line is the control melt index.



**Figure 8.8.** Confocal micrographs of IWS processed cheese prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) 17.3% protein and 0 wt% potato starch (B) 17.3% protein and 1 wt% potato starch, (C) 17.3% protein and 2 wt% potato starch, (D) 17.3% protein and 3 wt% potato starch and (E) 16.3% protein and 1 wt% potato starch.



**Figure 8.9.** Confocal micrographs of IWS processed cheese prepared on RVA obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) 15.3% protein and 2 wt% potato starch (B) 15.3% protein and 3 wt% potato starch.

### 8.4.6 Particle size measurement

The D [4,3] (the diameter of equivalent mean volume of the particles) is reported below in Table 8.4. The mean volume clearly increases with starch addition, at all protein levels. This contradicts the observations in the micrographs. A possible explanation for this is the sensitivity of the particle size measurement technique to high particle diameters. In other words, few large fat droplets scattered the light much more than by a big number of small fat droplets. In which case the resulting diameter is overestimated. To accurately compare the different samples, the position of the peak between 1 and 10  $\mu\text{m}$  (assumed most likely to be fat) was recorded along with the proportion of particles greater than 20  $\mu\text{m}$  (see Table 8.4). The latter increases markedly as the level of starch increases. This suggests the presence of large particles, related in some way to the starch. These particles may well overestimate the mean volume data but it is hard to see a clear trend in the corresponding peak positions. There may well be a downward trend but there is some uncertainty. Similarly, a trend between protein levels and fat particle size is hard to define.

**Table 8.4. Fat particle size (D [4,3]) for IWS processed cheese prepared on RVA**

Protein level	Starch level	D [4,3] ( $\mu\text{m}$ )	Peak ( $\mu\text{m}$ )	% Particle beyond 20 $\mu\text{m}$
17.3%	Control	3.87	2.45	1.28
	1%	7.07	1.32	8.30
	2%	11.32	2.01	15.68
	3%	22.63	2.11	33.9
16.3%	Control	3.85	3.08	0.65
	1%	5.87	2.15	4.60
	2%	13.81	3.01	19.11
	3%	25.75	1.84	37.47
15.3%	Control	8.45	4.53	10.75
	1%	9.98	3.18	10.43

## 8.5 Discussion

The viscosity, firmness and stress of the processed cheese increase with addition of starch. This is due to the water binding abilities of the starch, which increase the viscosity of the continuous phase. Leaching of the amylose fraction from the starch granules forms hydrogen bonds and gelify, resulting in the increase in the firmness and the elasticity of the IWS as mentioned in chapter 7. The confocal micrographs suggest that the fat particle size decreased as starch levels increased. This was expected, as the increase in viscosity would lead to more effective shearing during mixing and cooking. The reduction in fat particle size would then contribute to increased firmness and decreased melt as was observed.

However, the confocal micrographs were not consistent with the particle size measurements in the Malvern Mastersizer. This Malvern Mastersizer measurements indicated a sizeable increase in mean volume as starch level increased. Closer inspection of the data revealed a significant increase in the proportion of particles greater than 20  $\mu\text{m}$  as the starch levels increased.

To get a more realistic picture of the distribution of fat particles sizes in these products would require an alternative approach. It is difficult to obtain a confocal micrograph of processed cheese that is truly representative of the bulk of the product. It should be possible to capture micrographs from multiple locations in the product and then use software to analyse the distribution of fat particles sizes and determine the true mean volume. However, this is an extremely time consuming method.

It was also expected that reducing the protein while maintaining the fat level would result in larger fat particles because there would be less protein available to emulsify fat. Confocal micrographs were inconclusive but the Malvern data generally confirmed the prediction. This trend is consistent with the firmness and stress data, which decreased as protein levels were reduced.

The strain data is interesting. In the previous chapter, increasing the starch level at constant protein level had a little impact on the strain measurements - perhaps decreasing slightly. This was explained as the strain properties being dominated by the protein network rather than the starch/water combination. When the protein level is

reduced, the reduction in strain with increasing starch becomes more apparent. This probably reflects the fundamental change in structural components i.e. as the protein network is weakened the contribution and therefore impact, of the starch on the texture becomes more significant.

The melt index decreased with starch addition but was largely unaffected by reduction in protein. The reduction in melt is probably due to the starch binding water, increasing the viscosity and reducing flow during melt. The decrease in melting index, although still not fully understood is also likely due to the cross-linked amylose from the starch, and the dehydration of the protein matrix as suggested by Mounsey & O'Riordan (2001). If the confocal observations are correct, then the reduced fat globule size will contribute to the reduced melt. Acceptable melt indices for commercial IWS processed cheese are approximately 4 or higher. The RVA products greatly exceeded this (~8), and the melting index obtained with added starch is higher than the commercially accepted value of 4. However, at a manufacturing site, IWS processed cheese is often passed through a shear pump before packaging. The shearing pump reduces the fat particle size and also disrupts the protein matrix, reducing the meltability of processed cheese, as suggested by Savello et al, (1989). This could be an issue for processed cheese containing high starch levels.

## **8.6 Summary to Chapter**

It was found that a 1 wt% reduction in protein in IWS processed cheese could be offset by adding 1 wt% potato starch without compromising product firmness or seriously affecting melt. Similarly, a 2 wt% reduction could be achieved by adding 2 wt% potato starch. The lower protein/higher starch levels seemed to introduce some stickiness into the product. These findings need to be validated on a larger pilot-plant scale.

## 9. Pilot-plant Manufacture of IWS Processed Cheese

### 9.1 Introduction

The previous chapter describes how the RVA (Rapid Visco Analyser) was used to manufacture IWS processed cheese with potato starch replacing part of the protein. The RVA allowed a rapid assessment of the effect of protein and potato starch on product characteristics (Kapoor et al, 2004). For proper investigation, the findings from the RVA experiments needed to be validated on a larger scale. Ideally, the processing and final product behaviour seen in samples made using the RVA should correlate closely with the processing and product characteristics seen in samples made in the pilot-plant.

The RVA experiments showed that protein reduction of 1 wt% and 2 wt% in processed cheese were possible by replacement with an equivalent amount of potato starch. These findings were then investigated in the Fonterra Innovation (Palmerston North) pilot-plant. A significant difference in processed cheese manufacture on this larger scale is the inclusion of a shear pump. It was anticipated that its effect on the functional properties of the IWS processed cheese would be a key aspect of this study.

The main objectives of the work are:

- To make IWS processed cheese with the protein being reduced by 1 wt% and 2% respectively and replaced with equivalent amounts of potato starch. Thus the control processed cheese would contain 17.3% protein and 0 wt% starch, and the experimental processed cheeses would contain 16.3% protein and 1 wt% starch, and 15.3% protein and 2 wt% starch.
- To measure the impact on textural, microstructural and melt properties.
- To perform informal sensory evaluation on the IWS processed cheese prepared in the pilot-plant.
- To make recommendations for commercial production of the reduced protein IWS processed cheese.

## **9.1 Experimental approach**

A control formulation (17.3% protein) along with formulations containing 16.3% protein with 1 wt% potato starch, and 15.3% protein with 2 wt% potato starch were prepared in duplicate. The materials and methods are described in Chapter 3, Section 3.8.

In order to achieve the reductions in protein level, some of the young cheese was removed from the formulations. Cheese protein was replaced with lactose, fat with butter and moisture with added water. The ingredients and target compositions are shown in Tables 9.1 and 9.2.

Each of the formulations was prepared in duplicate. Each formulation allowed for 11% of the batch weight of water to be added as steam condensate during cooking.

**Table 9.1. Ingredients for IWS processed cheese with different target protein levels**

	<b>17.3% protein (Kg)</b>	<b>16.3% protein (Kg)</b>	<b>15.3% protein (Kg)</b>
High solids cheese- (young)	9.28	8.70	8.11
Cheddar (mature)	1.00	1.00	1.00
Butter	0.00	0.29	0.58
Lactose	0.30	0.45	0.60
Trisodium citrate	0.48	0.48	0.48
Citric acid	0.02	0.02	0.02
Sodium chloride	0.09	0.10	0.11
Potassium sorbate	0.03	0.03	0.03
Added water	2.15	2.26	2.39
Steam condensate	1.65	1.67	1.68
Potato starch	0.00	0.15	0.30
<b>Total weight</b>	<b>15.00</b>	<b>15.15</b>	<b>15.30</b>

**Table 9.2. Target composition for IWS processed cheese with different protein levels**

	<b>17.3% protein (Control)</b>	<b>16.3% protein</b>	<b>15.3% protein.</b>
Moisture (%)	47.0	46.6	46.3
Fat (%)	26.0	25.8	25.6
Protein (%)	17.3	16.2	15.0
Intact casein (%)	8.1	7.5	7.0
Salt (%)	1.8	1.8	1.8
Lactose (%)	2.0	3.0	3.9
Target pH	5.7	5.7	5.7

## 9.2 Results

Assessment of the textural and melt properties of the IWS processed cheeses with 17.3% protein, 16.3% protein and 1 wt% potato starch, and 15.3% protein and 2 wt% potato starch was carried out after 7 days of refrigerated storage of the slices. Results from the texture and melt characteristics measurements are reported below. The IWS processed cheese failed to peel cleanly from the wrapper with decrease in the protein level.

### 9.2.1 Chemical analysis

The chemical analysis (moisture, protein, fat and salt) data from the 17.3%, 16.3% and 15.3% protein IWS slices prepared in pilot-plant are tabulated in Table 9.3.

**Table 9.3. Moisture, total protein, fat and salt (%) in IWS processed cheese prepared on Blentech cooker in pilot-plant**

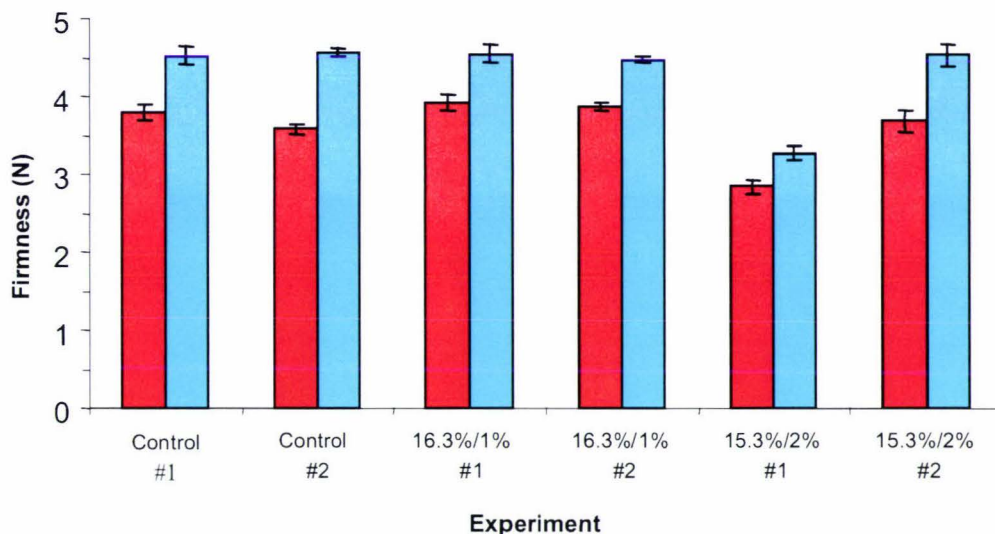
Target protein level.	Target Starch level (%)	Moisture (%)	Protein (%)	Fat (%)	Salt (%)
17.3% (Control)	0.0	48.8	18.3	24.9	1.8
16.3%	1.0	48.7	17.2	25.2	1.9
15.3%	2.0	49.0	16.0	24.4	1.8

All the parameters measured during the chemical analysis of the IWS processed cheese, particularly the moisture content, were found to be higher than the target values. The target compositions can be found in Table 9.2.

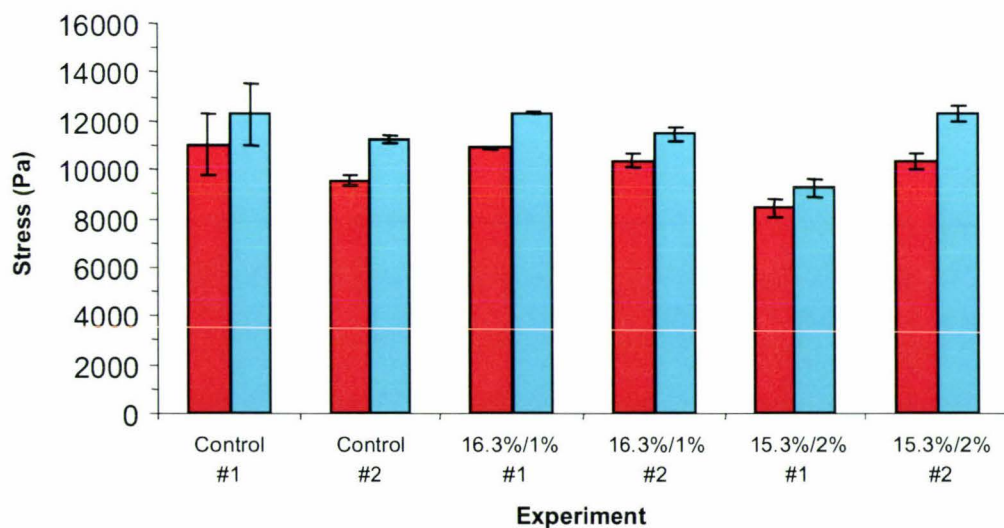
### 9.2.2 Firmness

The firmness data from the 17.3%, 16.3% and 15.3% protein IWS slices prepared in pilot-plant are plotted in Figure 9.1. There were increases in the firmness values of all sheared samples compared with those of the unshered samples. The firmness values were comparable for most samples. Note that sample containing 15.3% and 2% starch made in the first trial was lower than the other samples. This could be an experimental

error during the manufacture as its repeat (trial 2) gave product with comparable firmness to the control.



**Figure 9.1.** Firmness of IWS processed cheese prepared in the Blentech cooker (unsheared (■) and sheared (■)). Errors bars are  $\pm$  one standard deviation. [16.3%/1% refers to target protein level and potato starch level. #1 and #2 refers to trial-1 and trial-2].



**Figure 9.2.** Stress of IWS processed cheese prepared in the Blentech cooker (unsheared (■) and sheared (■)). Errors bars are  $\pm$  one standard deviation. [16.3%/1% refers to target protein level and potato starch level. #1 and #2 refers to trial-1 and trial-2].

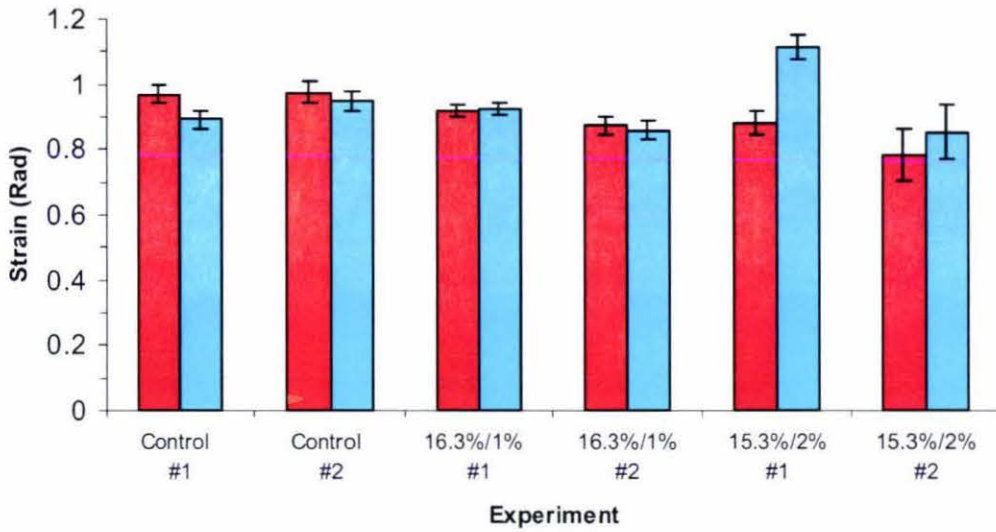
### **9.2.3 Stress and strain**

The stress data from the vane test performed on the 17.3%, 16.3% and 15.3% protein IWS slices prepared in pilot-plant are plotted in Figure 9.2. The stress values for unsheared samples were lower than those of the sheared samples at all protein levels. The stress values for the control and reduced protein processed cheese were similar (both sheared and unsheared) except for the first 15.3% protein trial. The overall trends from the stress results were similar to those from the firmness testing (Figure 9.1).

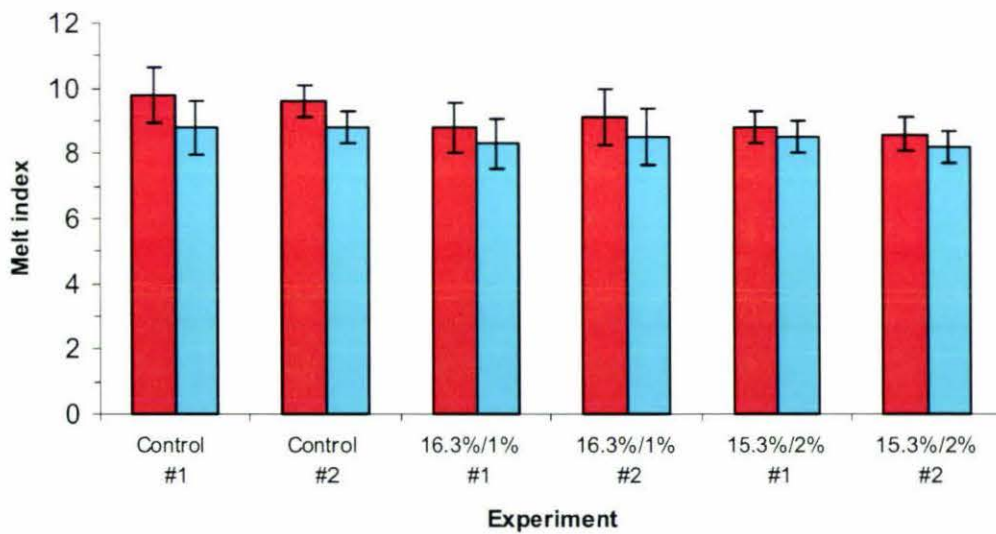
The strain data from Vane Test performed on the 17.3%, 16.3% and 15.3% protein IWS slices prepared in the pilot-plant are plotted in Figure 9.3. The strain values for the unsheared and sheared samples were generally very similar with the value for the sheared sample from the first 15.3% protein trial being the exception. There was significant variability in the strain data. Thus there was no significant difference between the strain values for all the samples. For samples prepared using the RVA, there was no statistically significant correlation between strain and starch concentration in the processed cheese system used in this investigation.

### **9.2.4 Melt index**

The melt index data from the 17.3%, 16.3% and 15.3% protein IWS slices prepared in pilot-plant are plotted in Figure 9.4. The unsheared samples had higher Melt Index values compared with those of the sheared samples. This was the case for all samples, at all protein levels. The melting index of the processed cheese appears to decrease with an increase in starch levels. However, there is significant data variability. This was also the case for the processed cheeses made in the RVA. As expected, the control samples had the highest melt index values.



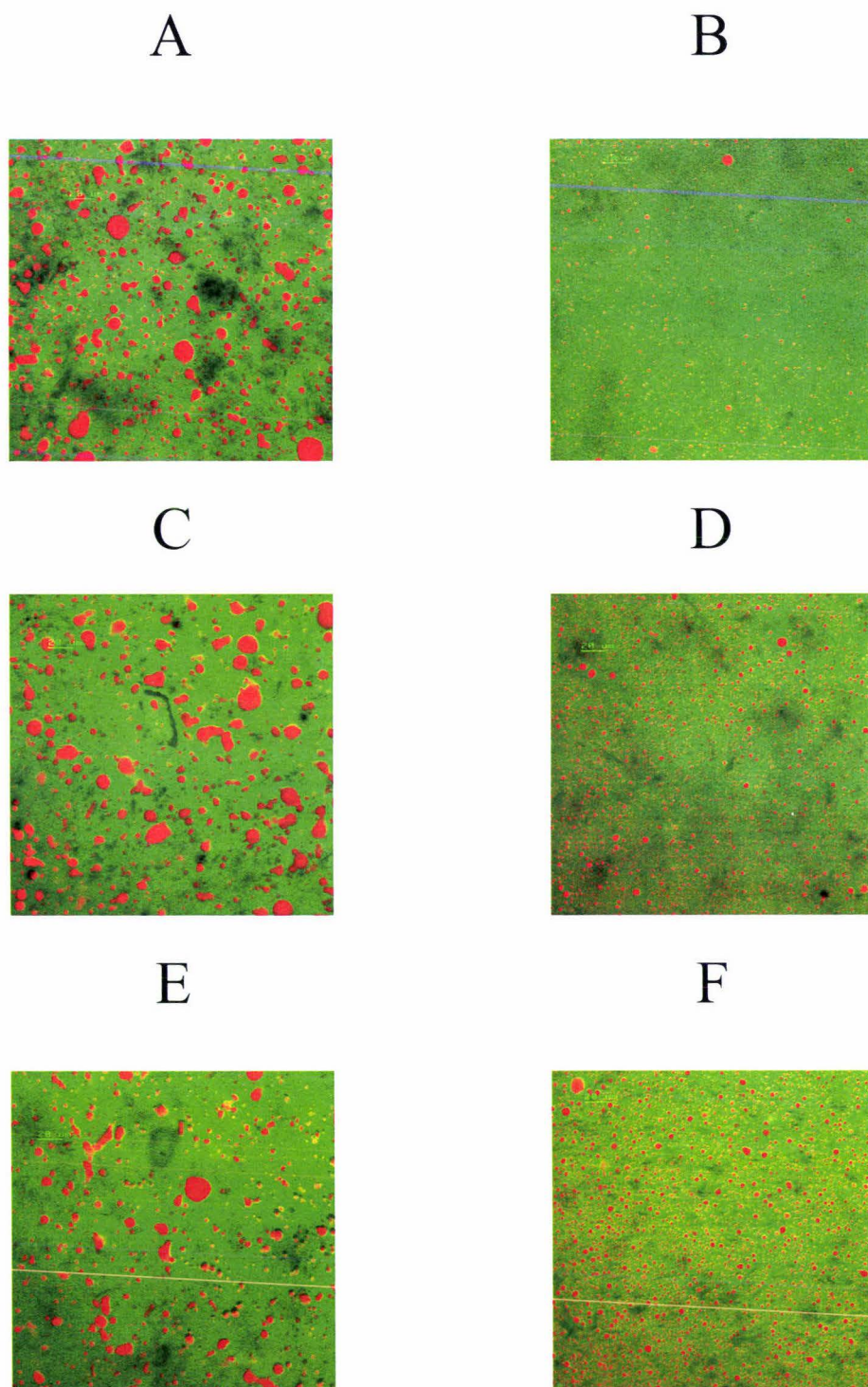
**Figure 9.3.** Strain of IWS processed cheese prepared in the Blentech cooker (unsheared (■) and sheared (■)). Errors bars are  $\pm$  one standard deviation. [16.3%/1% refers to target protein level and potato starch level. #1 and #2 refers to trial-1 and trial-2.



**Figure 9.4.** Melt index of IWS processed cheese prepared in the Blentech cooker (unsheared (■) and sheared (■)). Errors bars are  $\pm$  one standard deviation. [16.3%/1% refers to target protein level and potato starch level. #1 and #2 refers to trial-1 and trial-2].

### 9.2.5 Microstructural evaluation

Figure 9.5 shows the confocal micrographs of IWS processed cheese samples with potato starch and protein combinations. The micrographs illustrate how fat particles (red area) are entrapped within the protein matrix (green coloured continuous phase). For all the samples that were examined, the fat droplets were uniformly distributed. However, a wide range of particles of all sizes was observed. Processed cheese samples without shearing tended to show some irregular shaped and large fat droplets (Figure 9.5(A), (C) and (E)) compared with sheared samples for all protein levels (Figure 9.5(B), (D), (F)). There were no conclusive differences between the control sample (17.3% protein) (Figure 9.5(A) and (B)) and the reduced protein samples (Figure 9.5(C), (D), (E) and (F)). Previously (Chapter 8), confocal micrographs for samples prepared using the RVA showed a decrease in the globule size as the protein level decreased and the starch concentration increased (Figure 8.8 and Figure 8.9). For the equivalent samples prepared on the Blentech cooker, reduction in fat particle size was expected but was not observed.



**Figure 9.5.** Confocal micrographs of IWS processed cheese prepared in Blentech cooker obtained with a 40x oil immersion objective (magnification 400x). The micrographs are (A) control (unsheared), (B) control (sheared), (C) 16.3% protein and 1 wt% potato starch (unsheared), (D) 16.3% protein and 1 wt% potato starch (sheared), (E) 15.3% protein and 2 wt% potato starch (unsheared) and (F) 15.3% protein and 2 wt% potato starch (sheared).

### 9.2.6 Particle size measurement

Particle size data from the IWS processed cheese was obtained using the Malvern Mastersizer. The method is described in Chapter 3, section 3.6. The data is tabulated in Table 9.4.

**Table 9.4. Fat particle size (D [4,3]) for IWS processed cheese prepared on Blentech cooker in pilot-plant**

Formulation		D [4,3] ( $\mu\text{m}$ )	Peak ( $\mu\text{m}$ )	% Particle beyond 20 $\mu\text{m}$
17.3% protein and 0% starch	Unsheared	10.75	2.55	1.22
	Sheared	0.925	1.23	0.78
16.3% protein and 1% starch	Unsheared	18.45	2.71	3.68
	Sheared	1.06	1.59	2.16
15.3% protein and 2% starch	Unsheared	27.78	3.48	8.56
	Sheared	6.23	2.49	2.33
Commercial sample.	(Sheared)	0.97	1.01	0.45

When the molten processed cheese is passed through a shear pump, the mean volume (D [4,3]) of the particles is greatly reduced. The peak position and proportion of particles greater than 20  $\mu\text{m}$  also decreased. As starch level increases and protein level decreases, the mean volume, peak position and proportion greater than 20  $\mu\text{m}$ , all increase.

A commercial IWS processed cheese was included in the particle size testing. The particle size data was very similar to the sheared control. As the protein level dropped, significant differences became evident.

### 9.2.7 Sensory evaluation

Typically IWS processed cheese displays a high sheen, a clean peel from its plastic film, an even break when folded in half, a good emulsion when rubbed between finger and thumb (several rubs are required to break the fat out of the emulsion) and is usually slightly elastic (Fillery, 2004).

The pilot-plant prepared IWS processed cheese was evaluated for peel, shine, break, emulsion, mouth feel and flavour by several experienced cheese technologists from Fonterra, Palmerston North, during an informal sensory session. The panellists assessed each of the different samples one by one and recorded the different textural and flavour attributes that best described the samples. Textural attributes were based on the criteria identified above, while the flavour attributes were those usually associated with processed cheese. All the samples were kept under refrigeration until the evaluation. The results of the panel are reported in Table 9.5.

**Table 9.5. Summary of descriptors from informal grading of IWS processed cheese**

<b>Target protein level</b>	<b>Texture</b>	<b>Flavour/Mouth feel</b>
17.3% protein. (Control)	Firm, good shine, good emulsion, good peel and slightly pasty break.	Clean, fresh, cheesy and gummy.
16.3% protein.	Firm, good shine, sticky peel, pasty break and pasty texture.	Fresh, cheesy, creamy, milky, sweet and slightly metallic.
15.3% protein.	Pasty texture, ragged break and sticky peel.	Cheesy, creamy, slightly sweet, milky and gummy.

***Peel***

Clean peel from plastic film is an indicator that there is sufficient intact casein, satisfactory creaming and good structure. Both the control sample (unsheared and sheared) showed good peel characteristics. All of the reduced protein IWS showed a tendency to stick to the film. The samples with 15.3% protein had a much worse stickiness problem than did those with 16.3% protein.

***Shine***

Good shine is another indicator of good structure. Poor shine is an indicator of formulation problems or processing issues. The sheared samples were found to be whiter and shinier than the unsheared samples. The shine for the sheared samples with 16.3% and 15.3% protein was comparable to the sheared control samples.

***Break***

Processed cheese with good structure shows a good clean break. Uneven break indicates lack of intact casein or poor emulsion. All of the sheared samples were found to break better than the unsheared ones. The reduced protein samples had a softer, pastier break compared with the control samples.

***Body/emulsion***

Rubbing processed cheese between finger and thumb can give an indication of how stable the emulsion is i.e. how well the fat is held in the product. In all cases as expected, the unsheared samples were less well emulsified than the sheared samples. For all protein levels, the sheared samples had fairly stable emulsions.

***Flavour***

There were no issues with the flavour of any of the samples. All the samples were scored as having clean and fresh flavour. Mature cheese is the major contributor to the flavour of the processed cheese. To achieve protein reduction, the amount of young cheese in the formulation was reduced. Therefore there should be no significant deterioration in the flavour of the reduced protein processed cheese. Processed cheese samples with 15.3% protein cheese samples had a slight sweet or milky flavour, probably due to the excess lactose used for solids adjustment.

### 9.3 Discussion

The protein levels in the IWS processed cheese were higher than expected. The reason for this is not clear especially as the moistures are higher than expected. However, a spread of protein levels was achieved.

Firmness of the IWS processed cheese with 16.3% protein plus 1 wt% potato starch and 15.3% protein plus 2 wt% potato starch was comparable with the control sample (17.3% protein alone), as was predicted in Chapter 8. The firmness of processed cheese with added starch and reduced protein is thought to be mainly due to hydrogen bonding of amylose, which is leached out during the cooking process. This suggests both these formulations may be used to manufacture IWS processed cheese with a suitable firmness. The stress data confirmed the firmness data but more variability was observed. The strain data showed a slight decrease in strain values as protein is replaced by starch, as observed in previous chapters.

The Melt Index of processed cheese decreases with starch addition and with shearing. Replacing protein with starch tends to decrease the Melt Index values as seen in the previous chapters (Chapter 7 and 8) but overall melt remained satisfactory. The presence of starch could result in dehydration and disruption of the protein matrix, which in turn could lead to the decrease in the Melt Index (Mounsey & O'Riordan, 1999).

These observations were confirmed by the sensory evaluation. The control IWS processed cheese had good shine, break, body, mouth feel and flavour. The reduced protein cheese samples had good clean and fresh flavour and the firmness in the mouth was comparable with the control sample. The starch containing samples took a little longer to melt in the mouth. Cheese samples containing 15.3% protein plus 2 wt% potato starch had a slight gummy or pasty texture and also had a doughy mouth feel.

The confocal micrograph does not show any clear trend in terms of the fat particle size. It was expected that the fat particle size would increase with decrease in the protein level, but this is not evident in the confocal micrographs (see Figure 9.5). The fat particle size analysis showed that with increase in the starch addition rate there is an

increase in the fat particle size. The particle size data are similar to the data reported in Chapter 8. As the starch increases (and protein decreases), the mean volume increases. Again, this may be due to skewing of smaller fat particles by larger ones – the proportion of particles greater than 20  $\mu\text{m}$  increases as well.

The only hurdle to addition of starch is stickiness. While native starch can be used to maintain product firmness, removing protein seems to lead to stickiness. As more starch is introduced, the product gets stickier- as observed when peeling the IWS from its polypropylene wrapper.

#### **9.4 Summary to Chapter**

When starch was used to replace protein, the firmness of the control was retained in the resulting processed cheeses. The flavour of the starch containing products was satisfactory. Although the melt characteristics decreased, they remain within commercial acceptable limit. Use of a shear pump between the cooking and slice-forming steps in processed cheese manufacture appeared to greatly affect the functional properties. Shearing changes the fat globule size distribution in IWS processed cheese and thereby affects the functionality of the processed cheese, such as increasing the firmness of the product. However stickiness is a factor that needs to be addressed before commercialisation.

## 10. General Discussion

The purpose of the experiments described in this thesis was to study the effect of different starches on the rheological and functional properties of processed cheese. Studies on the effect of starch addition were conducted using two different formulations, a rennet casein-based model processed cheese spread and an Individually Wrapped Slice (IWS) processed cheese. In the case of model rennet casein spread, rheological measurements were carried out together with microstructural and particle size measurement to quantify the changes that occurred in the product due to starch addition. For the IWS processed cheese, in addition to the rheological and microstructural properties, functional properties such as firmness and melting were investigated. The IWS cheese was also manufactured at a pilot-plant scale to further investigate the possibility of taking processed cheese containing starch closer to a commercial reality. In this chapter we review the important findings and observations made in the framework of this thesis and try to provide a fundamental explanation where possible.

### 10.1 Processed cheese without starch addition

Processed cheese is basically an emulsion of fat or oil droplets dispersed in a continuous phase of a milk protein network. The network is achieved during the initial cooking of processed cheese, where the proteins (especially caseins) are dispersed into small units (sub-micelle level) due to the action of the emulsifying salts aided by the mechanical agitation. The calcium-phosphate bridges that hold the micelles together are broken down, allowing small protein units to dissociate. The emulsifying salt-protein interaction causes calcium chelation, ion exchange reaction and enhanced protein hydration. Unfolding and swelling of proteins takes place. Due to protein molecular chain entanglement, a stable structure is formed at the end of the processing (Lee et al, 2003). The mechanical agitation also contributes to the emulsification and formation of the oil droplets. As a result, oil droplets stabilised by milk proteins are homogeneously distributed in a protein network where there are protein-protein and protein-fat interactions. Thus, processed cheese is an emulsion where the oil droplets act as active-fillers, since they also interact with the continuous protein network through the protein (at the interface of the oil droplets)-protein (in the protein network) interaction.

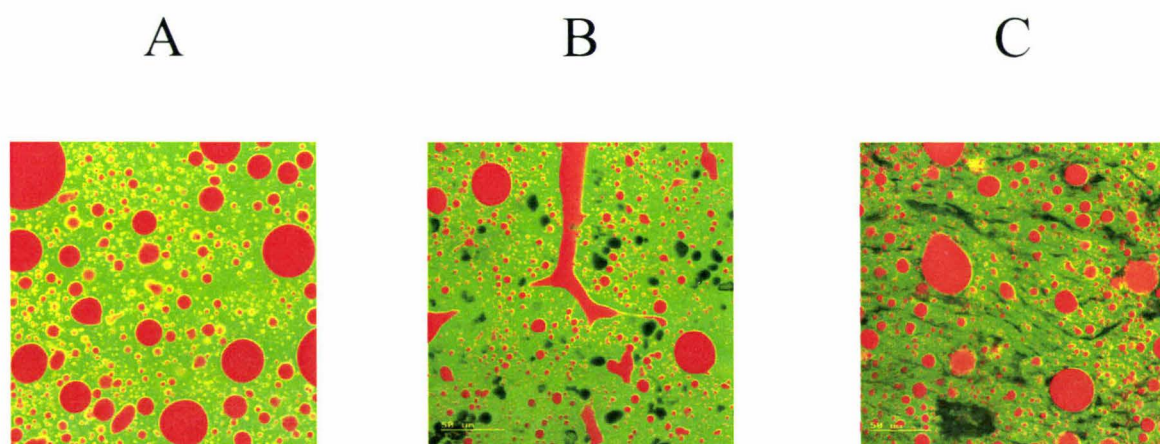
In this study, processed cheese samples were prepared on three different pieces of equipment, the Paar-Physica (starch cell), the RVA (Rapid Visco Analyser) and a Blentech cooker. Samples prepared on different machines showed differences, both in rheology and the particle size distribution, and to some extent, in functional properties. This is due to the difference in the shearing geometries, and the level of shear applied. The rheological measurements showed that the control samples (without starch addition) prepared on RVA showed higher complex modulus ( $G^*$ ) values (see Figure 5.2 and Figure 5.9) and lower fat globule size (see Figure 5.6 and Figure 5.13) than did those prepared on the Paar-Physica. The confocal micrographs (see Figure 5.7 and 5.14) confirmed these observations, by showing large, spherical fat droplets for the control samples prepared on the Paar-Physica compared to those prepared on RVA, which showed irregular shaped fat droplets. This is clearly due to the fact that the RVA delivers a higher shear regime than the Paar-Physica. This resulted in much smaller oil droplets and a resultant increase in their numbers. As the number of the oil droplets increases, their contribution to the protein network through fat globule-protein interactions is enhanced, thus the increase in the elasticity of the processed cheese. Furthermore as expected a decrease in the protein level (see Chapter 6, Figure 6.1) resulted in the decrease in the elasticity ( $G^*$  decreases) of the processed cheese. This is due to two effects. Firstly the decrease in proteins decreases the emulsification power, as fewer proteins are available to completely emulsify the oil droplet. Secondly lower protein concentrations results in a weaker protein network.

## **10.2 Effect of starch on model processed cheese**

Chapter 4 was dedicated to the scoping of the effects of starches on processed cheeses. Ten different starches namely, cornstarch, waxy cornstarch, high amylose cornstarch (HACS), rice starch, waxy rice starch, potato starch, wheat starch, resistant starch, acid converted starch and Perfectamyl, were used. In this chapter, rheological measurements showed that different starches behaved differently depending upon its type. However, for all the starches, their addition to model processed cheese resulted in the increase in the rheological attributes ( $G^*$  and strain) with the increase in starch concentration, with acid converted starch giving the highest increase in elasticity and HACS the lowest elasticity (Figure 4.6). However, although there is a satisfying correlation in the case of viscosity there was no direct correlation between the elastic behaviour of the starch in solution and their elastic behaviour in the model processed cheese (Figure 4.9).

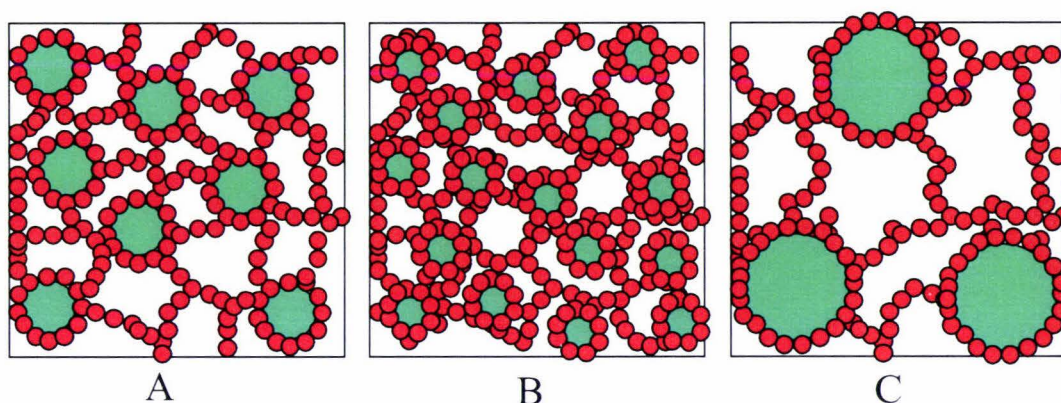
Furthermore, it clearly showed that the particle size of the oil droplets and the microstructures of the processed cheese were affected. These effects were also dependent on the type of equipment used to produce the samples. For instance, processed cheese made on the RVA had higher elasticity and smaller fat droplets than those made on the Paar-Physica. These differences between the RVA and the Paar-Physica could be due to the difference in the shearing geometry of the two pieces of equipment, with the RVA delivering the highest shear, resulting in smaller oil droplets, which as mentioned in Section 10.1 will increase the elasticity of the processed cheese.

Correlating the elasticity of the processed cheese containing starch to the elasticity of the starch in aqueous solution is difficult, because of the structure of the processed cheese formed during cooling (see Figure 10.1). Although intuitively it is tempting to assume that the viscosity of the processed cheese under shear is directly related to the viscosity of its continuous phase, the elasticity of processed cheese will depend on how the protein network is affected by the starch. For example microscopic observations on processed cheese containing HACS showed that the starch granules were not fully pasted (Figure 10.1(B)). Phase separation occurred in processed cheese containing waxy rice starch where elongated starch rice areas (dark area on the micrographs) are distributed through the protein continuous phase (Figure 10.1(C)).



**Figure 10.1. Confocal micrograph of (A) model processed cheese without starch addition, containing (B) 2 wt% HACS and (C) 2 wt% waxy rice starch. The processed cheeses were made in the Paar-Physica at 9.7% protein level.**

The protein reduction exercise undertaken in chapter 6, confirmed the above findings. Different starches had different effects on the processed cheese, and starch addition generally increases the elasticity of the processed cheese. As expected it has also demonstrated, that a decrease in protein level will decrease the elasticity of the processed cheese as the emulsifying properties and the strength of the gel network decreases (Fig. 10.2(C)). In addition the importance of the equipment used for the making of processed cheese was also confirmed since even at lower protein levels, the RVA produced more elastic cheeses with smaller fat droplets than did the Paar-Physica.



**Figure 10.2.** Schematic representation of a (A) processed cheese with (B) reduction in droplet particle size due to shear and (C) reduction in protein concentration.

In summary, processed cheese has a complex structure consisting of emulsified fat and a protein network. We do not fully understand the protein-protein and protein-fat interactions that are critical to the structure. Hence it is hard to understand/explain the impact of starch on this type of structure – even in a model system.

### 10.3 Individually Wrapped Slice (IWS) Processed Cheese

In the first chapters of this thesis (Chapters 4, 5 & 6), rennet casein was used as a source of protein in order to minimise sample variation as it gives uniformity in composition and consistency of product attributes. However, a commercial product such as IWS processed cheese is made with young (for its functionality) and mature (for its flavour) cheeses as a source of both proteins and fat. In addition, butter was used in the IWS processed cheese instead of the soya-oil used in the model-processed cheese. As well as the rheological and microstructural tests used for the model-processed cheese, functional testing such as melt index, vane test and cylinder test were also carried out.

Most of the observations made on the effect of starch on model-processed cheese are reiterated in the case of IWS processed cheese. There was an increase in viscosity and elasticity and this is due to the presence of starch molecules (amylose and amylopectin) and their water binding ability that results in the increase of the viscosity of the cheese continuous phase. The amylose, leached out during cooking of processed cheese, forms hydrogen bonds and gelifies, resulting in increased firmness. Different starches have also affected the IWS processed cheese differently, for example because potato starch has high swelling power and high amylose contents addition resulted in the increase of both the viscosity and the firmness of the IWS processed cheeses, while HACS contributed the least to the firmness and to the viscosity of the IWS processed cheeses, as it was not pasted during the cooking the cheese (as observed by confocal). However, the strain of the IWS processed cheeses made with the different starches was largely unaffected (Figure 7.4), which is an indication that the structure of cheese is dominated by a protein continuous network.

It was also found in the case of IWS processed cheese that the melting properties of the cheeses all decreased with the addition of starches. This reduction in melting index is possibly due to the lack of thermoplasticity of cross-linked, leached amylose, as suggested by Mounsey & O'Riordan (2001). The dehydration of the protein network due to the water competition from starch could be also another reason for the reduction in the meltability.

Similarly to model processed cheese, the protein reduction investigation (Chapter 8), performed only with the addition of potato starch, has shown that there was an increase in both the viscosity and firmness of the IWS processed cheese with starch addition. An important finding of this work was that when based only on the firmness or the stress of the IWS cheese, it was possible to accurately deduce the exact amount of starch needed to replace a given protein level (Figure 8.4 and Figure 8.5). And this could be achieved without a significant change in the large deformation (strain) of the IWS processed cheese (Figure 8.6), which was found to be largely independent of the amount of starch added, as the strain is dependent mainly on the protein network. However, a decrease in the melt index is observed, although within the commercially acceptable limits.

The trends in fat droplet size versus starch level and protein level were unclear. Confocal micrographs suggested fat droplet size decreased as starch increased. This is

understandable, as increased viscosity would lead to higher effective shear. This correlated with higher viscosity, firmness and stress while strain and melt decreased. Unfortunately the fat globule size data contradicts the confocal microscopy observations – the fat globule size increasing with added starch. This could be due to the limits of the particle size measurement technique used in this thesis (Section 8.5).

The storage time experiments up to 30 days (Chapter 5 and 6) have showed that the samples were stable with a small decrease in the product elasticity or firmness. This was not investigated in detail, as the reduction in firmness was less than 10%. It is possible that this is due to starch retrogradation or the coalescence of the oil droplets.

#### **10.4 Pilot-plant experiments**

Previous chapters (Chapters 4 to 8) covered the use of both the RVA and the Paar-Physica to manufacture model-processed cheese spreads and IWS processed cheeses. Although these types of equipment are commonly used to study starches, grain, batters and other foodstuffs (Kapoor et al, 2004) they behave very differently from commercial processed cheese manufacturing equipment. In a commercial set-up, cookers such as Blentechs or Stephans are used, with the addition of a shear pump in some cases. This makes the scale-up from a laboratory bench to a commercial cooker a challenge.

Chapter 9 covers the pilot-plant manufacture of IWS processed cheeses on a Blentech cooker with a shear pump. The control IWS processed cheese contained 17.3% protein. IWS processed cheeses with 16.3% protein and 1 wt% potato starch and 15.3% protein and 2 wt% potato starch were also made. In addition to the conventional functional tests (firmness, melt index, and microscopic observation), sensory evaluation of these processed cheeses was also performed.

This work clearly demonstrated that it was possible to manufacture IWS processed cheeses with part of the protein, replaced with potato starch, while maintaining similar rheological attributes (firmness) to those of the control. The IWS processed cheeses with potato starch also had an acceptable melting index. Although the maximum amount of protein replaced was ~12.0%, it should be possible to substitute more of the protein by using potato starch. However, sensory evaluation showed that although the reduced protein cheese samples had a good, clean, fresh flavour that was comparable

with the control, at high starch concentrations, the starch containing IWS processed cheeses had a slight gummy or pasty texture, a doughy mouth feel and tended to stick to the wrapper. This problem might be inherent to the use of native starch and thus would always be encountered if high levels of native starches were added to processed cheese.

## 11. Conclusions And Recommendations

### 11.1 Conclusions

The results obtained in this experimental work showed that it is possible to replace protein with potato starch in processed cheese. The properties of the processed cheese (rennet casein-based spreads and IWS processed cheese) were investigated using both confocal microscopy and particle size measurement, as well as rheological and functional tests. In the course of the present experimental work, the results obtained and reported here made it possible to conclude that:

1. Samples prepared on three different pieces of equipment showed differences in rheological, microstructural and functional properties. The differences arising might be due to the physiochemical properties of the food components and the effect of the differences in the geometry and shearing regime of the equipment used.
2. The three different tests used in the rheological evaluation of the samples - frequency sweep (small deformation), strain sweep (large deformation) and viscosity – yielded consistent, reproducible results that measured the differences between the samples. Addition of starch to processed cheese increases the rheological attributes such as complex modulus, strain and viscosity. The extent of this increase is totally dependent on the type and physio-chemical properties of the starch.
3. Confocal Laser Scanning Microscopy (CLSM) was a powerful and easy to use technique for the structural study of the samples. Microstructure observations showed the change in the microstructure properties of processed cheese upon starch addition. The microstructure showed marked differences, which were attributed to different processing conditions and also the physiochemical properties of the food components (fat, protein and starch). The confocal micrograph shows large and spherical fat droplets for control samples prepared on the Paar-Physica compared to those prepared on the RVA. This is clearly due to the fact that the RVA delivers a higher shear regime than the Paar-Physica. The cheese microstructure is affected by the processing conditions, which in turn affects the mechanical properties of the cheese samples.

4. Six different starches were used to reduce the protein level in processed cheese spreads to both 8.5% and 7.5% from the control value of 9.7% protein. It was found that reducing the level of protein in the processed cheese without starch addition influenced the rheological properties, the mean fat globule size and the microstructural properties of model processed cheese spreads. Starch addition at reduced protein levels compensated for the rheological changes.
5. The effect of different starches on the meltability and textural properties of IWS processed cheese was dependent on the type of starch. Potato starch, wheat starch and rice starch increased the firmness and viscosity of the processed cheese. However, as the firmness increased the melt index decreased. Strain values were largely unaffected by starch addition.
6. Potato starch gave the best results in terms of textural properties of the processed cheese, especially firmness. Protein substitution of 1% and 2% is possible by replacing it with equivalent amounts of potato starch in IWS processed cheese. The resulting processed cheeses prepared on the pilot-plant scale had similar firmness values to that of the control. The flavour of the starch containing products was satisfactory. Although melt index decreased, they remained within the commercial acceptable limits. Stickiness is an issue that needs to be addressed before commercialisation.

In conclusion, investigating the effects of starch addition on the rheological properties of processed cheese provided useful information for the processed cheese industry. Compared with milk protein, starch is relatively inexpensive and can be used to replace some of the young cheese used as a protein source in processed cheese formulations without any drastic effect on the firmness of the IWS processed cheese.

## **11.2 Recommendations**

1. To progress this piece of work, a better understanding of the fundamental interactions between protein, fat and starch is required. It is recommended that somebody:

- Conduct standard compression testing on trial products, as this would give additional structural information.
  - Investigate the use of other microscopy techniques, such as electron microscopy, which could enhance the amount of structural information and aid in understanding the interactions between different components (protein, fat and starch).
  - Conduct a creaming curve analysis on a viscometer, which would give information on the viscosity versus time behaviour of the molten processed cheese.
2. The optimum starch addition and protein replacement rates for a particular processed cheese must be determined. The range of starch options must include modified starches.
  3. The optimum processing conditions must be developed – this includes cook time, cook temperature and shear profile.
  4. The optimum emulsifying salt combination needs to be assessed. The current study used only TSC. Other emulsifying salts, such as Disodium Phosphate (DSP), may help to firm the product, increase the melting properties and reduce the stickiness.
  5. The effect of fat in processed cheese containing starch should also be considered. There may be a possibility to improve the melting properties of processed cheese, since this depend on both the starch and fat contents
  6. More commercial trials are required to assess in-process behaviour in commercial cookers. It would be interesting to see the impact of rework of processed cheeses containing starches. Storage trials are also needed to determine any differences from a typical commercial product.
  7. Assess the risks and impact of starch retrogradation on the product.

8. Other hydrocolloids such as alginates and carragenans should also be investigated and compared to starches.

## References

- Atwell, W.A., Hood, L.F., Lineback, D.F., Varriano-Marston, E. and Zobel, H.F. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Food World*, Volume 33, 306-308-310-311
- Bao, J. and Bergman, C.J. (2004). The functionality of rice starch. In *Starch in food – Structure, function and applications*, Eliasson, A-C. (ed), Woodhead Publishing Limited, Cambridge, 258-294
- BeMiller, J.N. and Whistler, R.L. (1996). Carbohydrates. In *Food Chemistry*, Fenemma, O.R. (ed), Marcel Dekker, Inc, New York, 191-204
- Berger, W., Klostermeyer, H., Merkenich, K. and Uhlmann, G. (1998). Processed cheese manufacture - a Joha Guide, BK Giulini Chemie GmbH & Co., Ladenburg
- Bergthaller, W. (2004). Developments in potato starches. In *Starch in food – Structure, function and applications*, Eliasson, A-C. (ed), Woodhead Publishing Limited, Cambridge, 241-257
- Blonk, J.C.G. and Aalst, H.V. (1993). Confocal scanning light microscopy in food research. *Food Research International*, Volume 26, 297-311
- Brummel, S.E. and Lee, K. (1990). Soluble hydrocolloids enable fat reduction in process cheese spreads. *Journal of Food Science*, Volume 55, 1290-1292
- Buleon, A., Colonna, P., Planchot, V. and Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules*, Volume 23, 85-112
- Caric, M. and Kalab, M. (1993). Processed cheese products. In *Cheese: chemistry, physics and microbiology. Volume 2. Major cheese groups*, Fox, P.F. (ed), Chapman & Hall, London, 467-505

- Caric, M., Gantar, M. and Kalab, M. (1985). Effects of emulsifying agents on the microstructure and other characteristics of process cheese - a review. *Food Microstructure*, Volume 4, 297-312
- Carpenter, R.N., Finnie, K.J. and Olsen, R.L (1998). Imitation cheese composition and products containing starch, United States Patent 5,807,601
- Chambre, M. and Daurelles, J. (2000). Processed cheese. In *Cheesemaking: from science to quality assurance*, Eck, A., & Gillis, J-C. (ed), Intercept, Londraes, 641-657
- Chang, P.K. (1979). Process cheese containing a modified whey solids. United States Patent 4,166,142
- Chiu, C-W. and Mason, W.R. (1998). Method of replacing fats with short chain amylose. United States Patent 5,711,986
- Corn starch (1964). Corn Industries Research Foundation, Inc. (ed), Washington, D.C.
- Croghan, M. and Mason, W. (1998). 100 years of food starch innovation. *Food Science & Technology Today*, Volume 12, 17-24
- Dimitreli, G. and Thomareis, A.S. (2004). Effect of temperature and chemical composition on processed cheese apparent viscosity. *Journal of Food Engineering*, Volume 64, 265-271
- Drake, M.A. Truong, V.D. and Daubert, C.R. (1999). Rheological and sensory properties of reduced-fat processed cheeses containing lecithin. *Journal of Food Science*, Volume 64, 744-747
- Duerrenberger, M.B., Handschin, S., Conde-Petit, B. and Escher, F. (2001). Visualization of food structure by confocal laser scanning microscopy (CLSM). *Lebensmittel Wissenschaft und Technologie*, Volume 34, 11-17
- Dunn, J.M. and Finocchiaro, E.T. (1997). Starch-based texturizing agents and method of manufacture. United States Patent 5,614,243

- Eastman, J.E. (1994). Starch hydrolysates as fat replacements. United States Patent 5,275,837
- Ellis, R.P., Cochrane, M.P., Dale, M.F.B., Duffus, C.M., Lynn, A., Morrison, I.M., Prentice, R.D.M., Swanston, J.S. and Tiller, S.A. (1998). Starch production and industrial use. *Journal of the Science of Food & Agriculture*, Volume 77, 289-311
- El-Neshawy, A.A., Farahat, S.M. and Wahbah, H.A. (1988). Production of processed cheese food enriched with vegetable and whey proteins. *Food Chemistry*, Volume 28, 245-255
- Fillery, S. (2004). Ambient Functionality of Processed cheese manufacture in a Rapid Visco Analyser – a master thesis, Massey University, Palmerston North, New Zealand
- Finney, E. (1972). Elementary concepts of rheology relevant to food texture studies. *Food Technology*, Volume 26, 68-77
- Finocchiaro, E.T. (1997). Non-fat, reduced fat and low fat cheeses and method of making. United States Patent 5,679,396
- Finocchiaro, E.T. (1996). Starch based texturizing agent. United States Patent 5,584,937
- Fox, P.F., O'Connor, T.P., McSweeney, P.L.H., Guinee, T.P. and O'Brien, N.M., (1996). Cheese: physical, biochemical, and nutritional aspects. *Advances in Food & Nutrition Research*, Volume 39, 163-328
- Fredriksson, H., Andersson, R., Eliasson, A.C., Aman, P. and Silverio, J. (1998). The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydrate Polymers*, Volume 35, 119-134
- Goel, P.K., Singhal, R.S. and Kulkarni, P.R. (1999). Studies on interactions of cornstarch with casein and casein hydrolysates. *Food Chemistry*, Volume 64, 383-389

Guinee, T.P. (2003). Role of protein in cheese and its products. In *Advanced dairy chemistry. Volume 1 Proteins, Part B*, Volume One, Fox, P.F. and McSweeney, P.L.H. (ed), Kluwer Academic, New York and London, 1147-1174

Guinee, T.P. (2002). Pasteurized processed cheese products. In *Encyclopedia of Dairy Sciences*, Volume 1, Roginski, H., Fuquay, J.W. and Fox, P.F. (ed), Academic Press, Amstredam and New York, 411-418

Guinee, T.P. (1987). Processed cheese products: physio-chemical aspects. In *Symposium on Industrial Aspects of Milk Protein*, Moorepark Research Centre, Fermoy, Co. Cork, Ireland, 111-178

Gunasekaran, S. and Ak, M.M., (2002). Linear Viscoelasticity of Cheese – Processed cheese. In *Cheese Rheology and Texture*, CRC Press, New York, 215-272

Gunasekaran, S. and Ak, M.M., (2000). Dynamic oscillatory testing of foods – selected applications. *Trends in Food Science & Technology*, Volume 11, 115-127

Hegenbart, S. (1996). Understanding starch functionality. *Food Product Design*, Volume 5, 23-24

Hill, S.E., Mitchell, J.R. and Ledward, D.A. (1998). Plant polysaccharide - Starch. In *Functional properties of food macromolecules*, Elsevier Applied Science Publishers, London and New York, 175-182

Hout, A.V.D. (2004). Hydrocolloids in Processed cheese – a master thesis, Massey University, Palmerston North, New Zealand

Hullinger, C.H., Patten, E.V. and Freck, J.A. (1973). Food application of high amylose starches. *Food Technology*, Volume 27, 22-24

Hutt, A. (2002). Processed cheese Rheology and the Vane test – a master thesis, Massey University, Palmerston North, New Zealand

- Jackson, L.K., Brown, D.M., Yeong-Ching, A.H. and Standholm, J.J. (1994). Method for manufacture of a low-fat cheese. United States Patent 5,374,443
- Jacobson, M.R., Obanni, M. and Bemiller, J.N. (1997). Retrogradation of starches from different botanical sources. *Cereal Chemistry*, Volume 74, 511-518
- Jay-lin, J. (1992). Preparation and food applications of physically modified starches. *Trends in Food Science & Technology*, Volume 3, 145-148
- Kapoor, R., Lehtola, P. and Metzger, L.E. (2004). Comparison of pilot-scale and Rapid Visco Analyzer process cheese manufacture. *Journal of Dairy Science*, Volume 87, 2813-2821
- Koisikowski, F.V. (1977). Cheese and Fermented Milk Foods. Ann Arbor, Edward Brothers, Inc., 337-340
- Krumhar, K.C., Phillips, A.N., Moffitt, K.R. and Damiano, D. (1998). Homogenized starch-based cheese product and methods of making same. United States Patent 5,731,026
- Kwak, H.S., Choi, S.S., Ahn, J. and Lee, S.W. (2002). Casein hydrolysate fractions act as emulsifiers in process cheese. *Journal of Food Science*, Volume 67, 821-825
- Laye, I., Lindstorm, T.R., Lowry, L., Mei, Fu-I., Zwolfer, M., Diaz-Castillo, O., Dolande, J., Havlik, S. and Rueda, V. (2003). Method for preparing process cheese containing increased levels of whey proteins. United States Patent 6,669,978
- Lee, S.K. (2004). Personal communication. Fonterra Research Centre, Palmerston North, New Zealand
- Lee, S.K., Buwalda, R.J., Euston, S.R., Foegeding, E.A. and McKenna, A.B. (2003). Changes in the rheology and microstructure of processed cheese during cooking. *Food Science & Technology Lebensmittel Wissenschaft & Technologie*, Volume 36, 339-345

Lee, S.K., and Klostermeyer, H. (2001). The effect of pH on the rheological properties of reduced-fat model processed cheese spreads. *Lebensmittel Wissenschaft & Technologie*, Volume 34, 288-292

Lee, S.K., Buwalda, R.J., Bracey, K.M., Hirst, R., Thresher, W. and Grant, D. (1999). Lactose in model processed cheese: its effect on the rheological and functional properties. NZDRI report, NZDRI-1999-018, Fonterra Research Centre, Palmerston North, New Zealand

Legg, A. (2005). Milk Protein Concentrates as ingredients in Processed cheese – a master thesis, Massey University, Palmerston North, New Zealand

Lindstorm, T.R., Laye, I., MacBlane, G., Mei, Fu-I. (2004). Processed cheese made with soy. United States Patent 2004001829

Luallen, T.E (2004). Utilizing starches in product development. In *Starch in food – Structure, function and applications*, Eliasson, A-C. (ed), Woodhead Publishing Limited, Cambridge, 393-424

Luallen, T.E. (2002). A comprehensive review of commercial starches and their potential in foods. In *Food additives*, Branen, A. L., Davidson, P. M., Salminen, S., Thorngate, J. H. III (ed), Marcek Dekker, New York, 757-807

Malvern Mastersizer E. (1992). User manual, Malvern Instruments Limited, Malvern, UK

Mann, E.J. (1997). Processed cheese. *Dairy Industries International*, Volume 62, 16-17

Mayer, H.K. (2001). Bitterness in processed cheese caused by an overdose of a specific emulsifying agent? *International Dairy Journal*, Volume 11, 533-542

Merkenich, K., Maurer-Rothmann, A., Walter, E., Scheurer, G. and Klostermeyer, H. (1993). Processed cheese preparations and method of making. United States Patent, 5,234,707

- Mizuno, R. and Lucey, J.A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interactions in casein micelles. *Journal of Dairy Science*, Volume 88, 3070-3078
- Morris, V.J. (1990). Starch gelation and retrogradation. *Trends in Food Science & Technology*, Volume 1, 2-6
- Mounsey, J.S. and O'Riordan, E.D. (2001). Characteristics of imitation cheese containing native starches. *Journal of Food Science*, Volume 66, 586-591
- Mounsey, J.S. and O'Riordan, E.D. (1999). Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. *Journal of Food Science*, Volume 64, 701-703
- Mulvihill, D.M. and McCarthy, A. (1994). Proteolytic and rheological changes during ageing of cheese analogues made from rennet caseins. *International Dairy Journal*, Volume 4, 15-23
- Murphy, P. (2000). Starch. In *Handbook of hydrocolloids*, Phillips, G.O. and Williams, P.A. (ed), Woodhead Publishing Limited, Cambridge, 41-65
- Osman, E.M., (1967). Starch in the Food Industry. In *Starch: Chemistry and Technology*, Volume II, Industrial Aspects, Whistler, R.L. and Paschall, E.F. (ed), Academic Press, New York and London, 163-216
- Pereira, R. (2000). Sensory, rheological and microstructural characteristics of model emulsified dairy systems – a doctoral thesis, Massey University, Palmerston North, New Zealand
- Pomeranz, Y. (1985). Carbohydrates - Starch. In *Functional properties of food components*, Academic Press, Orlando, Florida, 24-78
- Radley, R. A. (1976). The Food Industry. In *Industrial uses of starch and its derivatives*, Applied Science Publishers Ltd., London, 51-116

Savello, P.A., Ernstorm, C.A. and Kalab, M. (1989). Microstructure and meltability of model process cheese made with rennet and acid casein. *Journal of Dairy Science*, Volume 72, 1-11

Schaer, W. and Bosset, J.O. (2002). Chemical and physico-chemical changes in processed cheese and ready-made fondue during storage. A review. *Lebensmittel Wissenschaft & Technologie*, Volume 35, 15-20

Shimp, L.A. (1985). Process cheese principles. *Food Technology*, Volume 39, 63-64

Singh, N., Singh, J., Kaur, L., Sodhi, N.S. and Gill, B.S. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, Volume 81, 219-231

Stanley, K.D. and Harris, D.W. (1995). Method of preparing a starch hydrolysate, an aqueous starch hydrolysate dispersion, method of preparing a food containing a starch hydrolysate, and a food formulation containing a starch hydrolysate. United States Patent 5,378,491

Swenson, B.J., Wendorff, W.L. and Lindsay, R.C. (2000). Effects of ingredients on the functionality of fat-free process cheese spreads. *Journal of Food Science*, Volume 65, 822-825

Swinkels, J.J.M. (1985). Composition and properties of commercial native starches. *Starch Staerke*, Volume 37, 1-5

Taggart, P. (2004). Starch as an ingredient: manufacture and applications. In *Starch in food – Structure, function and applications*, Eliasson, A-C. (ed), Woodhead Publishing Limited, Cambridge, 363-392

Tan, Y.L. (2003). Effect of biopolymer addition on the properties of rennet-induced skim milk gels – a master thesis, Massey University, Palmerston North, New Zealand

Tester, R.F., Karkalas, J. and Xin, Q. (2004). Starch - composition, fine structure and architecture. *Journal of Cereal Science*, Volume 39, 151-165

- Tester, R.F. and Morrison, W.R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, Volume 67, 551-557
- Thomas, D.J. and Atwell, W.A. (1999). *Starches*. St Paul, Minnesota, Eagan Press, 94
- Thomas, M.A. (1970). Use of calcium co-precipitates in processed cheese. *Australian Journal of Dairy Technology*, Volume 25, 23-26
- Tunick, M.H. (2000). Rheology of dairy foods that gel, stretch, and fracture. *Journal of Dairy Science*, Volume 83, 1892-1898
- Walstra, P. (1965). Light scattering by milk fat globules. *The Netherlands Milk and Dairy Journal*, Volume 19, 93-109
- Watkinson, P. (2005). Personal communication. Fonterra Research Centre, Palmerston North, New Zealand
- Whistler, R. L. and BeMiller, J.N. (1997). Starch. In *Carbohydrate Chemistry for Food Scientists*, Eagan Press, St Paul, Minnesota, 117-138
- Yoder, D., Chang, S-G., Xu, A. and Domoras, T. (1996). Caseinate replacement composition for imitation cheese and process for making imitation cheese. United States Patent 5,486,375
- Zallie, J.P., and Chiu, C.W. (1990). Imitation cheeses containing enzymatically debranched starches in lieu of caseinates. United States Patent 4,937,091
- Zehren, V.L. and Nusbaum, D.D. (1992). Process cheese, D.Cooley & Co., New Holstein, Wisconsin, 364
- Zobel, H.F. (1988). Molecules to granules: a comprehensive starch review. *Starch Starke*, Volume 40, 44-50

Zwiercan, G.A., Lacourse, N.L. and Lenchin, J.M. (1987). Imitation cheese products containing high amylose starch as total caseinate replacement. United States Patent 4,695,475

Zwiercan, G.A., Lacourse, N.L. and Lenchin, J.M. (1985). Imitation cheese products containing modified starch as partial replacement and method of preparation. United States Patent 4,499,116

Websites:

[www.foodstarch.com](http://www.foodstarch.com)

[http://www.ifr.ac.uk/materials/fractures/images/confocal\\_microscope.gif](http://www.ifr.ac.uk/materials/fractures/images/confocal_microscope.gif)

## Appendix

Storage ( $G'$ ) and Loss ( $G''$ ) Modulus for different starches (source material for Figure 5.1, Page 74).

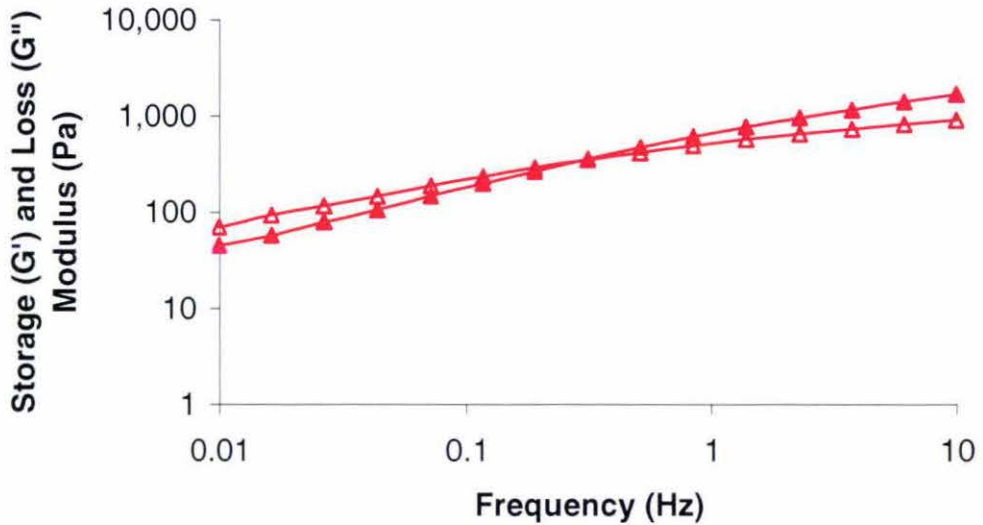


Figure 1. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are for 2 wt% rice starch ( $G'$ -▲,  $G''$ -△).

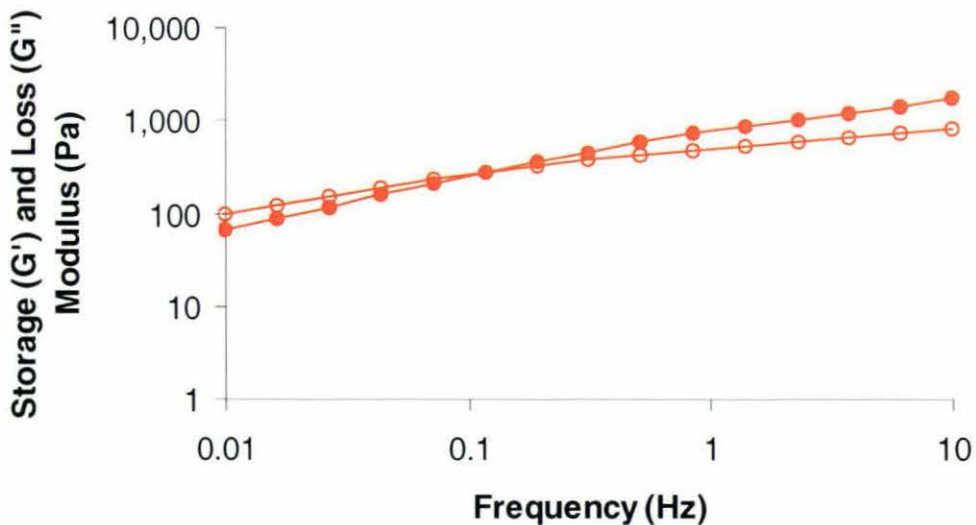


Figure 2. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are 2 wt% potato starch ( $G'$ -●,  $G''$ -○).

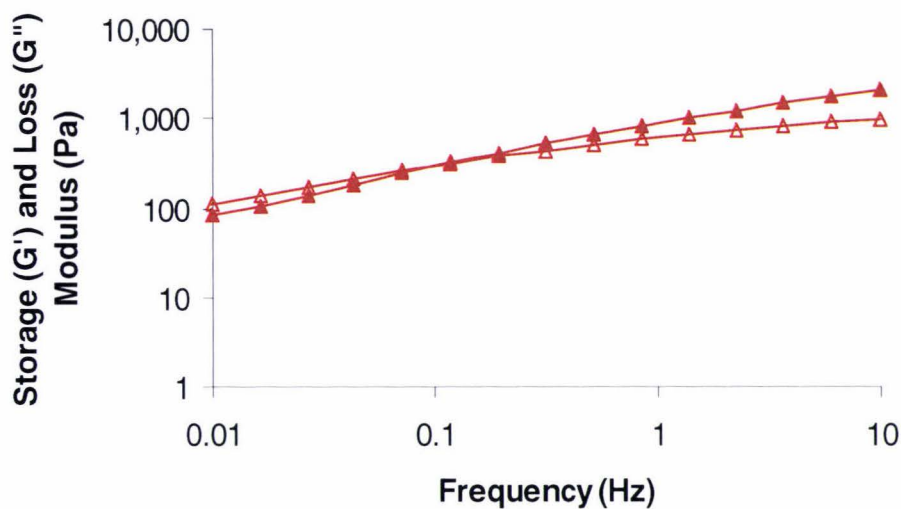


Figure 3. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are 2 wt% wheat starch ( $G'$ -▲,  $G''$ -△).

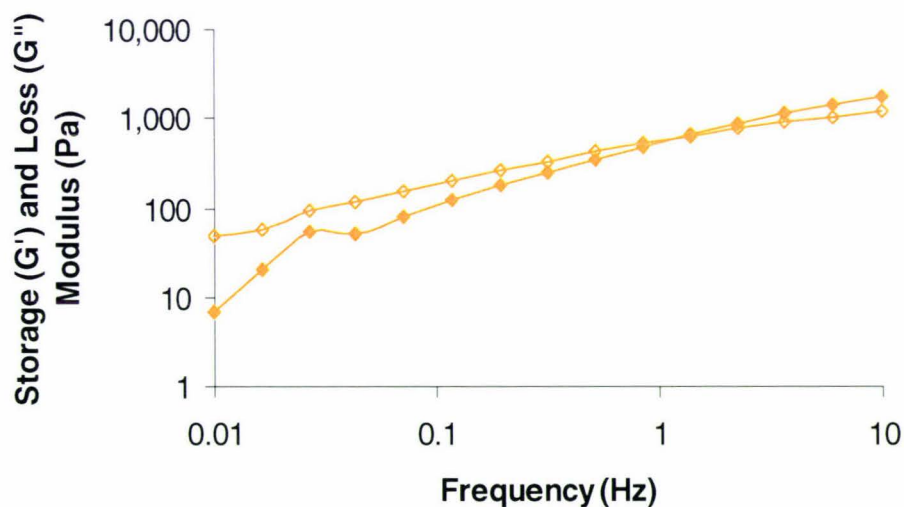


Figure 4. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are 2 wt% HACS ( $G'$ -◆,  $G''$ -◇).

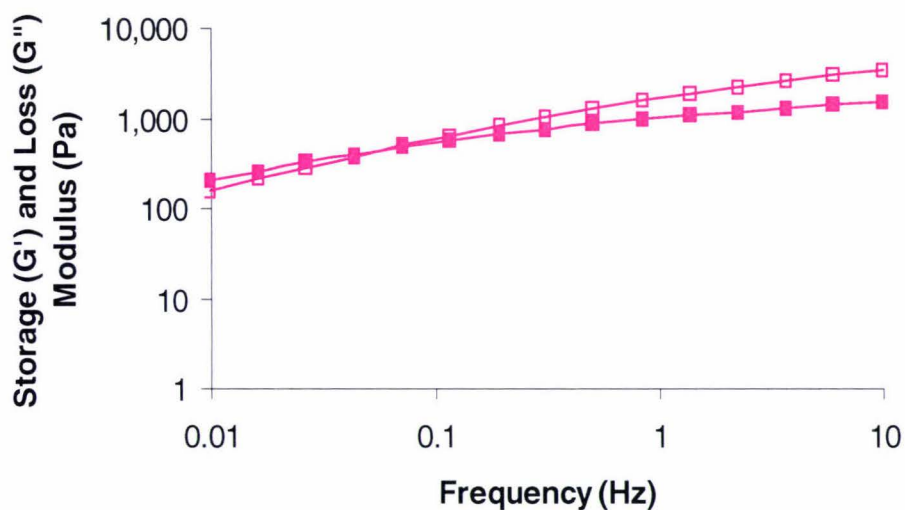


Figure 5. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are 2 wt % acid converted starch ( $G'$ -■,  $G''$ -□).

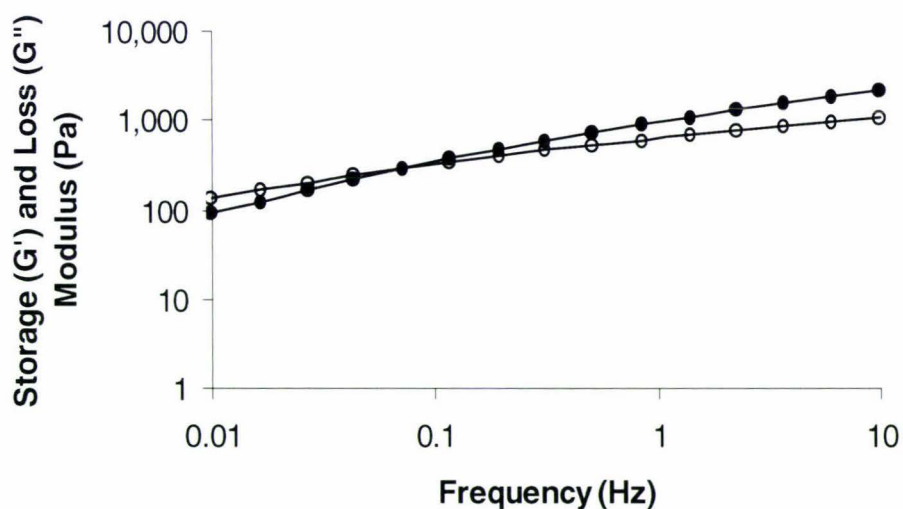


Figure 6. Storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) as a function of frequency of model processed cheese spreads prepared on Paar-Physica and measured at 25°C. Symbols are 2 wt % waxy corn starch ( $G'$ -●,  $G''$ -○).