Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognize the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.
Rheological, melting, microstructural, and oil droplets-interfacial properties of model processed cheese made with calcium caseinate and trisodium citrate or sodium pyrophosphate

Nur Anis Afifah Arzami

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Food Science, The University of Auckland, 2016.
Emulsifying salts play an important role in processed cheese manufacture, contributing to create a smooth and stable product. The aim of this thesis was to develop a fundamental understanding of the relationship between milk protein ingredients, specifically calcium caseinates, the different types of emulsifying salts, oil droplets and their interfacial properties, in a simplified model processed cheese. In this model processed cheese, which were manufactured on a lab scale using trisodium citrate (TSC) or tetrasodium pyrophosphate (TSPP), shear, protein and oil concentrations, emulsion stabilisers were varied. Rheology, particle size, melting measurements and microscopic observations were used to probe these systems.

This study showed that increasing shear rates, up to a critical value, during processed cheese processing decreased the size of the oil droplets up to ~0.2 µm and promoted oil droplet emulsification. Other than that, processed cheese made with 20% of protein (w/w) and 20% oil (w/w) using TSC presented optimal results for particle size (~0.4 µm), rheology ($G^*$ = ~14 kPa) and melting properties (78%). Increasing oil concentration up to 30%, increased the firmness ($G^*$ = ~13.5 kPa) and oil droplet particle size (~0.6 µm) but decreased the meltability (69%) of processed cheese samples made with TSC due to stronger oil-protein and protein-water interactions.

The results of the model processed cheese made with TSPP demonstrated similar trends with the results of processed cheese made with TSC in terms of rheology, particle size, microstructure and melting properties as a function of protein and oil concentrations. Comparison between model processed cheeses made with TSC and TSPP shows that optimal processed cheese are obtained when 20% protein and 20% oil are used.

The type of interactions that occurs between the oil droplets and the protein matrix depends on the nature of the surface active material stabilising the oil droplets. Model processed cheese containing oil droplets stabilised by calcium caseinate or whey protein isolate (WPI) emulsified either by TSC or TSPP demonstrated the higher $G^*$ values, smaller oil droplets, a more compact structure and lower melting properties. This is due to the fact that both WPI and calcium caseinate are active fillers, interacting positively with the casein network matrix producing more elastic processed cheeses. Model processed cheese containing oil droplets stabilised by lecithin either emulsified by TSC or TSPP exhibited the lowest $G^*$ values,
larger oil droplet particle size, loose structure and highest melting properties because oil droplets stabilised by lecithin may act as inert filler, not interacting with the casein network.

A preliminary study was done to investigate the casein micelle structures of skim milk (10% w/w) containing four different types of emulsifying salts which are sodium phosphate (SP), TSPP, sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) using small angle X-ray scattering (SAXS). SAXS data shows that the size of casein micelles decreased with the increase of the phosphate chain length according to the following order: control ≈ SP > TSPP > STPP > SHMP. The addition of TSPP or STPP or SHMP leads to alteration of the sub-micelles and the dissolution of the colloidal calcium phosphate nanoparticles (CPN). The dissociation of the casein micelle started to occur when the concentration of the emulsifying salts were: \( \geq 1\% \) SP, \( \geq 0.5\% \) TSPP, \( \geq 0.25\% \) STPP or \( \geq 0.25\% \) SHMP. Heat treatment only affected milk samples emulsified with SP (at 1% and 2% concentration).
Dedication

To my family, who has offered me love and support throughout the course of this thesis
In completion of this work, I would like to sincerely thank and express my deepest appreciation to my supervisor, A/Prof. Dr. Yacine Hemar, for his patience, scientific knowledge, systematic guidance, enthusiasm and encouragement throughout my PhD journey. I also would like to thank my advisor, Prof. Dr. Sylvie Marchesseau whose guidance and support from the first day of my PhD enabled me to develop an understanding of the project. Also, many thanks to my co-supervisor, Prof. Dr. Conrad Perera for his patience and encouragement throughout this course.

My appreciation also goes to Sreeni Pathirana who has offered much needed help, endless love, moral support and great advice throughout my study. I also share the credit of my work on the Scanning Electron Microscopy with Adrian Turner (School of Biological Sciences) who taught me how to use the critical point dryer and Hilary Holloway (Biomedical Imaging Research Unit) who guided me through on Confocal Laser Scanning Microscopy. I am also grateful to Catherine Hobbis (Department of Chemical and Materials Engineering) for showing me how to use the scanning electron microscope.

I would like to express my gratitude to Prof. Dr. Kevin Smith (Head of School), the members of staff of the School of Chemical Sciences (Anoma, Aisha, Carol, Tasdeeq and many more) for giving me a great time, excellent assistance and providing the learning environment necessary for me to reach a new level of excellence.

Equal thanks go to Westland Milk Product for providing calcium caseinate, Sigma-Aldrich for providing chemicals and Australian Synchrotron (mainly Nigel Kirby) for the assistance on SAXS experiments.

Many thanks to my colleagues and best friends, Elisa, Liza, Jessica, Fithri, Weam, Neo, Zhi, Zhao, Trang, Ray, Shengpu, Mona, Lydia, Fatima, Da, Jason, Jeffry, Mohammad, Sophia, Daphne, Sujeewa and many more who have been there with me through good times and bad. I thank you all for making the years so memorable.

I am sincerely grateful to Majlis Amanah Rakyat (MARA) for providing the PhD scholarship and also the opportunity to study in The University of Auckland.

I cannot find words to express my gratitude to my beloved parents, Arzami Adam and Maznah Mohamed who have sacrificed a lot of things for me to get this far in life. They have supported me both financially and emotionally in every step of my PhD. Also to my brother, Afiq for his support and unconditional love. I am truly blessed to have you as my family, you who have endured this long process with me, always offering support, patience, and understanding. I also would like to thank my fiancé who has been very patient, for his endless support and encouragement throughout this journey.

My sincere thanks to you all.
Table of Contents

ABSTRACT ................................................................................................................................. i
DEDICATION ............................................................................................................................. iii
ACKNOWLEDGEMENTS ........................................................................................................ iv
TABLE OF CONTENTS ............................................................................................................. v
LIST OF FIGURES .................................................................................................................... x
LIST OF TABLES ..................................................................................................................... xxi
LIST OF SYMBOLS AND ABBREVIATIONS .......................................................................... xxii
CO-AUTHORSHIP FORM ......................................................................................................... xxv

Chapter 1. Introduction ............................................................................................................. 1
1.1 Background ......................................................................................................................... 2
1.2 Research objectives ............................................................................................................ 3
1.3 Thesis structure .................................................................................................................. 5

Chapter 2. Literature Review .................................................................................................. 7
2.1 Cheese ................................................................................................................................ 8
  2.1.1 History of cheese-making ............................................................................................. 8
  2.1.2 Cheese manufacture ..................................................................................................... 9
2.2 Processed Cheese ................................................................................................................ 11
  2.2.1 Introduction and history ............................................................................................... 11
  2.2.2 Processed cheese and products .................................................................................. 12
  2.2.3 Advantages of processed cheese ................................................................................ 13
  2.2.4 Processed cheese legislation ...................................................................................... 14
  2.2.5 Ingredients of processed cheese ................................................................................ 15
  2.2.6 Processed cheese manufacture ................................................................................... 17
     2.2.6.1 Selection and calculation of raw materials ......................................................... 18
     2.2.6.2 Blending: Cleaning, cutting, mincing and mixing ........................................... 18
     2.2.6.3 Processing conditions ....................................................................................... 19
        2.2.6.3.1 Processing temperature ............................................................................ 19
        2.2.6.3.2 Mixing speed ......................................................................................... 20
        2.2.6.3.3 Homogenization .................................................................................... 20
        2.2.6.3.4 Packaging, cooling and storage ............................................................... 21
  2.2.7 Cheese analogues .......................................................................................................... 22
  2.2.8 Influence of pH on processed cheese ........................................................................ 24
2.3 Milk

2.3.1 Casein

2.3.2 Casein micelles

2.3.3 Caseinates

2.3.3.1 Sodium caseinate

2.3.3.2 Calcium caseinate

2.3.4 Whey proteins

2.3.4.1 Whey protein concentrate (WPC)

2.3.4.2 Whey protein isolate (WPI)

2.4 Emulsifying salts

2.4.1 Trisodium citrate (TSC)

2.4.2 Sodium phosphate (SP)

2.4.3 Sodium pyrophosphate (TSPP)

2.4.4 Sodium tripolyphosphate (STPP)

2.4.5 Sodium hexametaphosphate (SHMP)

2.4.6 Mechanisms of emulsifying salts during processed cheese manufacture

2.5 Emulsions

2.5.1 Surface active agents

2.5.1.1 Sodium dodecyl sulfate (SDS)

2.5.1.2 Polysorbate 20 (Tween 20)

2.5.1.3 Lecithin

2.6 Rheological Properties

2.6.1 General principles of rheology

2.7 Particle size measurements

2.7.1 General principles of particle size measurements by laser diffraction

2.8 Melting properties

2.8.1 Arnott melt test

2.8.2 Olson and Price melt test

2.8.3 Schreiber melt test

2.9 Protein content evaluation

2.9.1 Bradford Method

2.9.2 Electrophoresis

2.9.2.1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

2.9.3 Chromatography

2.10 Microstructural evaluation

2.10.1 Transmission electron microscopy (TEM)

2.10.2 Scanning electron microscopy (SEM)
2.10.3 Confocal laser scanning microscopy (CLSM) ......................................................... 61
2.11 Small angle X-ray scattering (SAXS) ..................................................................... 62
2.11.1 General principles of SAXS ................................................................................. 63
2.11.2 Some SAXS Applications ..................................................................................... 64
2.12 Concluding remarks ................................................................................................. 65

Chapter 3. Materials and Methods .................................................................................. 67
3.1 Materials ....................................................................................................................... 68
3.1.1 Chemicals for preparation of model processed cheese ........................................... 68
3.1.2 Chemicals for preparation of emulsions ................................................................. 68
3.1.3 Chemicals for preparation of milk samples ............................................................ 68
3.1.4 Minor chemicals ...................................................................................................... 68
3.2 Preliminary studies on model processed cheese ........................................................ 69
3.2.1 Method A ................................................................................................................. 69
3.2.2 Method B ................................................................................................................. 69
3.2.3 Method developed for this study ........................................................................... 70
3.3 Sample preparations .................................................................................................... 71
3.3.1 Manufacture of model processed cheese ............................................................... 71
3.3.2 Processed cheese with emulsions .......................................................................... 72
3.3.3 Preparation of milk samples ................................................................................... 73
3.4 Physical methods ......................................................................................................... 74
3.4.1 Rheological measurements .................................................................................... 74
3.4.2 Particle size measurement ....................................................................................... 75
3.4.3 Melting properties ................................................................................................... 76
3.4.4 Microstructural evaluations...................................................................................... 77
3.4.4.1 Scanning Electron Microscopy (SEM) ............................................................... 77
3.4.4.2 Cryo-Scanning Electron Microscopy (cryo-SEM) ........................................... 78
3.4.4.3 Confocal Laser Scanning Microscopy (CLSM) ................................................. 78
3.4.5 Small angle X-ray Scattering (SAXS) measurements ............................................ 79
3.5 Chemical methods ....................................................................................................... 80
3.5.1 Reversed phase high-performance liquid chromatography (RP-HPLC) ............... 80
3.6 Statistical analysis ....................................................................................................... 82

Chapter 4. Effect of processing shear and formulation on some physical properties of model
processed cheese made with trisodium citrate (TSC) .......................................................... 83
4.1 Introduction .................................................................................................................. 84
4.2 Materials and methods ........................................................................................................... 85
  4.2.1 Materials .......................................................................................................................... 85
  4.2.2 Preliminary studies on model processed cheese ............................................................. 85
  4.2.3 Sample preparation and experimental methods .............................................................. 85
4.3 Results and discussion .......................................................................................................... 87
  4.3.1 Preliminary studies on model processed cheese ............................................................. 87
    4.3.1.1 Creaming phase during model processed cheese manufacture ............................... 88
    4.3.1.2 Addition of NaCl ......................................................................................................... 89
    4.3.1.3 Comparison of microscopic images ......................................................................... 90
  4.3.2 Effect of shear ................................................................................................................. 92
  4.3.3 Effect of protein concentration ...................................................................................... 95
  4.3.4 Effect of oil concentration ............................................................................................. 103
4.4 Summary to chapter ............................................................................................................. 111

Chapter 5. Effect of formulation on the physical properties of model processed cheese made with sodium pyrophosphate (TSPP) ................................................................. 112
5.1 Introduction ......................................................................................................................... 113
5.2 Materials and methods ....................................................................................................... 115
  5.2.1 Materials ........................................................................................................................ 115
  5.2.2 Sample preparation and experimental methods ............................................................ 115
5.3 Results and discussion ....................................................................................................... 116
  5.3.1 Effect of protein concentration .................................................................................... 116
  5.3.2 Effect of oil concentration ............................................................................................. 125
  5.3.3 Comparison with TSC ..................................................................................................... 132
5.4 Summary to chapter ............................................................................................................ 139

Chapter 6. Effect of trisodium citrate (TSC) and sodium pyrophosphate (TSPP) on model processed cheese made with emulsions stabilised by different emulsifiers ........................................ 140
6.1 Introduction ......................................................................................................................... 141
6.2 Materials and methods ....................................................................................................... 142
  6.2.1 Materials ........................................................................................................................ 142
  6.2.2 Sample preparation ......................................................................................................... 142
  6.2.3 Experimental methods .................................................................................................... 143
6.3 Results and discussion ....................................................................................................... 144
  6.3.1 Effect of trisodium citrate (TSC) on model processed cheese made with emulsions stabilised by different emulsifiers ................................................................................. 144
List of Figures

Figure 2.1 General protocol of cheese manufacture. Source: Fox (2000) .................................................. 9

Figure 2.2 Schematic diagram of the processed cheese product manufacture. Source: Guinee (2011b). (Reproduced with permission from Guinee, 2011b © John Wiley and Sons)................................. 17

Figure 2.3 Creaming model: protein structure and viscosity change. Source: Lee et al. (2003). (Reproduced with permission from Lee et al., 2003 © LWT-Food Science and Technology). ............ 21

Figure 2.4 Different types of cheese substitutes. Source: Bachmann (2001). (Reproduced with permission from Bachmann, 2001 © International Dairy Journal) ...................................................... 23

Figure 2.5 Illustrations of casein micelle model structures which have been developed by various researchers over the years. (a) Sub-micelle model, (b) Modified sub-micelle model, (c) Nanocluster model, (d) The dual-binding model, (e) Dalgleish model and (f) Sponge model. Source: Anema (2014) and Bouchoux et al. (2010). (Reproduced with permission from Bouchoux et al., 2010 © Biophysical Journal) ................................................................. 27

Figure 2.6 Schematic diagram for caseinate production. Source: Jabara et al. (2004) .................................. 28

Figure 2.7 Chemical structure of trisodium citrate. .................................................................................. 32

Figure 2.8 Chemical structure of sodium phosphate .................................................................................. 33

Figure 2.9 Chemical structure of sodium pyrophosphate decahydrate .................................................. 34

Figure 2.10 Chemical structure of sodium tripolyphosphate ................................................................. 34

Figure 2.11 Chemical structure of sodium hexametaphosphate ............................................................... 35

Figure 2.12 Schematic diagram of possible reactions during the production of processed cheese; ES: emulsifying salts; Temp.: temperature. Source: Lucey et al. (2011). (Reproduced with permission from Lucey et al., 2011 © John Wiley and Sons) ................................................................. 36

Figure 2.13 Schematic illustration of processed cheese after cooling (A) and processed cheese after melting (B). Source: Lucey, Johnson, and Horne (2003). (Reproduced with permission from Lucey et al., 2003 © Journal of Dairy Science) ................................................................. 38

Figure 2.14 Chemical structure of sodium dodecyl sulfate (SDS) showing hydrophobic and hydrophilic region. Source: Caligur (2008) .................................................................................. 42

Figure 2.15 Chemical structure of polysorbate 20 (Tween 20) ............................................................... 43

Figure 2.16 Chemical structure of lecithin (phosphatidycholine) ............................................................ 44

Figure 2.17 Example of cone and plate geometry (left) and parallel plate geometry (right). Source: Lampman (2003) ................................................................................................................. 47

Figure 2.18 Example of particle sizes and shapes. A sphere shape (left) can be measured by its diameter (d). An uneven shape (right) has to be measured using various length and width ........... 48
Figure 2.19 Schematic diagram of the concept of equivalent spheres. *Source: Rawle (2003)*

Figure 2.20 Schematic diagram of a typical particle size distribution. *Source: Yang (2003)*

Figure 2.21 Schematic diagram of laser diffraction analysis. *Source: McGarvey et al. (1997)*

Figure 2.22 Cheese samples positioned on a glass tray before heat treatment (A) and after heat treatment (B). *Source: Arnott et al. (1957)*

Figure 2.23 Cheese samples inserted into a glass tube before heat treatment (A) and melted cheese samples in the glass tube after heat treatment (B). *Source: Gunasekaran & Ak (2002)*

Figure 2.24 Cheese samples positioned on a petri dish before heat treatment (A) and melted cheese samples on petri dish after heat treatment (B). Concentrically numbered graph was placed under the petri dish for measurement purposes. *Source: Gunasekaran & Ak (2002)*

Figure 2.25 Example of SDS-PAGE patterns of proteins from different goat milk cheeses. *Source: Park and Jin (1998)*. (Reproduced with permission from Park and Jin, 1998 © Journal of Food Science)

Figure 2.26 Example of reversed-phase HPLC chromatograms of different bovine milks. (A) milk powder; (B) half skimmed UHT milk; (C) half skimmed pasteurised milk; (D) raw milk. *Source: Bordin, Cordeiro, de la Calle, and Rodriguez (2001)*. (Reproduced with permission from Bordin et al., 2001 © Journal of Chromatography A)

Figure 2.27 Example of fluorescence images of processed cheese treated with sodium chloride (left) and processed cheese made with trisodium citrate (right). F: fat globule; P: protein network. *Source: Sutheerawattananonda et al. (1997)*. (Reproduced with permission from Sutheerawattananonda et al., 1997 © Journal of Dairy Science)

Figure 2.28 Example of TEM image of protein network in analogue cheese grain. *Source: Geng et al. (2011)*. (Reproduced with permission from Geng et al., 2003 © International Dairy Journal)

Figure 2.29 Example of SEM image of traditional cheese (left) and analogue processed cheese (right). *Source: Cunha, Dias and Viotto (2010)*. (Reproduced with permission from Cunha et al., 2010 © Food Research International)

Figure 2.30 Example of CLSM images of cheese made from unhomogenised milk (left) and cheese made from homogenised milk (right). NGF represents non-globular fat. Markers- red: fat; green: protein network. *Source: Ong et al. (2011)*. (Reproduced with permission from Ong et al., 2011 © LWT- Food Science and Technology)

Figure 2.31 Schematic diagram of typical SAXS experiment. *Source: Pauw (2013); Stawski & Benning (2013)*

Figure 2.32 Schematic diagram of SAXS setup and the description of scattering vector (q). *Source: Borsali and Pecora (2008)*

Figure 2.33 Schematic illustrations of SAXS applications. *Source: Bernadó et al. (2010); Holt, de Kruif, Tuinier, and Timmins (2003); O’Kane et al. (1994)*
Figure 3.1 Temperature profile of model processed cheese which includes heat treatment phase and creaming phase. Note that model processed cheese made without the creaming phase is cooled just after the heat treatment phase………………………………………………………………………….. 70

Figure 3.2 Vorwerk Thermomix TM 31 blender cooker used for making model processed cheese… 71

Figure 3.3 Steps to produce model processed cheese made from emulsions……………………………………… 73

Figure 3.4 Controlled-stress rheometer (Paar Physica MCR 301), used for rheological measurements. ………………………………………………………………………………………………………… 74

Figure 3.5 Malvern Mastersizer 2000, used for particle size measurements ……………………………………… 75

Figure 3.6 Steps for meltability measurements. The model processed cheese shown in the figure is made with 15% oil and 20% protein………………………………………………………………………………… 77

Figure 3.7 SAXS/WAXS beamline in Australian Synchrotron (Melbourne, Australia) …………………… 80

Figure 3.8 Typical setup for reversed phase HPLC (HP Series 1100), used for chromatography measurements………………………………………………………………………………………… 82

Figure 4.1 Processed cheese samples emulsified using TSC, with seven different oil concentrations ranging from 0 to 30% (left to right) with constant amount of protein concentration (20%)……………… 86

Figure 4.2 Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese between this study (left) and study from Lee et al. (2004) (right) (Reproduced with permission from Lee et al., 2004. © International Journal of Food Science and Technology). ( …………………………………………………………………………………………………………………… 87

Figure 4.3 Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) of model processed cheese with creaming phase and without creaming phase emulsified with TSC or SHMP. ( ▲ ) $G'$ processed cheese emulsified with TSC with creaming phase; ( △ ) $G''$ processed cheese emulsified with TSC with creaming phase; ( △ △ ) $G'$ processed cheese emulsified with SHMP with creaming phase; ( △ △ △ ) $G''$ processed cheese emulsified with SHMP with creaming phase; ( △ △ △ △ ) $G'$ processed cheese emulsified with TSC without creaming phase; ( △ △ △ △ △ ) $G''$ processed cheese emulsified with TSC without creaming phase; ( △ △ △ △ △ △ △ ) $G'$ processed cheese emulsified with SHMP without creaming phase; ( △ △ △ △ △ △ △ △ △ △ ) $G''$ processed cheese emulsified with SHMP without creaming phase. Protein concentration: 20%, oil concentration: 20%……………………………………………………………………………………………………………. 88

Figure 4.4 Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) of model processed cheese emulsified with TSC or SHMP and made with 1% NaCl or without NaCl. ( ▲ ) $G'$ processed cheese emulsified with TSC made with 1% NaCl; ( △ ) $G''$ processed cheese emulsified with TSC made with 1% NaCl; ( △ △ ) $G'$ processed cheese emulsified with TSC without NaCl; ( △ △ △ ) $G''$ processed cheese emulsified with TSC without NaCl; ( △ △ △ △ ) $G'$ processed cheese emulsified with SHMP made with 1% NaCl; ( △ △ △ △ △ ) $G''$ processed cheese emulsified with SHMP made with 1% NaCl; ( △ △ △ △ △ △ △ ) $G'$ processed cheese emulsified with SHMP without NaCl; ( △ △ △ △ △ △ △ △ △ △ ) $G''$ processed cheese emulsified with SHMP without NaCl. Protein concentration: 20%, oil concentration: 20%………………………………………………………………………………………………………………… 90

Figure 4.5 Comparisons of microscopic images of model processed cheese made with 20% protein and 20% oil using CLSM (a), Cryo-SEM (b) and SEM (c)……………………………………………………………………………………………………………….. 91
**Figure 4.6** Evolution of the complex modulus ($G^*$) of model processed cheese samples (20% protein, 20% oil) manufactured in a lab cooker (Thermomix) with different speeds (1 to 8) (rheological parameters: 20°C, 1Hz, applied strain of 10%). Data represent means ($n = 2$) and error bars correspond to standard deviations. ................................................................................................................................. 92

**Figure 4.7** Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese manufactured in a lab cooker (Thermomix) with different speeds (1 to 10). Data represent means ($n = 2$) and error bars correspond to standard deviations. ................................................................................................................................. 93

**Figure 4.8** Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 5, 15 and 30% of protein concentration with (20%) and without oil addition. (○) $G'$ 5% protein, 0% oil; (□) $G'$ 5% protein, 0% oil; (■) $G'$ 15% protein, 0% oil; (▲) $G'$ 15% protein, 0% oil; (△) $G'$ 30% protein, 0% oil; (◆) $G'$ 30% protein, 0% oil; (○) $G''$ 5% protein, 20% oil; (□) $G''$ 5% protein, 20% oil; (▲) $G''$ 15% protein, 20% oil; (△) $G''$ 15% protein, 20% oil; (◆) $G''$ 30% protein, 20% oil. ........................................................................................................................................ 95

**Figure 4.9** Evolution of complex modulus ($G^*$) of model processed cheese samples manufactured with different protein concentrations (2.5 to 40%) and different oil concentrations (0 to 30%). (■) No oil; (○) 20% oil; (▲) 30% oil. Data represent means ($n = 2$) and error bars correspond to standard deviations. ................................................................................................................................. 96

**Figure 4.10** Large deformation ($G'$ and $G''$) as a function of strain of model processed cheese containing 30% of oil manufactured with 5, 10 and 20% of protein concentration. (○) $G'$ 5% protein, 30% oil; (□) $G'$ 5% protein, 30% oil; (▲) $G'$ 10% protein, 30% oil; (△) $G'$ 10% protein, 30% oil; (◆) $G'$ 20% protein, 30% oil; (○) $G'$ 20% protein, 30% oil; (□) $G'$ 20% protein, 30% oil. ........................................................................................................................................ 97

**Figure 4.11** The evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (○) of model processed cheese manufactured with different protein concentrations (2.5 to 40%) (oil concentration: 30%). Data represent means ($n = 2$) and error bars correspond to standard deviations. ................................................................................................................................. 99

**Figure 4.12** Oil droplet particle size distribution for model processed cheese manufactured with 5, 10 and 40% of protein concentration (oil concentration: 20%). (■) 5% protein, 20% oil; (○) 20% protein, 20% oil; (▲) 40% protein, 20% oil. ........................................................................................................................................ 100

**Figure 4.13** Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%). Data represent means ($n = 3$) and error bars correspond to standard deviations. ................................................................................................................................. 101

**Figure 4.14** The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration: 20%). Data represent means ($n = 3$) and error bars correspond to standard deviations. ................................................................................................................................. 102

**Figure 4.15** Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 0, 10 and 20% of oil concentration at 20% protein concentration. (■) $G'$ 0% oil; (○) $G'$ 0% oil; (△) $G'$ 10% oil; (▲) $G'$ 10% oil; (△) $G'$ 20% oil; (◆) $G'$ 20% oil. ........................................................................................................................................ 104
Figure 4.16 Complex modulus ($G^*$) of processed cheese samples as a function of oil concentration (protein concentration: 20%). Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 4.17 Evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (○) of model processed cheese manufactured with 20% of protein and different oil concentration. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 4.18 Oil droplet particle size distribution for model processed cheese manufactured with (■) 5% oil; (○) 20% oil and (▲) 30% oil concentration (protein concentration: 20%).

Figure 4.19 Mean average oil droplet particle sizes ($D(3, 2)$ in $\mu m$) of processed cheese manufactured with 20% of protein as a function of oil concentration (5 to 30%). Data represent means ($n = 3$) and error bars correspond to standard deviations.

Figure 4.20 Scanning electron (SEM) micrographs of model processed cheese made with 20% of protein and different concentrations of oil a) 5%, b) 10%, c) 15%, d) 20%, e) 30%. Scale bar = 2$\mu m$.

Figure 4.21 The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%). Protein concentration: 20%. Data represent means ($n = 3$) and error bars correspond to standard deviations.

Figure 4.22 Melting pictures of model processed cheese manufactured with different oil concentration (0 to 30%). Protein concentration: 20%.

Figure 5.1 Processed cheese samples made with TSPP, with seven different oil concentrations ranging from 0 to 30% (left to right) with constant amount of protein concentration (20%)…

Figure 5.2 Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 5, 15 and 30% of protein concentration with (20%) and without oil addition. (●) $G'$ 5% protein, 0% oil; (■) $G'$ 5% protein, 0% oil; (▲) $G'$ 15% protein, 0% oil; (■) $G''$ 15% protein, 0% oil; (●) $G''$ 5% protein, 0% oil; (○) $G'$ 30% protein, 0% oil; (▲) $G'$ 30% protein, 0% oil; (■) $G''$ 30% protein, 0% oil; (▲) $G''$ 5% protein, 20% oil; (○) $G'$ 5% protein, 20% oil; (■) $G'$ 15% protein, 20% oil; (●) $G''$ 15% protein, 20% oil; (▲) $G'$ 30% protein, 20% oil; (○) $G''$ 30% protein, 20% oil.

Figure 5.3 Evolution of complex modulus ($G^*$) of model processed cheese samples manufactured with different protein concentrations (2.5 to 40%) and different oil concentrations (0 to 30%). (■) No oil; (●) 5% oil; (▲) 10% oil; (●) 15% oil; (○) 20% oil; (▲) 30% oil. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 5.4 Large deformation ($G'$ and $G''$) as a function of strain of model processed cheese containing 30% of oil manufactured with 5, 10 and 20% of protein concentration. (●) $G'$ 5% protein, 30% oil; (▲) $G''$ 5% protein, 30% oil; (■) $G'$ 10% protein, 30% oil; (○) $G''$ 10% protein, 30% oil; (●) $G'$ 20% protein, 30% oil; (▲) $G''$ 20% protein, 30% oil.

Figure 5.5 The evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (○) of model processed cheese as a function of protein concentrations (2.5 to 40%) (oil concentration: 30%). Data represent means ($n = 2$) and error bars correspond to standard deviations.
Figure 5.6 Oil droplet particle size distribution for model processed cheese manufactured with 5, 20 and 40% of protein concentration (oil concentration: 20%). (→) 5% protein, 20% oil; (→) 20% protein, 20% oil; (→) 40% protein, 20% oil .................................................................122

Figure 5.7 Mean average oil droplet particle sizes (D(3, 2) in µm) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations.................................................................123

Figure 5.8 The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations .................................................................124

Figure 5.9 Evolution of elastic modulus (G') and loss modulus (G'') as a function of frequency of model processed cheese manufactured with 0, 10 and 20% of oil concentration and 20% protein concentration. (→) G' 0% oil; (→) G'' 0% oil; (→) G' 10% oil; (→) G'' 10% oil; (→) G' 20% oil; (→) G'' 20% oil.................................................................125

Figure 5.10 Complex modulus (G*) of processed cheese samples as a function of oil concentration (protein concentration: 20%). Data represent means (n = 2) and error bars correspond to standard deviations.................................................................126

Figure 5.11 Evolution of critical strain γc (■) and critical shear stress σc (●) of analogue processed cheese as a function of oil concentration. Protein concentration is 20%. Data represent means (n = 2) and error bars correspond to standard deviations.................................................................127

Figure 5.12 Oil droplet particle size distribution for model processed cheese manufactured with (→) 5% oil; (→) 20% oil and (→) 30% oil concentration (protein concentration: 20%)....128

Figure 5.13 Evolution of mean average oil droplet sizes (D(3, 2) in µm) of processed cheese manufactured with 20% of protein as a function of oil concentration (5 to 30%). Data represent means (n = 3) and error bars correspond to standard deviations.................................................................129

Figure 5.14 Scanning electron (SEM) micrographs of model processed cheese made with 20% of protein and different concentrations of oil: a) 5%, b) 10%, c) 15%, d) 20%, e) 30%. Scale bar = 2 to 5µm.................................................................130

Figure 5.15 The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%). Data represent means (n = 3) and error bars correspond to standard deviations.................................................................131

Figure 5.16 Evolution of complex modulus (G*) of model processed cheese samples as a function of protein concentrations (2.5 to 40%) at different oil concentrations (0 to 30%) using TSC and TSPP. (■) TSC no oil; (□) TSPP no oil; (▲) TSC 20% oil; (△) TSPP 20% oil; (●) TSC 30% oil; (◇) TSPP 30% oil. Data represent means (n = 2) and error bars correspond to standard deviations.......132
Figure 5.17 Mean average oil droplet particle sizes (D(3, 2) in µm) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%) using TSC (●) and TSPP (♦). Data represent means (n = 3) and error bars correspond to standard deviations............. 133

Figure 5.18 The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%) using TSC (●) and TSPP (♦). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration : 20%). Data represent means (n = 3) and error bars correspond to standard deviations......................................................... 134

Figure 5.19 Evolution of the complex modulus (G*) of processed cheese samples as a function of oil concentration (protein concentration: 20%) using TSC (●) and TSPP (♦) as emulsifiers. Data represent means (n = 2) and error bars correspond to standard deviations............................................. 136

Figure 5.20 Mean average oil droplet particle sizes (D(3, 2) in µm) as a function of oil concentration (0 to 30%) for processed cheese manufactured with 20% protein using TSC (●) and TSPP (♦). Data represent means (n = 3) and error bars correspond to standard deviations......................................................... 137

Figure 5.21 The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%) using TSC (●) and TSPP (♦). Data represent means (n = 3) and error bars correspond to standard deviations......................................................... 138

Figure 6.1 Processed cheese samples made with emulsions (lecithin) and emulsified using TSPP, with six different oil concentrations ranging from 5 to 30% (left to right) with constant amount of protein concentration (20%)........................................................................................................ 143

Figure 6.2 Evolution of elastic (G') and loss (G'') modulus as a function of frequency for model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). (■) G' of PC made with emulsion stabilised by calcium caseinate; (□) G' of PC made with emulsion stabilised by calcium caseinate; (▲) G' of PC made with emulsion stabilised by WPI; (▲) G'' of PC made with emulsion stabilised by WPI; (♦) G' of PC made with emulsion stabilised by lecithin; (♦) G'' of PC made with emulsion stabilised by lecithin. ......................................................... 144

Figure 6.3 Complex modulus (G*) of model processed cheese samples manufactured with different types of emulsions with added TSC as a function of oil concentration (protein concentration: 20%). (■) G* of PC made with emulsion stabilised by calcium caseinate; (▲) G* of PC made with emulsion stabilised by WPI; (♦) G* of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations......................................................... 146

Figure 6.4 Reversed-phase HPLC elution profiles of model processed cheese containing oil droplets stabilised by WPI manufactured without heat treatment (a) and with heat treatment (b). Gradient 30% to 45%B, 30 min. A280 = Absorbance at 280 nm, α-LA = α-lactalbumin, β-LG = β-lactoglobulin, κ-CN = κ-casein. ................................................................................................................ 147

Figure 6.5 Evolution of critical shear stress σ, of model processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (0 to 30%) (protein concentration: 20%). (■) σ, (shear stress) of PC made with emulsion stabilised by calcium caseinate; (▲) σ, (shear stress) of PC made with emulsion stabilised by WPI; (♦) σ, (shear stress) of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations......................................................... 148
**Figure 6.6** Evolution of critical strain $\gamma_c$ of model processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (0 to 30%) (protein concentration: 20%). (■) $\gamma_c$ (% strain) of PC made with emulsion stabilised by calcium caseinate; (▲) $\gamma_c$ (% strain) of PC made with emulsion stabilised by WPI; (●) $\gamma_c$ (% strain) of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations. 149

**Figure 6.7** Oil droplet particle size distribution for model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). (■) PC made with emulsion stabilised by calcium caseinate; (▲) PC made with emulsion stabilised by WPI; (●) PC made with emulsion stabilised by lecithin; (●) WPI emulsion- before being added into PC. 151

**Figure 6.8** Mean average oil droplet particle sizes ($D(3, 2)$ in $\mu$m) of processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (oil concentration: 0 to 30%) (protein concentration: 20%). (■) $D$ 3.2 of PC made with emulsion stabilised by calcium caseinate; (▲) $D$ 3.2 of PC made with emulsion stabilised by WPI; (●) $D$ 3.2 of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations. 152

**Figure 6.9** Scanning electron (SEM) micrographs of model processed cheese made with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). a) PC made with emulsion stabilised by calcium caseinate, b) PC made with emulsion stabilised by WPI, c) PC made with emulsion stabilised by lecithin. Scale bar = 20$\mu$m. 153

**Figure 6.10** The height melting percentage of model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 0 to 30%) (protein concentration: 20%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (■) % Melt of PC made with emulsion stabilised by calcium caseinate; (▲) % Melt of PC made with emulsion stabilised by WPI; (●) % Melt of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations. 154

**Figure 6.11** Evolution of elastic ($G'$) and loss ($G''$) modulus of model processed cheese manufactured with different types of emulsions with added TSP as a function of frequency (oil concentration: 20%) (protein concentration: 20%). (■) $G'$ of PC made with emulsion stabilised by calcium caseinate; (▲) $G''$ of PC made with emulsion stabilised by calcium caseinate; (●) $G'$ of PC made with emulsion stabilised by WPI; (▲) $G''$ of PC made with emulsion stabilised by WPI; (●) $G'$ of PC made with emulsion stabilised by lecithin; (▲) $G''$ of PC made with emulsion stabilised by lecithin. 156

**Figure 6.12** Complex modulus ($G^*$) of model processed cheese samples manufactured with different types of emulsions with added TSP as a function of oil concentration (protein concentration: 20%). (■) $G^*$ of PC made with emulsion stabilised by calcium caseinate; (▲) $G^*$ of PC made with emulsion stabilised by WPI; (●) $G^*$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations. 157
Figure 6.13 Evolution of critical shear stress $\sigma_c$ of model processed cheese manufactured with different types of emulsions with added TSPP as a function of oil concentration (0 to 30%) (protein concentration: 20%). (⚫) $\sigma_c$ of PC made with emulsion stabilised by calcium caseinate; (▴) $\sigma_c$ of PC made with emulsion stabilised by WPI; (★) $\sigma_c$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations. ........................................ 158

Figure 6.14 Evolution of critical strain $\gamma_c$ of model processed cheese manufactured with different types of emulsions with added TSPP as a function of oil concentration (0 to 30%) (protein concentration: 20%). (⚫) $\gamma_c$ of PC made with emulsion stabilised by calcium caseinate; (▴) $\gamma_c$ of PC made with emulsion stabilised by WPI; (★) $\gamma_c$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations. ........................................ 159

Figure 6.15 Oil droplet particle size distribution for model processed cheese manufactured with different types of emulsions with added TSPP (oil concentration: 20%) (protein concentration: 20%). (⚫) PC made with emulsion stabilised by calcium caseinate; (▴) PC made with emulsion stabilised by WPI; (★) PC made with emulsion stabilised by lecithin; (■) WPI emulsion- before being added into PC. .......................................................................................................................... 160

Figure 6.16 Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese manufactured with different types of emulsions with added TSPP as a function of oil concentration (oil concentration: 0 to 30%) (protein concentration: 20%). (⚫) $D(3, 2)$ of PC made with emulsion stabilised by calcium caseinate; (▴) $D(3, 2)$ of PC made with emulsion stabilised by WPI; (★) $D(3, 2)$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations. ........................................................................................................ 161

Figure 6.17 Scanning electron (SEM) micrographs of model processed cheese made with different types of emulsions with added TSPP (oil concentration: 20%) (protein concentration: 20%). a) PC made with emulsion stabilised by calcium caseinate, b) PC made with emulsion stabilised by WPI, c) PC made with emulsion stabilised by lecithin. Scale bar = 20µm. ............................................................... 163

Figure 6.18 The height melting percentage of model processed cheese manufactured with different types of emulsions with added TSPP (oil concentration: 0 to 30%) (protein concentration: 20%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (⚫) % Melt of PC made with emulsion stabilised by calcium caseinate; (▴) % Melt of PC made with emulsion stabilised by WPI; (★) % Melt of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations................................................................. 164

Figure 6.19 Complex modulus ($G^*$) of model processed cheese samples manufactured with different types of emulsions with added TSC or TSPP as a function of oil concentration (protein concentration: 20%). (■) $G^*$ of PC made with emulsion stabilised by calcium caseinate with added TSC; (▴) $G^*$ of PC made with emulsion stabilised by WPI with added TSC; (◆) $G^*$ of PC made with emulsion stabilised by lecithin with added TSC; (□) $G^*$ of PC made with emulsion stabilised by calcium caseinate with added TSPP; (△) $G^*$ of PC made with emulsion stabilised by WPI with added TSPP; (◇) $G^*$ of PC made with emulsion stabilised by lecithin with added TSPP. Data represent means (n = 2) and error bars correspond to standard deviations ................................................................................... 165
Figure 6.20 Mean average oil droplet particle sizes \(D(3, 2)\) in \(\mu m\) of processed cheese manufactured with different types of emulsions (oil concentration: 5 to 30\%) (protein concentration: 20\%). (■) \(D\) 3,2 of PC made with emulsion stabilised by calcium caseinate with added TSC; (▲) \(D\) 3,2 of PC made with emulsion stabilised by WPI with added TSC; (◆) \(D\) 3,2 of PC made with emulsion stabilised by lecithin with added TSC; (□) \(D\) 3,2 of PC made with emulsion stabilised by calcium caseinate with added TSPP; (▲) \(D\) 3,2 of PC made with emulsion stabilised by WPI with added TSPP; (◆) \(D\) 3,2 of PC made with emulsion stabilised by lecithin with added TSPP; (●) \(D\) 3,2 of emulsion only- before being added into PC. Data represent means \((n = 3)\) and error bars correspond to standard deviations

Figure 6.21 The height melting percentage of model processed cheese manufactured with different types of emulsions (oil concentration: 0 to 30\%) (protein concentration: 20\%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (■) % Melt of PC made with emulsion stabilised by calcium caseinate with added TSC; (▲) % Melt of PC made with emulsion stabilised by WPI with added TSC; (◆) % Melt of PC made with emulsion stabilised by lecithin with added TSC; (□) % Melt of PC made with emulsion stabilised by calcium caseinate with added TSPP; (▲) % Melt of PC made with emulsion stabilised by WPI with added TSPP; (◆) % Melt of PC made with emulsion stabilised by lecithin with added TSPP. Data represent means \((n = 3)\) and error bars correspond to standard deviations

Figure 7.1 SAXS profile of different emulsifying salts on 10\% (w/w) milk samples. Emulsifying salts concentration is 0.5\% (w/w). The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to size of casein micelles, level 1 is assigned to sub-micelles, and level 2 is related to calcium phosphate nanoclusters. (▼) Control milk; (●) Milk with SP; (■) Milk with TSPP; (◇) Milk with STPP; (□) Milk with SHMP

Figure 7.2 SAXS profile of different concentrations of sodium phosphate (SP) on 10\% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▼) Control milk; (●) Milk with 0.1% SP; (■) Milk with 0.25% SP; (◇) Milk with 0.5% SP; (□) Milk with 1% SP; (○) Milk with 2% SP

Figure 7.3 SAXS profile of different concentrations of sodium pyrophosphate (TSPP) on 10\% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 corresponds to to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 is assigned to calcium phosphate nanoclusters. (▼) Control milk; (●) Milk with 0.1% TSPP; (■) Milk with 0.25% TSPP; (◇) Milk with 0.5% TSPP; (□) Milk with 1% TSPP; (○) Milk with 2% TSPP

Figure 7.4 SAXS profile of different concentrations of sodium tripolyphosphate (STPP) on 10\% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▼) Control milk; (●) Milk with 0.1% STPP; (■) Milk with 0.25% STPP; (◇) Milk with 0.5% STPP; (□) Milk with 1% STPP; (○) Milk with 2% STPP
Figure 7.5 SAXS profile of different concentrations of sodium hexametaphosphate (SHMP) on 10% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▼) Control milk; (■) Milk with 0.1% SHMP; (▲) Milk with 0.25% SHMP; (○) Milk with 0.5% SHMP; (□) Milk with 1% SHMP; (●) Milk with 2% SHMP.

Figure 7.6 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium phosphate (SP) in 10% (w/w) milk samples. The temperature for 1% and 2% SP samples was at 20°C while 1% and 2% SP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (∼24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (■) Milk with 1% SP at 20°C; (□) Heat treated milk with 1% SP; (▲) Milk with 2% SP at 20°C; (△) Heat treated milk with 2% SP.

Figure 7.7 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium pyrophosphate (TSPP) in 10% (w/w) milk samples. The temperature for 1% and 2% TSPP samples was at 20°C while 1% and 2% TSPP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (∼24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (■) Milk with 1% TSPP at 20°C; (□) Heat treated milk with 1% TSPP; (▲) Milk with 2% TSPP at 20°C; (△) Heat treated milk with 2% TSPP.

Figure 7.8 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium tripolyphosphate (STPP) in 10% (w/w) milk samples. The temperature for 1% and 2% STPP samples was at 20°C while 1% and 2% STPP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (∼24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (■) Milk with 1% STPP at 20°C; (□) Heat treated milk with 1% STPP; (▲) Milk with 2% STPP at 20°C; (△) Heat treated milk with 2% STPP.

Figure 7.9 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium hexametaphosphate (SHMP) in 10% (w/w) milk samples. The temperature for 1% and 2% SHMP samples was at 20°C while 1% and 2% SHMP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (∼24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (■) Milk with 1% SHMP at 20°C; (□) Heat treated milk with 1% SHMP; (▲) Milk with 2% SHMP at 20°C; (△) Heat treated milk with 2% SHMP.
List of Tables

Table 2.1 Different categories of pasteurized cheese. *Source: Guinee (2011b).* (Reproduced with permission from Guinee, 2011b © John Wiley and Sons)................................................................................................................13

Table 2.2 List of optional ingredients permitted in pasteurized processed cheese products and their main functions. *Source: Guinee (2011b).* (Reproduced with permission from Guinee, 2011b © John Wiley and Sons)................................................................................................................16

Table 2.3 Emulsifying salts and their properties during cheese processing. *Source: Guinee et al. (2004) and Nollet & Sinha (2007).................................................................................................................................39

Table 3.1 The ingredients, compositions of ingredients and type of cooker used to manufacture model processed cheese by Cavalier-Salou and Cheftel (1991)..........................................................................................................................69

Table 3.2 The ingredients, compositions of ingredients and type of cooker used to manufacture model processed cheese by Lee et al. (2004)..........................................................................................................................70
List of Symbols

Å  Angstrom  
x g  times gravity  
$G'$  elastic modulus  
$G''$  loss modulus  
$G^*$  complex shear modulus  
$G'-G''$  $G'$-$G''$ crossover  
$\gamma_c$  critical strain  
$\sigma_c$  critical shear stress  
$I$  Relative intensity  
$I_{IF}$  interfacial scattering intensity  
$I_{IS}$  internal structure scattering intensity  
keV  kilo electron volts  
Pa  Pascal  
pK_{ass}  ion association constant  
$q$  scattering vector  
rpm  revolution per minute  
$\lambda$  wavelength  
$\theta$  scattering angle  
$\Sigma_{SPAc}$  sum of standardized peak areas in control sample  
$\Sigma_{SPAh}$  sum of standardized peak areas in heated sample

List of Abbreviations

$\alpha_s$-CN  $\alpha_s$-casein  
$\beta$-CN  $\beta$-casein  
$\kappa$-CN  $\kappa$-casein  
$\alpha$-LA  $\alpha$-lactalbumin  
$\beta$-LG  $\beta$-lactoglobulin  
BSA  bovine serum albumin  
Ca  calcium  
Ca(OH)$_2$  calcium hydroxide
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-P</td>
<td>calcium phosphate</td>
</tr>
<tr>
<td>CCP</td>
<td>colloidal calcium phosphate</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscopy</td>
</tr>
<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
</tr>
<tr>
<td>CPN</td>
<td>colloidal calcium phosphate nanoparticles</td>
</tr>
<tr>
<td>cryo-SEM</td>
<td>Cryo-scanning electron microscopy</td>
</tr>
<tr>
<td>CTAB</td>
<td>cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ESEM</td>
<td>Environmental scanning electron microscopy</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GA</td>
<td>gum Arabic</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>LAOS</td>
<td>large amplitude oscillatory shear</td>
</tr>
<tr>
<td>LMP</td>
<td>low-methoxyl pectin</td>
</tr>
<tr>
<td>MPC</td>
<td>milk protein concentrate</td>
</tr>
<tr>
<td>MSNF</td>
<td>milk solids non-fat</td>
</tr>
<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>NaN₃</td>
<td>sodium azide</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>NPC</td>
<td>native phosphocaseinate powder</td>
</tr>
<tr>
<td>PC</td>
<td>model processed cheese</td>
</tr>
<tr>
<td>POR</td>
<td>protein to oil ratio</td>
</tr>
<tr>
<td>Pt</td>
<td>platinum</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reversed phase high performance liquid chromatography</td>
</tr>
<tr>
<td>SAOS</td>
<td>small amplitude oscillatory shear rheology</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small angle X-ray scattering</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SP</td>
<td>sodium phosphate</td>
</tr>
<tr>
<td>SPA</td>
<td>standardized peak areas</td>
</tr>
<tr>
<td>SHMP</td>
<td>sodium hexametaphosphate</td>
</tr>
<tr>
<td>STPP</td>
<td>sodium tripolyphosphate</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TSC</td>
<td>trisodium citrate</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>TSPP</td>
<td>sodium pyrophosphate</td>
</tr>
<tr>
<td>UF</td>
<td>ultra-filtrated milk</td>
</tr>
<tr>
<td>VTD</td>
<td>vacuum transfer device</td>
</tr>
<tr>
<td>WAXS</td>
<td>Wide angle X-ray scattering</td>
</tr>
<tr>
<td>WPC</td>
<td>whey protein concentrate</td>
</tr>
<tr>
<td>WPI</td>
<td>whey protein isolate</td>
</tr>
</tbody>
</table>
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 7- Small angle X-ray scattering (SAXS) investigation on the effect of different emulsifying salts on reconstituted milk

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Senior author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>80</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Prof. Dr. Yacine Hemar</td>
<td>Experimental and proof reading</td>
</tr>
<tr>
<td>Prof. Dr. Sylvie Marchesseau</td>
<td>Experimental and proof reading</td>
</tr>
<tr>
<td>Wei Ping Pai</td>
<td>Experimental</td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:
- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Prof. Dr. Yacine Hemar</td>
<td></td>
<td>25/01/2016</td>
</tr>
<tr>
<td>Prof. Dr. Sylvie Marchesseau</td>
<td></td>
<td>26.01.16</td>
</tr>
<tr>
<td>Wei Ping Pai</td>
<td></td>
<td>28/01/16</td>
</tr>
</tbody>
</table>
1

Introduction
1.1 Background

Processed cheese has found great interest among cheese manufacturers due to its stability, simplicity, easily available and open to any new development ideas. The needs of processed cheese among consumers have risen tremendously over the years making it one of the most sought food products in the market (Schmit, Dong, Chung, Kaiser, & Gould, 2002). Furthermore, the increase of demand among consumers for reduced-fat processed cheese has increased the reduced-fat cheese supply in the cheese market (Gould, Cornick, & Cox, 1994). This calls for extensive research to be done on processed cheese where various types of ingredients can be incorporated into the processed cheese and the product stability to be investigated (Drake, Truong, & Daubert, 1999; Goyal & Goyal, 2012; Macků, Buňka, Pavlínek, Leciánová, & Hrabě, 2008; McMahon, Alleyne, Fife, & Oberg, 1996; Raval & Mistry, 1999).

The presence of emulsifying salts in processed cheese is one of the most studied among cheese scientists (Lu, Shirashoji, & Lucey, 2008; Mizuno & Lucey, 2005b). Each emulsifying salt demonstrates unique binding ability which reacts differently depending on the types of ingredients presence in processed cheese and processed cheese manufacturing conditions. Monovalent cations (sodium) and multivalent anions (citrates or phosphates) are the active compounds in these chelating salts.

In processed cheese, the emulsifying salt chelate the calcium, disrupts the structural casein network and solubilizes casein (Lee, Anema, & Klostermeyer, 2004). These compounds influenced the separation of large hydrophobic aggregates of casein into smaller units which increase casein hydration and exposes its polar and nonpolar groups, allowing casein to interact with water, proteins and fat under agitation and heating, and during the cooling step forming a network (Cunha, Grimaldi, Alcântara, & Viotto, 2013; Lee et al., 2004). The degree of hydration of the para-casein and the size distribution of the emulsified fat globules influences the structure and rheology of the processed cheese (Guinee, Caric, & Kalab, 2004). Moreover, the type of emulsifying salts contributes to the degree of fat emulsification (Savello, Ernstrom, & Kalab, 1989). Currently, citrates and phosphates are widely used in the manufacture of processed cheese (Dimitreli & Thomareis, 2008).
This makes the study of emulsifying salts in processed cheese interesting and hundreds of studies have been done since its first introduction over a century ago (Awad, Abdel-Hamid, El-Shabrawy, & Singh, 2002; Černíková et al., 2010; Cunha & Viotto, 2010; Dimitreli, Thomareis, & Smith, 2005; Gupta, Karahadian, & Lindsay, 1984). The aim of this thesis is to develop a simplified model processed cheese on a lab scale, using different types of emulsifying salts in order to develop further understanding of the behaviour; mainly their rheological, melting and microstructural properties.

1.2 Research objectives

The main goal of this PhD thesis was to create a fundamental understanding of the relationship between milk protein specifically casein from calcium caseinates with different types of emulsifying salts and oil droplets in model processed cheese system through the variations of protein concentration, oil concentration, emulsion stabilisers. The relationships were investigated using rheology, particle size, melting and microscopic investigation. Initially, model processed cheese ingredients and manufacturing process was selected based on the literature review. The review was done to oversee which method will be the best to obtain model processed cheese on a lab scale. Then, the types of emulsifying salts to be studied also were selected using the literature. The first objective deals with determination of the impact of shear, different protein concentrations and oil concentration on the rheological properties, oil droplet particle size, meltability and microstructure in a model processed cheese spread system manufactured with trisodium citrate (TSC). Different shear rates ranging from 100 rpm to 10,100 rpm were chosen based on the capacity of the processed cheese equipment. Nine different concentrations of proteins ranging from 2.5 to 40% and seven different oil concentrations ranging from 0 to 30% were also chosen based on the processed cheese machine capability. This objective allows determination of the best parameters to use in subsequent chapters.

Once this objective was completed, similar types of investigations were done on the model processed cheese, but using (in addition to TSC) another emulsifying salt (sodium pyrophosphate). This second objective is set to investigate the influence of different protein (calcium caseinate) concentrations and oil concentrations on the rheological properties, oil droplet particle size, meltability and microstructure of the model processed cheese spread system manufactured with sodium pyrophosphate (TSPP). TSPP was also chosen based on
the literature where many studies reported that model processed cheese emulsified with TSPP demonstrated higher firmness than model processed cheese emulsified with other emulsifying salts, making TSPP interesting for further studies. Comparisons were made between the two emulsifying salts; TSC and TSPP.

The third objective deals with the effect of different surface agents, used to stabilise the oil droplets, on the properties of the model processed cheese. Three types of food-grade surface active agents, namely calcium caseinate, whey protein isolate (WPI) and lecithin were chosen. The model processed cheese made with emulsions stabilised by these surface agents were prepared by the addition of either TSC or TSPP. These processed cheeses were also analysed using rheological, particle size, melting and microstructural properties. Protein evaluation using reversed phase high performance liquid chromatography (RP-HPLC) was also performed to confirm the extent of WPI denaturation during model processed cheese preparation.

After the third objective is completed, a preliminary study is performed using synchrotron small-angle X-ray scattering (SAXS) to probe the structure of casein when different emulsifying salts are added to skim milk (final objective). Skim milk was chosen as it is a well-studied milk system, compared to calcium caseinates. The different emulsifying salts were: sodium phosphate (SP), TSPP, sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP); and the effect of heating was also considered.
1.3 Thesis structure

Chapter 1 gives a brief background on processed cheese development using emulsifying salts and the objectives of the thesis.

Chapter 2 reports an extensive review on the history of cheese, cheese manufacture, processed cheese history, advantages and legislation. This chapter also discusses about milk proteins, the addition of emulsifying salts in processed cheese, emulsions and surface active agents. The fundamental theories of different processed cheese evaluation techniques such as rheological properties, particle size measurements, melting properties, microstructural evaluation, protein content determination and small angle X-ray scattering methods were also covered in this chapter.

Chapter 3 describes the materials and experimental methods used in this thesis. This chapter is sub-divided into six sections: (1) materials for sample preparation; (2) methods for preliminary studies on model processed cheese; (3) sample preparation for analyses; (4) methods for physical analysis; (5) methods for chemical analysis and (6) method for statistical analysis. Note that a brief section of materials and methods was also included in Chapter 4 to 7 where the materials and experimental methods used are referenced back to those described in Chapter 3 as appropriate.

Chapter 4 investigates the impact of shear, protein concentration and oil concentration on the rheological properties, oil droplet particle size, meltability and microstructure of model processed cheese with trisodium citrate (TSC). The results of the preliminary studies on model processed cheese which includes model processed cheese ingredients, salt addition and microstructural evaluation were also discussed in this chapter.

Chapter 5 deals with the effect of protein concentration and oil concentration on the rheological properties, oil droplet particle size, meltability and microstructure of model processed cheese with sodium pyrophosphate (TSPP). The results obtained in this chapter were compared with the previous chapter (Chapter 4) and the effect of each emulsifying salt on the model processed cheese system was investigated.
Chapter 6 focusses on engineering interfacial properties by changing the interfacial properties of the oil droplets. Three types of food-grade surface active agents (calcium caseinate, WPI and lecithin) were chosen based on the literature review. The emulsions obtained were included into model processed cheese system. Rheological properties, oil droplet particle size, meltability and microstructure of model processed cheese emulsified with either TSC or TSPP were analysed. Comparison between TSC and TSPP on model processed cheese made with emulsion was carried out.

Chapter 7 reports the analysis of SAXS measurements on milk emulsified with four different types of emulsifying salts at different emulsifying salts concentrations. This chapter also discussed the results obtained from SAXS evaluations on heat treated and non-heat treated milk samples emulsified with different emulsifying salts.

Chapter 8 discusses the overall experiments, summarises the main findings and presents a general conclusions. This chapter also provides information on the possible future works for model processed cheese.
2

Literature Review


Chapter 2

2.1 Cheese

2.1.1 History of cheese-making

For many centuries, cheese-making has been used to preserve the nutritional value of milk. These dairy products are derived from milk with the presence of rennet and represented a wholesome and interesting foodstuff. After the elimination of cheese whey, the major milk proteins and milk fat left may provide a good source of energy for human requirements. Cheese also provides calcium and minerals for human being. Cheese-making processes started almost over 9000 years ago when random and accidental infection and souring of milk by unrecognized lactic acid-producing bacteria was applied. This process involves metabolic conversion of lactose into lactic acid and it largely avoids later growth of spoilage organisms and pathogens. In the beginning of the last century, cheese makers had developed modern practice of using carefully selected pure strains of these bacteria, encompassing the starter cultures, which were intentionally added to cheese milk in standard amounts depending on the type of cheese required (Rose, 1982).

The characteristics of cheese variety are administered by composition of the starter culture, temperature of manufacture, the coagulant used to gel the milk (enzyme chymosin or a microbial substitute) and by the secondary microflora which may present as chance contaminants or introduced into the cheese making process purposefully (Rose, 1982).

Besides, cheese variety also depends on calcium concentration during the cheese-making process. Renneting cleaves the κ-casein in milk where one peptide bond (Phe\textsubscript{105}-Met\textsubscript{106}) is hydrolyzed, releasing its hydrophilic C-terminal. The residual casein is coagulated depending on the calcium (Ca\textsuperscript{2+}) content (Fox, 2004). The calcium content in cheeses may vary depends on the pH, curd moisture content and degree of whey elimination (Guinee, 2011b). Hard cheeses such as Parmigiano, Grana Padano, Pecorino, Cheddar, Gruyere, Emmental, and Mimolette are rich in fat, protein and calcium content (Kongo & Malcata, 2016b).
2.1.2 Cheese manufacture

The general protocol of the production of mature cheese from fresh milk is summarised in Figure 2.1 below:

Milk
   Selection
   Pre-treatment
   Standardization

Cheese milk
   Addition of:
   - Starter culture (acidification)
   - CaCl₂ (optional)
   - Coagulation (rennet or acid [produced in situ or pre-formed] or heat/acid)

Coagulum (gel)
   - Cut coagulum
   - Stir / Heat
   - Acidification (rennet-coagulated cheeses)
   - Separation of curd from whey

Curd
   - Acidification
   - Special operations (e.g., cheddaring, stretching)
   - Molding
   - Pressing (some varieties)

Fresh Cheese
   - Salting (most varieties)
   - Ripening (most rennet-coagulated cheeses)

Mature Cheese

Figure 2.1 General protocol of cheese manufacture. Source: Fox (2000)

It is interesting to know that the casein can be coagulated in different ways in cheese technology. The main method is by adding a coagulating enzyme, which destabilizes the casein network. This method is used for most of the cheese varieties and can be seen as clot in the cheese vat. Rennets which can be derived from calf stomachs are the first coagulants used. Nowadays, the coagulants can be derived from many sources including plants and fungi (Law & Tamime, 2011). Other methods can also be used by decreasing the pH using acid and the involvement of high heat treatment to precipitate both casein and serum/whey proteins to
form a clot. Such methods were implemented for Ricotta and Queso Blanco cheeses (Law & Tamime, 2011).

According to Fox, McSweeney, Cogan and Guinee (2004), the majority of rennet-coagulated cheeses can be sub-divided into two well-defined phases which are manufacture (preparation of milk, acidification, coagulation, dehydration, pressing and salting which normally takes about 5 to 24 hours) and ripening (2 weeks to 2 years). Fox et al. (2004) also stated that, cheese manufacture is a dehydration process in which the fat and casein in milk are concentrated between 6- and 12-fold, depending on the variety. The degree of dehydration is controlled by the extent and combination of the operation steps in cheese making, in addition to the chemical composition of the milk. Biochemical changes that occur during ripening are controlled by the levels of moisture, salt, pH and the cheese microflora which will be responsible for the flavour, aroma and texture of the finished products. Thus, manufacturing steps greatly determine the nature and quality of the finished cheese. However, flavour characteristics and texture of the individual cheese varieties are developed during the ripening phase (Fox et al., 2004). Therefore, these cheeses differ from each other according to the coagulation process, the fat, protein, calcium and pH content, and the time of ripening (Fox, 2000).

The cheeses also may differ in terms of shelf life which can be varied from days to months. In the beginning of the last century, a specific cheese has been invented to increase the product’s shelf life which is now commonly known as the processed cheese. This technology signifies an interesting pathway to preserve milk products and also assist the products to be accessible towards distant markets (e.g. for countries with tropical climates) with its numerous end-use applications (Kapoor & Metzger, 2008).
2.2 Processed Cheese

2.2.1 Introduction and history

The idea of this technology was originated from the Swiss national dish, “Fondue”, where cheese is melted and heated gently under continuous stirring with the addition of wine which contains tartrate that has an emulsifying effect (Fox, 1999). Processed cheese today is a dairy product manufactured from natural cheese of different maturities under constant stirring and upon heating with the addition of suitable emulsifying salts until a homogenous mass is obtained (Sádlíková et al., 2010).

Initially, in 1885, processed cheese was manufactured without emulsifying agent. However, after the introduction of citrates and phosphates, the functions of emulsifying salts in the industrial production of processed cheese were recognised. The first processed cheese was developed by Walter Gerber and Fritz Stettler in Switzerland in 1911 and the first production started in 1912 in Europe using citrates on the basis of a Swiss patent (Fox, 1999). The initial idea of this process was to improve shelf-life of cheese shipped to warmer climates.

In 1916, processed cheese was developed independently by James Lewis Kraft in the USA who blended and heated natural cheeses with citrates and orthophosphates (used as emulsifying agents) and transferred the homogenous warm cheese into glass jars or cans before cooling. Addition of emulsifying salts (sodium phosphate) was described in a patent few years later by Garstin (Garstin, 1921). This newly developed product also solved the problem of long-term storage of hard cheeses because prolonged storage of these kind of cheeses in their natural form would undergo excessive proteolysis, lipolysis and other detrimental changes (Fox, 1999). For 90 years, processed cheese has evolved to a wide range and diversity in many countries in accordance to different legislative and consumer requirements. Natural cheeses, other dairy and non-dairy ingredients may be included in the blend such as spices, herbs, onions, mushrooms, shellfish or meat to increase the processed cheese flavours (Russell & Gould, 2012).
Today, these cheeses have different names according to different types of processed cheese which have been developed throughout the century such as processed cheese, pasteurized processed cheese, spreadable processed cheese, cheese spread, cheese food, cheese preparation, cheese product, imitation cheese, cheese analogue, etc. On the other hand, the formats of processed cheese include blocks, triangles, slices, tubs, glass jars, tubes and aerosol cans (Tamime, 2011). Processed cheese-making is a straightforward process which involves complex chemical and physical phenomena. The goals of this technology are to establish parameters that create a desirable product (in terms of flavour, body, texture, melt, stretch properties and shelf-life) and to build up a manufacturing protocol so that these parameters are routinely met every time cheese is made (Law & Tamime, 2011). A processed cheese with a smooth, compact, firm body which can be easily cut into slices is generally considered to be a good processed cheese.

### 2.2.2 Processed cheese and products

Over the years, processed cheese products have evolved to lots of varieties, with standards of identity (involving composition and levels and types of permitted ingredients) that varies from country to country. In the United Kingdom, there are two categories of processed cheese products which are processed cheese and processed cheese spread. In Germany, four categories of processed cheese have been introduced. These include processed cheese, processed cheese preparation, cheese preparation and cheese composition (Guinee et al., 2004). There are three main categories of processed cheese products in the USA which are pasteurized process cheese, pasteurized process cheese food and pasteurized process cheese spread. The processing temperature of pasteurized process cheese differs from 80 to 85°C and the pH of the end product ranges from 5.4 to 5.6. Table 2.1 summarises the characteristics of these different categories of processed cheese and related products.

Spreadable processed cheese type has to be cooled as soon as possible and at a slower rate compared to block processed cheese. This is due to the effect of cooling on processed cheese because cooling may soften the product where interaction between emulsified caseins (protein-protein interaction) can occur to form a gel (Tamime, 2011). Hui et al. (2007) stated that, processed cheeses with porous gels (weak cross-links) can be obtained by increasing the cooling rate of processed cheese whereas processed cheese with a firmer gels (strong cross-links) and lower meltability can be produced by lowering the cooling rate. However, a slow
cooling rate can induce the Maillard reaction and also enhance the growth of spores. It is advisable to store processed cheese below 10ºC even though formation of crystals may occur to prolong the shelf life of the processed cheese (Tamime, 2011).

Table 2.1 Different categories of pasteurized cheese. Source: Guinee (2011b). (Reproduced with permission from Guinee, 2011b © John Wiley and Sons).

<table>
<thead>
<tr>
<th>Product</th>
<th>Ingredients (g 100g⁻¹)</th>
<th>Chemical composition (g 100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurized Process Cheese</td>
<td>Contains natural cheeses or enzyme modified cheeses, emulsifying agents (≤3), acidulants, milk fat (≤5), water salt, colours, spices, flavourings, mould inhibitors (≤0.3), anti-sticking agent (lecithin ≤0.03).</td>
<td>Moisture (≤40), fat (≥30) and pH ≥ 5.3</td>
</tr>
<tr>
<td>Pasteurized Process Cheese Food</td>
<td>Natural cheeses and enzyme modified cheeses (&gt;5 of the final products), all the ingredients allowed in processed cheese, also milk, skimmed milk, buttermilk and cheese whey</td>
<td>Moisture (≤44), fat (≥23) and pH ≥ 5.0</td>
</tr>
<tr>
<td>Pasteurized Process Cheese Spread</td>
<td>Natural cheeses and enzyme modified cheeses (&gt;51% w/w of the final products), all the ingredients allowed in the processed cheese food, also food gums, sweeteners, nisin (≤250 ppm)</td>
<td>Moisture (44-60), fat (≥20) and pH &gt; 4.0</td>
</tr>
<tr>
<td>Pasteurized Blended Cheese</td>
<td>Cheese; cream, anhydrous milk fat, dehydrated cream (less than 5%, w/w, in finished product); water, salt, food-grade colours, spices and flavourings; mould inhibitors (≤0.2% w/w).</td>
<td>Moisture (≤43), fat in dry matter (≥47) and pH &gt; 4.0</td>
</tr>
<tr>
<td>Pasteurized Cheese Spread</td>
<td>Similar to pasteurized processed cheese spread, except that emulsifying salts is not permitted</td>
<td>Moisture (40-60), fat (≥20), pH &gt; 4.0</td>
</tr>
</tbody>
</table>

2.2.3 Advantages of processed cheese

According to Caric and Kalab (1999), there are several advantages of processed cheese compared to natural cheeses. Processed cheese can cut down the refrigeration cost during storage and transport which are very important in hot climates. In addition, processed cheese has a better keeping quality, with less apparent changes during prolonged storage. Other than that, there is major diversity of type and flavour intensity of processed cheese spanning from mild to sharp, including native cheese flavour and specific spices. The packaging of
processed cheese also can be adjustable over a range of usages which are economical and imaginative. Finally, processed cheese is suitable for home use as well as for snack restaurants for example in cheeseburgers, hot sandwiches, spreads and dips for fast foods.

2.2.4 Processed cheese legislation

Due to the expanding range of processed cheese products throughout the world, Codex Alimentarius Commission which implements the joint FAO/WHO foods standards programme, developed originally in 1978, has failed to make improvement in updating its original standards for processed cheese and related products that developed originally in the last decade or so (Hickey, 2011). According to Peaslee and Xydis (1979), processed cheese products can be made with added dairy products (including powdered milk, whey-concentrates or casein). Mineral salts, spices or aromas and vitamins (authorized by internal legislation) can also be added to processed cheese. The addition of emulsifying salts in processed cheese was allowed up to no more than 3% of the total weight. Tamime (2011) stated that, the Annex regulation 2742/90 (EU, 1990a) specifies that the use of casein in the manufacture of processed cheese is only permitted to a maximum level of 5g 100g\(^{-1}\). Since the casein manufacturing subsidy has been abolished, there has been no decision as to whether this regulation is still necessary. Different regulation is set for the use of milk protein concentrate (MPC) in natural cheeses and processed cheese. In opposite to processed cheese products, Food and Drug Administration (FDA) regulations prohibit the use of MPC in natural cheeses. The important differences in the production processes between natural and processed cheese may influence the processed cheese manufacturers to use MPC rather than ultra-filtrated (UF) milk. The primary ingredient for processed cheese processing are natural cheese either ingredient cheese or barrel cheese whereas the production process for natural cheese begins with liquid milk. The difference of primary ingredients between both processes influence the production facilities. The production facility for natural cheese involves infrastructure that can store and process large volumes of liquid ingredients. On the other hand, processed cheese facilities may not have the capability to store and process large quantities of liquid ingredients (Jabara, Lipovsky, Coleman, & Payne, 2004).
2.2.5 Ingredients of processed cheese

Processed cheese products can be manufactured by using different ingredients. Table 2.2 summarises the ingredients used in pasteurized processed cheese products. According to (Guinee & Kilcawley, 2004), the ingredients of processed cheese are prepared by subjecting cheese to either a minimal primary processing involving macrostructural changes by the application of some physical treatment (e.g., diced, grated or shredded cheese) or to a more complicated secondary processing involving processes (e.g. heating and shearing) and agents (e.g. enzymes, emulsifying salts) which result in marked transformations in microstructure, composition, levels of proteolysis and lypolysis, texture, flavour and physical form.

Tamime (2008) confirmed that, all the different ingredients used in processed cheese largely influence the development of its microstructure, texture and functional properties. It is important to look at the degree of maturity of the cheese base because it greatly determines the microstructure, rheology and textural properties of the final product. The use of young cheese results in stable emulsions with high water-binding capacity. The final product obtained with the processing of young cheese hardens easily during storage and has good slicing properties with a firm body. To study the impact of the cheese maturity on the final properties of the processed cheese, Weiserová et al. (2011) used Edam cheese block of 8 weeks of maturity (50% w/w of dry matter, 30% w/w of fat, water and 3% of selected emulsifying salts) to make processed cheese spreads. Gempala and Brennan (2008) on the other hand, used Cheddar cheese (24.8% protein, 36.7% fat, 1.3% carbohydrate) with skim milk powder, butter, salt, trisodium citrate, selected starches and water.

The addition of skimmed milk powder or lactose into the blend, normally created processed cheese products that are susceptible towards enzymatic browning during storage. The addition of skimmed milk powder is better than lactose since skimmed milk powder enhance the stability of the processed cheese whereas lactose normally leads to lower spreadability and lower water activity. Excess of lactose may also produce mixed crystals during storage (Tamime, 2008).
Table 2.2 List of optional ingredients permitted in pasteurized processed cheese products and their main functions. *Source: Guinee (2011b).* (Reproduced with permission from Guinee, 2011b © John Wiley and Sons).

<table>
<thead>
<tr>
<th>Ingredient Type</th>
<th>Main function/effect</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy ingredients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk fat</td>
<td>- Standardization of composition. - Contributes to flavour, texture and cooking characteristics.</td>
<td>Cream, anhydrous milk fat, dehydrated cream, butter.</td>
</tr>
<tr>
<td>Milk proteins</td>
<td>- Standardization of composition. - Assist in ‘creaming’ (thickening of blend during manufacture) and formation of product.</td>
<td>Casein, caseinates, whey proteins, milk proteins concentrates, skim milk powder.</td>
</tr>
<tr>
<td>Lactose</td>
<td>- Low-cost filler; may affect texture.</td>
<td>Whey powder, skim milk powder.</td>
</tr>
<tr>
<td>Cheese base</td>
<td>- Substitute for young cheese. - Similar in behaviour to milk proteins, it contributes to thickening during manufacture, texture and cooking properties.</td>
<td>Typically, high dry-matter milk solids (≈60%, w/w) prepared by evaporation of milk ultrafiltrates to which starter culture and rennet have been added.</td>
</tr>
<tr>
<td><strong>Stabilisers</strong></td>
<td>- Impart desired texture and cooking characteristics</td>
<td>Emulsifying salts. Hydrocolloids.</td>
</tr>
<tr>
<td><strong>Acidifying agents</strong></td>
<td>- Assist control of the pH of final product</td>
<td>Food grade organic acids.</td>
</tr>
<tr>
<td><strong>Flavourings</strong></td>
<td>- Impart flavour to processed cheese foods and spreads, especially where much young cheese, cheese base, or milk proteins are used</td>
<td>Enzyme-modified cheese, starter distillate, wood smoke extracts, spices.</td>
</tr>
<tr>
<td><strong>Flavour enhancers</strong></td>
<td>- Accentuate flavour</td>
<td>NaCl, yeast extract</td>
</tr>
<tr>
<td><strong>Condiments</strong></td>
<td>- Affect appearance, flavour and texture and product differentiation</td>
<td>Sterile preparations of meat, fish, vegetables, nuts and/or fruits</td>
</tr>
<tr>
<td><strong>Sweetening agents</strong></td>
<td>- Increase sweetness, especially in products targeted to young children</td>
<td>Sucrose, dextrose, corn syrup, hydrolysed lactose</td>
</tr>
<tr>
<td><strong>Colours</strong></td>
<td>- Impart desired colour</td>
<td>Annato, paprika, artificial colours</td>
</tr>
<tr>
<td><strong>Preservatives</strong></td>
<td>- Retard mould growth; prolong shelf life</td>
<td>Nisin, potassium sorbate, Ca- or Na- propionate</td>
</tr>
</tbody>
</table>
Guinee et al. (2004) stated that, softer and more spreadable products can be produced with the addition of 3-5% of skim milk powder to the processed cheese blends. However, higher level of skim milk powder addition (7-10%) can lead to textural defects such as crumbliness, decrease in cohesiveness and may remain undissolved. If the skim milk powder is first reconstituted and precipitated by proteolytic enzymes or citric acid, then the curd is added into the blend, and high level of skim milk powder can be used to produce good processed cheese products (Guinee et al., 2004).

2.2.6 Processed cheese manufacture

![Diagram of processed cheese manufacture](image)

**Figure 2.2** Schematic diagram of the processed cheese product manufacture. Source: Guinee (2011b). (Reproduced with permission from Guinee, 2011b © John Wiley and Sons).

Various steps are involved in processed cheese manufacture. These include (i) formulation with selection and calculation of levels of raw materials, (ii) blending including cleaning, cutting and mincing of cheese and mixing with the other ingredients, (iii) processing corresponding to the heat treatment under continuous stirring and the creaming phase, homogenization, and (iv) packaging (hot fill and portioning) and storage. Figure 2.2
summarises the main steps involved in processed cheese manufacture and the following paragraphs present in detail the different steps of the manufacture.

Similar to traditional cheese manufacture, in processed cheese manufacture, the most important issue to be taken into account is the yield of the product obtained (Tunick, 2014). For example, by using 100 grams of milk, approximately 10 grams of Cheddar cheese can be produced. The cheese industry will make precise and accurate calculations on the amount of ingredients added to gain more profit and avoid losses, and the procedures are commonly adjusted to cater these needs (Tunick, 2014).

2.2.6.1 Selection and calculation of raw materials

The selection process and formulation are very important for a good final process cheese quality. Two factors must be considered in selecting the cheese for the blend. The first factor is the characteristics of the natural cheese chosen, such as the type, age, maturity, pH, fat, total solids and the physical properties. As cheese is the major ingredient in all processed cheese (>51 g/100g of dry matter, DM), its characteristics have major influence on the properties of the final product (Guinee, 2011b). The second factor is the desired properties for the processed cheese, for example, in terms of firmness and spreadability (Gouda & El-Nour, 2003). According to Kapoor and Metzger (2008), different ingredients influence the physicochemical properties, flavour and the functional properties of processed cheese in various ways. In order to ensure that the processed cheese products conforms to the legal standards of the specific type of processed cheese, the amount of cheese and other ingredients in the blend are calculated according to their fat and dry matter contents (Gouda & El-Nour, 2003). The two important parameters which play a key role in the production of high-quality processed cheese; the chemical composition of the cheese (concentrations of casein, peptide (proteolysis), fat, calcium and pH) and the nature of the emulsifying salts will be discussed in Section 2.4.

2.2.6.2 Blending: Cleaning, cutting, mincing and mixing

After the selection and calculation of raw materials needed, the mechanically or manually removing the surface defects of cheeses is done before the size reduction of cheese. The latter step consists of cutting cheese into small slices as a preparation for the mincing machines.
The cheese slices are then shredded into fine pieces and grounded using roller mills until they were soft and smooth. This process maximizes the surface area of the cheese, facilitates heat transfer to the blend and enhances emulsifying capability, water absorption and dispersion of the cheese protein during the process (Gouda & El-Nour, 2003). All the others ingredients are blended with the cheese in blenders/cookers or in pilot plants equipment.

2.2.6.3 Processing conditions

After the blending step, the pasteurization or sterilization and the emulsification of the mix are completed using processed cheese plants equipped with agitator. The primary functions of this process are to destroy any potential pathogenic microorganisms and to promote the formation of a stable emulsion with a uniform appearance and texture (Guinee, 2011a).

Formation of emulsion and properties of processed cheese can be controlled by its processing conditions such as cook time and temperature, amount of mixing and cooling rate (Kapoor & Metzger, 2008). Different types of processed cheese can be produced with different processing time and temperature. For example, using traditional kettle, block-processed cheeses require 8 to 15 minutes at 85-95°C while processed cheese spreads need 8 to 10 minutes at 80–85°C. The time-length for cheese processing is related to the speed of agitation. As Stephan kettles are equipped with very fast agitators, a period of 4-5 minutes is adequate to obtain processed cheese spread (Gouda & El-Nour, 2003).

2.2.6.3.1 Processing temperature

The minimum cook temperature and time for processed cheese is 65.5°C for 30s (Kapoor & Metzger, 2008). Nowadays, processed cheese manufacturers use two different types of cookers; batch and continuous, with different designs and operating conditions (pasteurization or sterilisation system) with direct steam injection. The indirect heating of the cheese blend has disappeared from the industry nowadays. The cooking temperature used by these manufacturers ranges from 70°C to 140°C depending on the type of processed cheese manufactured. According to Tamime (2011), increasing the processing temperature from 70 to 95°C for a processing time of 4 minutes for a given block processed cheese formulation (fat ~24.5g 100 g⁻¹, protein ~20 g 100 g⁻¹, moisture ~49%, pH ~5.75), increased the firmness and the elastic modulus \( G' \) at 25°C (at ~6 Pa per 1°C) and reduced the flow and fluidity of the
melted processed cheese (indicated by a linear decrease of about -0.037 units per 1°C in the maximum loss tangent at temperatures above 50°C). On the other hand, increasing temperature from 90°C to 140°C, as used in ultra-high temperature treatment (UHT) of long-life processed cheeses markedly reduces the viscosity (Lee, Buwalda, Euston, Foegeding, & McKenna, 2003).

### 2.2.6.3.2 Mixing speed

Batch cooking systems can be divided into low shear and high shear equipment. The type of mechanical action can have a critical impact on the final product texture and mouth-feel (Dixon, 2011). Purna, Pollard, & Metzger (2006) manufactured pasteurized processed cheese food (44% moisture and 25% fat) at 85°C for 6 min at 2 mixing speeds (450 and 1050 rpm). They found that, viscosity and firmness of processed cheese samples increased with the increased of mixing speed.

### 2.2.6.3.3 Homogenization

This step is optional in processed cheese manufacturing. Homogenization of cheesemilk is usually done for processed cheese spreads with high fat content and is done directly after processing (Gouda & El-Nour, 2003). Smaller fat globules with altered interfaces and more stable emulsion resistant to the creaming phase are the positive results of the homogenization on the final cheese macrostructure. Lee et al. (2003) studied the viscosity changes in processed cheese during cooking. Figure 2.3 demonstrated the creaming model proposed by (Lee et al., 2003).

Based on this creaming model, once the processed cheese mixture started cooking (Time A) with the presence of emulsifying salts (melting salt) and under constant stirring (homogenization), the individual casein particles started to disintegrate. Detailed functions of emulsifying salts during processed cheese manufacture will be discussed further in Section 2.4 of this chapter. As cooking progressed to Time B, the disintegrated casein started to interact with each other via casein-casein interaction forming a protein gel network structure which is ideal for processed cheese spreads.
Prolonged cooking up to Time C causes extensive protein re-association which eventually breaks the protein gel network structure and produce a large lump of compacted proteins. This phenomenon is known as overcreaming which increases the viscosity of processed cheese (e.g. block processed cheese) (Lee et al., 2003).

2.2.6.3.4 Packaging, cooling and storage

After the cooking and creaming phase, the hot molten blend must be rapidly cooled and stored at 4°C to prolong shelf-life. However, during this step, interactions between proteins and the surface of emulsified fat globules may occur (Marchesseau, Gastaldi, Lagaude, & Cuq, 1997). Some fat crystallisation and aggregation of calcium-phosphate complex could also contribute to the structure of the process cheese (Guinee, 2011b). Rayan, Ernstrom, and Kalab (1980), Caric, Gantar, and Kalab(1985) and Savello et al. (1989) have demonstrated the positive correlation between the degree of emulsification and the firmness/elasticity of the final product, and the inverse relation between the degree of emulsification and flowability of process cheese. Indeed, spreadable type of cheese has to be cooled as soon as possible (30-60 min) compared to block processed cheese that needed slower cooling rates (10-15h).

After the heat treatment, the hot-flowing mass of processed cheese is transported to the filling machines by using stainless-steel buckets or special pumps so that the processed cheese can be sealed easily (Gouda & El-Nour, 2003). According to Tamime (2011), primary and
secondary packaging should be impermeable and able to protect the product against moisture loss (dehydration), fat oxidation, mechanical damage during storage, product contamination and off-flavours absorption from the environment. Processed cheese must be stored below 20ºC but above 0ºC so that freezing of the product could be avoided (Gouda & El-Nour, 2003).

### 2.2.7 Cheese analogues

Cheese substitutes or imitations can be classified as analogue cheese products. Cheese analogues or known as model processed cheese can include partly or wholly substitute or imitate cheese and in which milk fat, milk protein or both are partially or wholly replaced by non-milk based components, usually of vegetable origins. According to Guinee et al. (2004) blending various edible oils/fats, proteins, other ingredients and water into a smooth homogenous blend with the aid of heat, mechanical shear and emulsifying salts can produce analogue cheese products commonly known as cheese-like products. Bachmann (2001) stated that, in order to manufacture cheese substitutes, there are two basic types of processes involved. Figure 2.4 shows the two different types of cheese imitations.

The first type is filled cheese where a liquid (milk) is used and involves conventional cheesemaking methods. The second basic type is by blending various ingredients using similar techniques as those used in processed cheese manufacture. This type is referred to as cheese analogues. The majority of cheese imitations are produced using the blending process. However, there are some disadvantages of the filled cheese process. Handling fairly large volumes of low solids stream is one of the filled cheese process disadvantages. Cheese analogue can be regarded as an engineered product since the production of this kind of cheese involves the use of fat and/or protein sources other than those native to milk with a suitable processing regime together with a flavour system that is developed to be almost similar to the natural product (Bachmann, 2001).

There are several advantages in making cheese analogue. First, cheaper raw materials such as casein and maltodextrin are used instead of normal milk solids. Second, ease of manufacture and less equipment involved such as blender and processing kettle. Finally, producing cheese analogue needs fewer manpower requirements (Shah, Jana, Aparnathi, & Prajapati, 2010). Moreover, normal processed cheeses consist of a mixture of natural cheeses of different types.
and degrees of maturation which is a difficult parameter to control. Processed cheese analogues on the other hand, are versatile dairy systems that do not incorporate natural cheese as an ingredient.

**CHEESE SUBSTITUTE TYPES**

**FILLED CHEESES**
- Skimmed milk, butterfat, vegetable oil

**CHEESE ANALOGUES**
- (a) Synthetic e.g. soya protein, artificial flavour
- (b) Partial dairy e.g. casein/ates, soya oil, artificial flavour
- (c) Dairy e.g. casein/ates, butter oil,

Conventional cheese manufacturing methods (matured products)

Processed cheese manufacturing methods (non-matured products)

**Figure 2.4** Different types of cheese substitutes. *Source: Bachmann (2001).* (Reproduced with permission from Bachmann, 2001 © International Dairy Journal).

The texture of processed cheese analogues can be easily manipulated to suit specific consumer needs. Satisfactory model system for research can be achieved by using processed cheese analogues since analogues allow for better compositional uniformity over time and over different batches (Pereira, Bennett, Hemar, & Campanella, 2001). For example, caseinate plus various fats by a single extruder pass can produce gelled and emulsified cheese analogues, with textures ranging from soft spreads to hard blocks. Processing parameters and composition of the cheese analogues may influence the melting ability and the extents of fat emulsification and casein “reassociation” (Cheftel, Kitagawa, & Quéguiner, 1992). The similarities between processed cheese products and analogue processed cheeses are the use of many similar ingredients (e.g. emulsifying salt, stabilisers, non-cheese dairy ingredients such as colours, flavours, flavour enhancers, etc), the absence of a ripening period, similar manufacturing technology (application of heat and shear to the formulated blend, hot filling, packing and cooling) and variety of textures, flavours, cooking properties and packaging formats (Guinee et al., 2004).
Lee et al. (2004) produced model processed cheese spreads using different ingredients involving rennet casein, trisodium citrate, citric acid, sodium chloride, sunflower oil and water. Another group of researchers, Trivedi et al. (2008) used a combination of rennet casein, soy oil, lactose, trisodium citrate, sodium chloride and citric acid with the addition of six different starches to create specific model processed cheese. Research on processed cheese spreads also was done by Cunha and Viotto (2010). The ingredients to produce model processed cheese in their research are calcium caseinate, butter oil, four different types of emulsifying salts (sodium citrate, sodium hexametaphosphate, sodium tripolyphosphate and tetrasodium pyrophosphate) and technological additives such as food grade sodium chloride and lactic acid.

2.2.8 Influence of pH on processed cheese

In processed cheese manufacture, pH is one of the key factors that may influence the physical properties of the processed cheese (Lu et al., 2008). Marchesseau et al. (1997) studied in detail on the influence of pH variations (pH 5.2 to 6.7) on the protein interactions and the microstructure of processed cheese. According to their study, the microstructure of processed cheese made at lower pH (pH 5.2) displayed big protein aggregates (3 to 10 μm) with small fat globules (~1 μm). At pH 5.2, high levels of protein interactions occur due to charge-charge interactions of the proteins at the pH value near the isoelectric point of casein (pH 4.6) forming large protein aggregates. As the pH of the processed cheese increased from 5.7 to 6.0, the caseins were more negatively charged allowing various types of interactions to occur (e.g. protein-protein, protein-fat and protein-water interactions). Processed cheese made at these pH (5.7 to 6.0) demonstrated three-dimensional protein network with homogenously distributed fat globules. On the other hand, small condensed aggregates (~0.5 to 1 μm) which disrupt the protein matrix were observed for processed cheese made at high pH (pH 6.7). At pH 6.7, the protein-protein and protein-fat interactions in the processed cheese system weakens due to the greater water adsorption of the proteins. This results in processed cheese with less compact microstructure (Marchesseau et al., 1997).
2.3 Milk

The main ingredient in cheese processing is milk. In processed cheese or analogue cheese, the addition of milk components is either in powder form or liquid form or any other forms of milk derivatives such as casein or caseinates. Milk is composed of carbohydrates, water, proteins, lipids, salts, minerals and other minor components (O’Mahony & Fox, 2014). The main focus in this discussion is milk protein because it plays an important role in determining cheese quality. Casein is the most abundant fraction in milk protein which accounts around 80% of the milk protein. Another 20% of the milk protein consists of whey protein (Miller, Jarvis, & McBean, 2006).

2.3.1 Casein

Through electrophoresis, casein can be divided into 4 major groups which are alpha- (\(\alpha\)), beta- (\(\beta\)), gamma (\(\gamma\)) and kappa- casein (\(\kappa\)). The isoelectric pH of casein is 4.6 (Miller et al., 2006). There are three groups of \(\alpha\)-casein in milk which includes \(\alpha_s1\), \(\alpha_s2\) and \(\alpha_s3\). Only \(\alpha_s1\) and \(\alpha_s2\) are present in the casein micelles of the milk. Phosphorylated serine or threonine residues are present in all the casein. Due to the presence of negative charges in these proteins, calcium ions are easily attached (Dalgleish, 2014). The post-secretory hydrolysis of \(\beta\)-casein results in the formation of \(\gamma\)-casein. The amount of \(\gamma\)-casein is very low compared to other caseins which is only 5% in good quality cow’s milk. The amount of \(\gamma\)-casein must not exceed 5% because large amounts of \(\gamma\)-casein can affect the proteolytic enzymes in milk which bring difficulties for food industries to process the milk into some products (Early, 1998). Another type of casein which is \(\kappa\)-casein has only one serine phosphate ester group and is not affected by calcium addition (Vaclavik & Christian, 2007).

2.3.2 Casein micelles

In milk, many thousands of individual casein molecules form a dynamic macromolecule which is called “casein micelle”. These casein micelles co-exist with colloidal particles known as calcium colloidal phosphate (CCP) that could link the acid and phosphorylated milk proteins in the form of aggregates (De Kruif, Huppertz, Urban, & Petukhov, 2012). The average diameter size of casein micelles is around 200 nm (Dalgleish, 2014). The casein
micelle structure has become a debate among researchers for the past decades but the detailed structure of casein micelle remains a mystery (Anema, 2014; Dalgleish, 2014; De Kruif et al., 2012). Several models of casein micelles have been proposed. Figure 2.5 illustrates the different casein micelles models.

Based on light scattering and microscopy techniques, sub-micelle model was proposed (Figure 2.5a). In this model, aggregation between the casein proteins through hydrophobic bonds formed the submicelle which is then linked by CCP. The surface of the micelle is protected by $\alpha_s$- and $\kappa$-caseins (Walstra & Jenness, 1984; Horne, 2002; Phadungath, 2005). Some researchers disagreed with this model and came up with modified sub-micelle model (Figure 2.5b) where the CCP was uniformly distributed within the casein micelle (Walstra, 1999). Years later, the nanocluster model was developed (Figure 2.5c). In the nanocluster model, $\alpha_s$- and $\beta$-caseins surrounded the CCP nanoparticles and are randomly distributed within the casein micelle (De Kruif & Holt, 2003). The dual-binding model (Figure 2.5d) was proposed based on polymerization which enhanced the association of micellar structure through cross-linking of caseins hydrophobic regions across CCP nanoclusters (Horne, 1998).

Due to the lack of structural information of casein micelles in the dual-binding model, Dalgleish developed another model (Figure 2.5e) where $\beta$-casein is present in the internal pores of the cross-link CCP nanoclusters surrounded by hairy layers of $\kappa$-casein (Dalgleish, 2014). On the other hand, Bouchoux, Gésan-Guiziou, Pérez, and Cabane (2010) came up with a model where the casein micelle looks like a sponge with a triple hierarchical structure (Figure 2.5f). In this model, the casein micelle consists of hard regions (filled with calcium phosphate (Ca-P) nanoclusters) connecting together forming a porous material.
Figure 2.5 Illustrations of casein micelle model structures which have been developed by various researchers over the years. (a) Sub-micelle model, (b) Modified sub-micelle model, (c) Nanocluster model, (d) The dual-binding model, (e) Dalgleish model and (f) Sponge model. Source: Anema (2014) and Bouchoux et al. (2010). (Reproduced with permission from Bouchoux et al., 2010 © Biophysical Journal).
2.3.3 Caseinates

Caseinates, either sodium, potassium or calcium, are widely used in the food industry for many purposes such as emulsifier, stabiliser and water binder (Early, 1998). Figure 2.6 summarises the production of caseinates.

![Schematic diagram for caseinate production](image)

**Figure 2.6** Schematic diagram for caseinate production. *Source: Jabara et al. (2004).*

The addition of acid into milk causes the casein protein to precipitate at pH 4.6, producing acid casein. After the injection of acid, acid-milk mixture enters the coagulator where indirect heat is applied with minimal mixing to maximize casein precipitation. The casein curd is centrifuged twice to remove whey, lactose and minerals, and to reduce moisture to about 50%. According to Codex standards, the protein concentration in acid casein has to be ≥90%. Alkali is added into the insoluble acid casein, producing caseinates which is more soluble in water (Jabara et al., 2004). The use of sodium hydroxide results in sodium caseinate, while the use of calcium hydroxide results in calcium caseinate.

The idea of adding caseinates in cheese is due to the economic value because caseinates are less expensive compared to raw milk. Furthermore, the addition of caseinates offers benefits in cheese processing such as increased cheese yield, gel strength, consistency and meltability (Miralles, Krause, Ramos, & Amigo, 2006).
2.3.3.1 Sodium caseinate

Sodium caseinate is made by the reaction of the curd of acid casein with alkali solution, sodium hydroxide (NaOH) (Ranken, 2012). Sodium bicarbonate and sodium phosphate may also be used, but the reagents are more expensive in larger quantities (Varnam & Sutherland, 2012). The solubility of sodium caseinate is greater than potassium caseinate and calcium caseinate (Zayas, 2012). Many studies have been done using sodium caseinate in cheese making. Lobato-Calleros, Aguirre-Mandujano, Vernon-Carter and Sanchez-Garcia (2000) found that the addition of sodium caseinate in fresh cheese significantly influences the viscoelastic characteristics of the cheese. Lee and Klostermeyer (2001) also used sodium caseinate as part of the ingredients of a low-fat model processed cheese.

2.3.3.2 Calcium caseinate

Calcium caseinate which is high in protein (mainly caseins) and are very low in lactose content is produced by reacting the curd of acid casein with calcium hydroxide (Ca(OH)\(_2\)) followed by drying (Belyamani, Prochazka, Assezat, & Debeaufort, 2014; Burešová, Masaříková, Hřivna, Kulhanová, & Bureš, 2016; Ranken, 2012). Acid casein is finely ground using colloid mills to allow rapid dissolution. Dilute Ca(OH)\(_2\) solution is added into the casein slurry and passed into dissolving vats with indirect heating and continuous agitation. Compared to sodium caseinate, the reaction between acid casein and Ca(OH)\(_2\) solution in calcium caseinate production proceeds more slowly and highly temperature dependent which has to be closely monitored. The solution is then passed into the dryer (either spray dryer or roller dryer), producing calcium caseinate (Varnam & Sutherland, 2012). Calcium caseinate powders have low particle density, low bulk density and high tendency to dust (Early, 1998). Similar to sodium caseinate, calcium caseinate has also been widely used for processed cheese making. Calcium caseinate consists of water-soluble phosphate groups and non-polar fat soluble groups at each end of the protein structure. The addition of emulsifying salts during cheese making process, will chelate the calcium and increase the hydrosolubility of the caseinate thus improving its emulsifying properties (this will be discussed further in Section 2.4.6) (Bachmann, 2001). Due to calcium caseinate low solubility, model processed cheese made with calcium caseinate is poorly emulsified and show lower melting properties.
Chapter 2

compared to sodium caseinate. However, the use of sodium caseinate in processed cheese may cause irregular melting patterns which are undesirable for the final products (Hokes, Mangino, & Hansen, 1982).

Cavalier-Salou and Cheftel (1991) prepared imitation cheese using calcium caseinate or sodium caseinate as the protein source and found that analogue cheese produced with calcium caseinate has lower firmness and higher degree of casein dissociation compared to cheese prepared with sodium caseinate provided that sodium citrate (1%) and disodium hydrogen phosphate (2%) were used as the emulsifying salts. Nolan, Holsinger, and Shieh (1989), found that the addition of 1% calcium caseinate to fresh raw milk before pasteurization increased the viscosity of imitation Mozarella cheese. Pellegrino, Cattaneo, Masotti, & Psathas (2010) also had successfully prepared imitation Halloumi cheese using 5% calcium caseinate and combination of calcium caseinate and skim milk powder.

2.3.4 Whey proteins

Whey proteins account for 20% of the total protein in milk and consist of α-lactalbumin (α-lac), β-lactoglobulin (β-lac), serum albumin (SA), and immunoglobulins (Igs). Three of the whey proteins fractions which are α-lac, β-lac and Igs have the ability to function as immune system boosters and preventing diseases (Bowden, 2007). During cheese-making process, whey which is in liquid form is separated from the casein in the cheese matrix. In the old days, whey was considered as an unwanted by-product of cheese and was thrown away carelessly. However, nowadays things have changed and food industries found other usages of whey such as converting the whey into whey protein concentrate and whey protein isolate (Tunick, 2014). Whey proteins exhibit unique properties which are hydrophilic, heat sensitive (denatured at ~75°C) and less sensitive to calcium (Phadungath, 2005).

2.3.4.1 Whey protein concentrate (WPC)

Whey protein concentrate (WPC) is produced by removing fat from whey, filtering using microfiltration and drying into a powder form (Tunick, 2014). WPC contains at least 20 to 25% of protein and can have up to 89% by protein concentration by microfiltration. WPC are suitable for use in a variety of applications such as infant formula, yoghurt, coffee whiteners,
meat and dairy desserts. The ability of WPC to form stable foams, binding water, adding nutritive value to foods, prolonging shelf life and exhibiting gelling properties has attracted interests from the food industries (Miller et al., 2006; Tunick, 2014).

### 2.3.4.2  Whey protein isolate (WPI)

Whey protein isolate (WPI) which can be manufactured using similar procedure as WPC contains a minimum of 90% of protein and a very minimal amount of fat, lactose and minerals. WPI is less commonly used in the food industries compared to WPC. WPI also provide functional characteristics as WPC and is usually used for low pH nutritional beverages, clear beverages, protein fortified foods and sports nutrition products. Heating WPI in the presence of acid or proteolytic enzymes can produce whey protein hydrolysates (Miller et al., 2006).

### 2.4  Emulsifying salts

Emulsifying salts are of major importance in processed cheese production. Inhomogenities of processed cheese mass will occur without the existence of emulsifying salt during processing (Buňka et al., 2014). Their essential role is to supplement the emulsifying capability of cheese proteins. This is accomplished by removing calcium from the protein system, and peptizing, hydrating, swelling, solubilizing, and dispersing the protein. It also help (through the proteins) to emulsify fat and stabilize the emulsion, controls pH and contribute in forming an appropriate structure after heat treatment and upon cooling (Chen & Liu, 2012).

It is known that some emulsifying salts have better emulsifying properties than others. Emulsifying salts consisting of monovalent cation and polyvalent anion possess the best emulsifying characteristics. Emulsifying salts mostly used in processed cheese manufacture can be classified into three types namely monophosphates, polyphosphates, and citrates. It is necessary to combine two or more salts into mixtures to achieve optimal emulsifying and melting characteristics as well as to produce a homogeneous and stable processed cheese (Awad, Abdel-Hamid, El-Shabrawy, & Singh, 2004). Emulsifying salts have the ability to influence Ca$^{2+}$ binding, pH adjustment, casein dispersion, fat emulsification and structure formation of the processed cheese (Tamime, 2011). Different types of emulsifying salts
promote different degree of the ability for emulsification. Guinee et al. (2004) stated that electron microscopy and oiling-off studies can determine the effectiveness of emulsifying ability of different type of emulsifying salts. Buňka et al. (2014) found that the variations of emulsifying salts concentration affect the adhesiveness, cohesiveness and hardness of processed cheese spreads. Most of the emulsifying salts which are important to the processed cheese technology will be discussed further in the following paragraphs.

2.4.1 Trisodium citrate (TSC)

Trisodium citrate (TSC) also known as sodium citrate is the salt of citric acid (Tamime, 2011). It has a molecular weight of 294.10 g/mol (dehydrate) with melting a point >300ºC. The chemical structure of trisodium citrate is shown in Figure 2.7.

![Figure 2.7 Chemical structure of trisodium citrate.](image)

Trisodium citrate is commonly used in food products as acidity regulators in food formulations, emulsifiers in processed cheese and a cure accelerator in meat products (Saltmarsh & Barlow, 2013). A few researches have been done to study the effect of trisodium citrate on processed cheese physical properties. A study done by Purna et al. (2006), showed that variations in trisodium citrate concentrations may influenced the processed cheese melt and flow characteristics.

Swenson, Wendorff, & Lindsay (2000), studied the impact of ingredients on fat-free processed cheese. According to their research, processed cheese with added trisodium citrate demonstrated properties similar to full-fat cheese with the highest meltability. On the other hand, a study done by Gupta and Reuter (1992) demonstrated that processed cheese manufactured with trisodium citrate was smoother compared to other processed cheeses. With 2.5% of trisodium citrate concentration (45.2% moisture content), the processed cheese gave a very good sensory characteristics.
2.4.2 Sodium phosphate (SP)

Sodium phosphate (SP) is also known as sodium orthophosphate or trisodium phosphate and the chemical structure is shown in Figure 2.8.

![Figure 2.8 Chemical structure of sodium phosphate.](image)

This compound has a molecular weight of 163.94 g/mol with a melting point of 1583°C. Sodium phosphate is widely used as food additives in frozen custards, fruit jellies and ice cream (Maga & Tu, 1994). Koide, Yoneda, and Musashi (1983) patented a process to produce cream cheese-like food using orthophosphate as part of the ingredients. About 1.4 to 28.0 mg (per gram of casein) of orthophosphate was used to achieve smooth texture, glossy surface and stable pH during the fermentation process. Sodium phosphate was also used as emulsifier in processed cheese. According to a study done by Chen and Liu (2012), processed cheese manufactured with orthophosphate has larger particle size compared to processed cheese manufactured with trisodium citrate. Besides that, the colour of the processed cheese prepared with orthophosphate was more yellowish compared to processed cheese made with other emulsifying salts.

2.4.3 Sodium pyrophosphate (TSPP)

Other names for sodium pyrophosphate are tetrasodium diphosphate or tetrasodium pyrophosphate (TSPP). The chemical structure of sodium pyrophosphate is shown in Figure 2.9. The molecular weight of this compound is 446.06 g/mol with a melting point of 79.5°C in decahydrate form and 988°C in anhydrous form. Sodium pyrophosphate functions as a thickener, emulsifier, stabilizer and food additive in various food products. Lee, Hendricks,
and Cornforth (1998) used sodium pyrophosphate to study the physico-chemical properties of re-structured beef.

![Chemical structure of sodium pyrophosphate decahydrate.](image)

**Figure 2.9** Chemical structure of sodium pyrophosphate decahydrate.

Dybing and Smith (1998) studied the used of sodium pyrophosphate to coagulate whey proteins at different pH and temperature. Such coagula can be used to produce cheese. According to Mizuno and Lucey (2005b), the addition of sodium pyrophosphate in non-fat pasta filata cheese decreased the soluble protein content, stretchability and meltability of the cheese. Ozcan, Lucey, and Horne (2008) studied the effect of different concentrations (0.05 to 0.2%) of sodium pyrophosphate on yogurt gels. The results of their study showed that 0.1% TSPP concentration can decrease whey separation in yogurt by 35%. Increasing TSPP concentration in yogurt may increase the size of the clusters and pores in yogurt gels.

### 2.4.4 Sodium tripolyphosphate (STPP)

Sodium tripolyphosphate (STPP) is also known as sodium triphosphate and the chemical structure is shown in Figure 2.10. This compound has a molecular weight of 367.86g/mol with a melting point of 622ºC. Other than food applications, STPP is also widely used in detergents, ceramics, leather tannings and rubber manufacture.

![Chemical structure of sodium tripolyphosphate.](image)

**Figure 2.10** Chemical structure of sodium tripolyphosphate.
In food products, research has been done by adding STPP in trevally surimi to improve the gelling properties (Arfat & Benjakul, 2013). STPP also was reported to inhibit the growth of \textit{Streptococcus lactis} and \textit{Streptococcus cremoris} in milk (Kadis & Babel, 1962) which is interesting for cheese studies. There are few studies of the addition of STPP in processed cheese. Some researches combine the use of STPP in processed cheese with other phosphate salts such as orthophosphate, diphosphate and polyphosphate (Sádlíková et al., 2010). Recent study done by Li, Xia, Zhou, and Xie (2013) uses STPP as part of ingredients in soy cheese spreads. Chen and Liu (2012) studied on the physico-chemical properties of Mozarella based processed cheese. They reported that processed cheese made with STPP is softer compared to processed cheese made with TSPP.

2.4.5 **Sodium hexametaphosphate (SHMP)**

Sodium hexametaphosphate (SHMP) has various other names such as Graham’s salt, hexasodium metaphosphate, sodium polyphosphate, glassy sodium, Calgon S, hexasodium salt and metaphosphoric acid. The molecular weight of this compound is 611.77 g/mol with a melting point of 628ºC and the chemical structure is shown in Figure 2.11.

![Figure 2.11 Chemical structure of sodium hexametaphosphate.](image)

Sodium hexametaphosphate play an important role as emulsifier and sequestrant in processed cheese. According to Dybing, Parsons, Martin, and Spurgeon (1982), increasing sodium hexametaphosphate concentrations in milk can produce higher Cottage cheese yield. Buňka et al. (2014) reported that, higher concentrations of sodium hexametaphosphate produce harder and more cohesive processed cheese. Nagyová et al. (2014) used different chain length of
sodium polyphosphate to study the textural properties of processed cheese which has been stored in the fridge for 2, 9 and 30 days. According to their study, processed cheese became firmer with lower amount of polyphosphate. Sodium hexametaphosphate also was commonly used as blends with other emulsifying salts in processed cheese manufacture (Kaliappan & Lucey, 2011; Sádlíková et al., 2010).

### 2.4.6 Mechanisms of emulsifying salts during processed cheese manufacture

The presence of emulsifying salts during processed cheese manufacture can influence calcium chelation, casein dispersion, creaming, fat emulsification and gel formation (Lucey et al., 2011). These reactions which occur during processed cheese manufacture are shown in Figure 2.12.

![Figure 2.12](image.png)

**Figure 2.12** Schematic diagram of possible reactions during the production of processed cheese; ES: emulsifying salts; Temp.: temperature. *Source: Lucey, Maurer-Rothman and Kaliappan (2011).* (Reproduced with permission from Lucey et al., 2011 © John Wiley and Sons).

Emulsifying salts possess the ability to bind with metal ions and induce the formation of soluble complexes. The metal-binding ability of the emulsifying salts depends on its chain length. For example, monophosphates such as sodium phosphate demonstrated weaker
binding capability compared to polyphosphates (e.g. sodium hexametaphosphate) (Sádlíková et al., 2010). During the heating process of processed cheese manufacture (with the aid of shearing), the addition of emulsifying salts induces calcium (Ca\(^{2+}\)) chelation also known as calcium binding or calcium sequestration. The calcium binding equation is shown in equation 2.1.

\[
Ca^{2+} + \text{emulsifying salts} \leftrightarrow Ca^{2+} - \text{emulsifying salts complex} \\
\leftrightarrow Ca^{2+} - \text{emulsifying salts precipitate}
\]  

(2.1)

In this equation (2.1), the complex formed is soluble and the precipitate formed is insoluble. The efficacy of calcium binding depends on type, valency, temperature and ionic strength of the emulsifying salt. Orthophosphates ionise easily at any concentrations. Ionisation of polyphosphates on the other hand, decreases with longer chain length (Lucey et al., 2011). Emulsifying salt containing a monovalent cation and a polyvalent anion are best to complete ion exchange. The effectiveness normally increases with the valency of the anion. In shorter chain phosphates, the sequestering ability is strongly influenced by pH. At higher pH values, more complete dissociation of sodium phosphate molecules occur which contributes to a higher valency anion thus, increases the ion-exchange function (Taylor, 1996).

Emulsifying salts removes the cross-links of calcium phosphate (Ca-P) to reveal charged phosphoserine residues by chelating the Ca\(^{2+}\) from the casein-bound Ca-P. This action enhances the casein particles charge repulsion, increases the pH and the solubility of the mixture which later contributes to casein dispersion (Cunha & Viotto, 2010). The dispersed protein then acts as an active emulsifier which promotes fat emulsification and assists in the formation of a homogenous product. Increasing shear rates during processed cheese manufacture can enhance fat emulsification and also improve the viscosity of the final product (Lucey et al., 2011).

Creaming stage may occur due to the protein-protein interactions during cooking of processed cheese. These interactions occur via non-polar groups which have been exposed during casein dispersion and help in the formation of firm processed cheese. Once processed cheese is cooked, cooling stage took place. During the cooling stage, casein association occur via hydrogen bonding. Processed cheese cooled at slower rate tends to be firmer compared to processed cheese cooled at faster rate. This phenomenon occur because cooling at slower rate
will give casein more time to rearrange themselves and associate with each other. Formation of stiffer gels also occurs due to the increase of the rate of firming caused by calcium bridging between micelles (Sandra, Ho, Alexander, & Corredig, 2012). Schematic illustration of processed cheese after the cooling process and after melting is shown in Figure 2.13.

Figure 2.13 Schematic illustration of processed cheese after cooling (A) and processed cheese after melting (B). Source: Lucey, Johnson, and Horne (2003). (Reproduced with permission from Lucey et al., 2003 © Journal of Dairy Science).

Addition of calcium complexing agents with controlled pH, can improve heat stability while the addition of soluble calcium salts decreases the heat stability of milk (Taylor, 2007). Guinee et al. (2004) indicated that, the addition of orthophosphates and citrates created soft processed cheeses which undergo a slight oiling-off or ‘sweating’ during heating. However, the addition of these phosphates produces processed cheese with desirable melting properties (e.g. good flowability, moistness and surface sheen). Condensed phosphates such as STPP and SHMP on the other hand produce harder processed cheeses with very little or no oiling-off on heating and have low melting properties (little or no flowability, skin formation and crusting, dull and dry surface appearance). Table 2.3 shows the properties of emulsifying salts that are commonly used in pasteurized cheese products and their properties during cheese processing.
Table 2.3 Emulsifying salts and their properties during cheese processing. *Source: Guinee et al. (2004) and Nollet & Sinha (2007).*

<table>
<thead>
<tr>
<th>Group</th>
<th>Commonly used form</th>
<th>Calcium sequestration</th>
<th>Buffering action</th>
<th>Paracasein hydration</th>
<th>Fat emulsification</th>
<th>Bacteriostatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrates</td>
<td>TSC</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Nil</td>
</tr>
<tr>
<td>Orthophosphates</td>
<td>DSP or TSP</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Condensed phosphates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrophosphates</td>
<td>DSPP</td>
<td>Medium</td>
<td>Medium</td>
<td>Very high</td>
<td>Very high</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>TSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-phosphates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PSTP</td>
<td>High to very high</td>
<td>Low to very low</td>
<td>High to low</td>
<td>Very high to low</td>
<td>High to very high</td>
</tr>
<tr>
<td></td>
<td>STPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Processed cheese products and analogue processed cheeses with the addition of different emulsifying salts showed similar trend on calcium sequestration. The trend is as follows: sodium aluminium phosphate ≈ trisodium citrate (slightly) > disodium orthophosphate >> sodium tripolyphosphate ≈ tetrasodium pyrophosphate > higher chain sodium polyphosphate. Firmness showed the opposite effect. Based on this trend, better emulsification and structural-forming properties of condensed phosphates can be observed as they showed greater calcium sequestration and hydration effects (Guinee et al., 2004).
2.5 Emulsions

An emulsion is a system which contains two immiscible liquids (commonly oil and water) that dispersed with one another (Dalgleish, 2003; McClements & Demetriades, 1998). In dairy systems such as milk and cream, oil-in-water emulsion holds the basic concept of their consistency where the oil droplets are dispersed in the aqueous phase. On the other hand, margarine and butter are water-in-oil emulsion where the water droplets are dispersed in the oil phase (McClements, 2004).

Processed cheese is also an oil-in-water emulsions (gel state) where fat (oil) is dispersed in a continuous phase (protein/water mixture) (Gliguem, Lopez, Michon, Lesieur, & Ollivon, 2011). However, mixing both oil and water phases together results in a rapid phase separation without the intervention of homogenization and emulsification (Abd El-Rahman, Madkor, Ibrahim, & Kilara, 1997; Dickinson, 1997; Goff, 1997). Therefore, the fat and water phases require substances such as emulsifier and stabiliser in order to bind them together and stabilise the emulsion formed. This is where the role of emulsifying salts takes place. As discussed in Section 2.4 of this chapter, emulsifying salts play a vital role in maintaining a smooth and homogenous processed cheese product at the end of the manufacture (Černíková et al., 2010; Dimitreli et al., 2005). The effect of emulsifying salts is to disperse the proteins well in order to allow them to adsorb to the oil-water interface. It is the proteins which are the stabiliser and not the emulsifying salts.

An emulsion can be categorized based on its stability over time. Most emulsions will likely to experience instability for example phase inversion, creaming, sedimentation, flocculation and coalescence (Dalgleish, 2003; McClements, 2004). Phase inversion occurs when the oil in water emulsion change its physical behavior to become water in oil emulsion or vice versa. Creaming occurs when less dense emulsion droplets tend to clog together on the top layer of the food emulsions (Mueth, Crocker, Esipov, & Grier, 1996; Robins, 2000). Sedimentation on the other hand, occurs due to higher density of the droplets grouped together and settling at the bottom of the food emulsion (McClements & Demetriades, 1998). Both creaming and sedimentation are due to gravitational separation. Droplet aggregation is another type of emulsion instability which involves flocculation and coalescence (Robins, 2000). Aggregation of emulsion droplets within the food emulsion is known as flocculation while
coalescence occurs when emulsion droplets come together forming a larger single droplet (Kumar, Narsimhan, & Ramkrishna, 1996).

The central part of a typical food emulsion (droplet) is surrounded by an interfacial layer called the interface or interfacial region and the surrounding liquid is known as the continuous phase. The central part of the droplets usually attracts non-polar molecules. Polar molecules tend to be positioned in the aqueous phase while the amphiphilic molecules are located at the interface. The interfacial region plays a vital role in determining the physico-chemical properties of food emulsions. Therefore, many studies have been done to determine the interfacial properties (structure, thickness, composition, rheology and charge of the interface) of food emulsions (Dickinson, 1999; Murray, 2002; Wilde, 2000; Ye & Singh, 2001).

2.5.1 Surface active agents

Surface active agents or surfactants are compounds which have both hydrophilic (water loving/polar) and lipophilic (fat loving/non-polar) molecules (amphiphilic compounds) which make them water soluble and oil soluble at the same time (Block, 2001; Pletnev, 2001). Surface active substances are also known as emulsifier agents because these substances may assist in oil-in-water or water-in-oil emulsions formation by dispersing two different immiscible liquids. Surfactants can be categorized into four groups which are anionics, cationics, non-ionics and amphoterics or zwitterionics (Block, 2001). In the food industry, non-ionics surfactants such as glycerides and esters are commonly used as food emulsifiers to obtain a stable final product (Dickinson, 2003).

Surfactants also have been widely used to understand the formation of emulsions which contributes to food structure stabilisation (Kralova & Sjöblom, 2009). However, there is certain level of surfactants which is permitted to be used in food industry to avoid toxicity effect on human body. Tangsuphoom and Coupland (2008) investigated the influence of several surface active agents on the stability of coconut milk emulsions. Their study proved that the addition of small-molecule surfactant before homogenization highly improved the stability of the coconut milk emulsions. Other examples of small-molecule surface active agents are sodium dodecyl sulfate (SDS), polysorbate 20 (Tween 20) and lecithin. There are
also macromolecular emulsifiers which are commonly used in food industry such as sodium caseinate, calcium caseinate and whey protein isolate (WPI).

2.5.1.1 Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate (SDS) is also known as sodium lauryl sulfate (SLS). SDS is an anionic surfactant with chemical formula $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$ and molecular weight of 288.38 g/mol. SDS can be derived from plant origin such as coconut and palm oils or animal origin (Ramanathan, 2005). As one of the most commonly used surfactant, SDS also contains hydrophobic (which is also lipophilic) region and hydrophilic region as shown in Figure 2.14.

![Figure 2.14](Image) Chemical structure of sodium dodecyl sulfate (SDS) showing hydrophobic and hydrophilic region. Source: Caligur (2008).

SDS has been used in cleaning, body cleansers, pharmaceuticals and skin products (Singer & Tjeerdema, 1993). In food industry, SDS also has been added as part of ingredients (food additive and emulsifier) (Burdock, 1997). Apart from that, SDS also has been widely used in protein analysis such as in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for biological and biomedical research (Schägger & von Jagow, 1987). In particle size evaluations, SDS has the ability to disrupt the group of droplets formed in an emulsion by displacing other particles from the emulsion droplets surface (Tangsuphoom & Coupland, 2008).

The incorporation of SDS in protein-stabilised emulsions can improve the stability of the final product (Kong, Beattie, & Hunter, 2003). Studies done by Kelley and McClements (2003) demonstrated that the addition of 0.01% SDS to emulsions stabilised by bovine serum albumin (BSA) prior to heating improved the thermal stability of the emulsions due to the
increased of the electrostatic repulsion between the oil droplets thus, preventing droplet flocculation.

2.5.1.2 Polysorbate 20 (Tween 20)

Polysorbate 20 (Tween 20) also known as polyoxyethylene sorbitan monolaurate is a non-ionic surfactant which has been widely used as detergent, emulsifiers, solubilisers and stabilisers in cosmetics products, pharmaceutical, food and other industries (Smolinske, 1992). Tween 20 has a molecular weight of 1227.54 g/mol and its chemical structure is shown in Figure 2.15.

![Chemical structure of polysorbate 20 (Tween 20).](image)

**Figure 2.15** Chemical structure of polysorbate 20 (Tween 20).

Polysorbate 20 is among the polysorbitans group which consist of various numbers up to polysorbate 85. In food industries, polysorbates 20, 60, 65 and 80 were commonly used as emulsifiers, dough conditioners, stabilizers and defoaming agents (Smolinske, 1992). Dickinson, Ritzoulis, and Povey (1999) studied the stability of emulsions stabilized by sodium caseinate and Tween 20. Their study provided important knowledge to the food industry because the relationship of mixed systems having anionic surfactants was associated to interfacial and surfactant-protein interactions. According to their research, combinations of sodium caseinate and Tween 20 at certain extent may disrupt the stability of the oil-in-water emulsions. There is a complicated relationship between the two surface-active agents and the emulsion stability. However, when adequate amount of Tween 20 was added, the creaming of the emulsion was greatly increased which suggests that Tween 20 may have displace the majority of the protein from the surface of the emulsion droplet (Dickinson et al., 1999).
2.5.1.3 Lecithin

Lecithin can be found in fatty substances of human, animal and plant tissues. The name “phosphatidylcholine” has been used interchangeably with lecithin. In 1846, a pharmacist named Théodore-Nicolas Gobley from France made a scientific research on hen’s egg yolk. He successfully isolated lecithin which is a specific phospholipid and years later, he established the complete chemical formula of phosphatidylcholine (Hensing, 2004). The chemical structure of lecithin is shown in Figure 2.16.

![Chemical structure of lecithin (phosphatidylcholine).](image)

Through his extensive research, he found the existence of lecithin in chicken, sheep and human brain. He also found similar viscous matter in fish roe, sea urchin, starfish and other biological fluid (Hensing, 2004). Nowadays, lecithin has been widely produced industrially by chemical extraction or mechanical extraction. For industrial production, sources of lecithin may come from soybeans, sunflower, rapeseed, eggs and milk.

The most commonly used lecithin is soybean lecithin which is a by-product from the soybean oil (Rydhag & Wilton, 1981). Soybean lecithin is popular because it is easily available with excellent emulsifying properties, taste and colour (Van Nieuwenhuyzen, 1976).

In food industry, lecithin is commonly used as emulsifiers, stabilisers and food additives (Erickson, 1990). Food emulsions may experience instability over time. The liquid film...
between individual droplets in the emulsions rapidly drains thus, the emulsion droplets coalesce with each other. The presence of lecithin may enhance the food emulsions stability by adsorbing at the droplet surfaces and providing a barrier to prevent coalescence (Kong et al., 2003).

Studies also have been done to investigate the function of lecithin on cheese (Dabour, Kheadr, Benhamou, Fliss, & LaPointe, 2006; Drake et al., 1999; Hicks, O’Leary, & Holbrook, 1985; Sipahioglu, Alvarez, & Solano-Lopez, 1999). These molecules increased the cheese yield up to 1.9% and received good remarks in sensory properties (Drake et al., 1999; Hicks et al., 1985).

2.6 Rheological Properties

In 1929, Professor Bingham from Lafayette College, Indiana introduced rheology as the study of the flow of a matter and deformation when a force is applied. Starting from the study of lubricants, rubber, asphalts, plastics and paints, the scope of rheological studies has now become wider and has been divided into different kinds of scientific disciplines (Barnes, Hutton, & Walters, 1989). In dairy product studies, cheese is categorized as a viscoelastic material.

The relationship of strain-stress for cheese demonstrates a pattern of solids and liquids. Therefore, in determining rheological properties of cheese, two types of moduli must be taken into accounts which are the elasticity and viscosity behaviours (Guinee, 2011a). The definition of elasticity and viscosity started way back in the 17th centuries based on Hooke’s and Newton’s discoveries. Based on Robert Hooke’s “True Theory of Elasticity”, he defined elasticity as “the power of any spring is in the same proportion with the tension”. According to Newton who published “Principia” in 1687, viscosity is defined as “the resistance which arises from the lack of slipperiness of the parts of the liquid, other things being equal, is proportional to the velocity with which the parts of the liquid are separated from one another”. This definition has been simplified as the resistance of a liquid to flow (Barnes et al., 1989). Rheology serves many applications such as a quality control assessment in food processing (Karoui & Dufour, 2006).
2.6.1 General principles of rheology

Oscillatory shear methods are normally used to determine the rheological properties of complex fluid such as emulsions, biological gels, melts and other viscoelastic materials. Oscillatory shear methods can be divided into large amplitude oscillatory shear (LAOS) and small amplitude oscillatory shear (SAOS) (Deshpande, Krishnan, & Kumar, 2010). SAOS is the most commonly used method to investigate the rheological properties of cheese (Muliawan & Hatzikiriakos, 2007). A study done by Ak et al. (1996) uses SAOS to determine the influence of storage at low temperature on mozzarella cheese. Ma, James, Zhang, and Emanuelsson-Patterson (2011), found that SAOS is better to evaluate melting properties of Mozzarella cheese compared to Schreiber test. In SAOS, the strain applied and the stress response determines the material functions to measure the material behaviour (Deshpande et al., 2010). Sinusoidal strain which is applied on cheese can be described following equation 2.2 below:

\[ \gamma = \gamma_0 \sin(\omega t) \]  

(2.2)

where \( \gamma \) is shear strain, \( \gamma_0 \) is amplitudes of strain, \( \omega \) is the frequency oscillation and \( t \) refers to time.

For instance, the proportions of stress and strain amplitudes influence the storage or elastic (\( G' \)) and loss or viscous (\( G'' \)) moduli as shown in equation 2.3 below:

\[ \sigma = G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t) \]  

(2.3)

where \( \sigma \) is critical shear stress.

For a viscoelastic material, \( G' \) and \( G'' \) cross-over occurs at the frequency, \( \omega = 1/\lambda \). A complex modulus, \( G^* \) is also commonly used to describe viscoelasticity of cheese. This is shown by equation 2.4 below:

\[ |G^*| = \sqrt{G'^2 + G''^2} \]  

(2.4)
Besides having the knowledge of the concept of rheology, the correct choice of measurement method is needed based on the cheese nature. Cone and plate geometry and parallel plate geometry (Figure 2.17) are normally used in cheese studies (Patarin, Galliard, Magnin, & Goldschmidt, 2014).

Figure 2.17 Example of cone and plate geometry (left) and parallel plate geometry (right). Source: Lampman (2003).

Several advantages of rheological method especially on cheese studies have been reported (Karoui & Dufour, 2006; Lee, Klostermeyer, & Anema, 2015; Montesinos-Herrero, Cottell, O’Riordan, & O’Sullivan, 2006; Muliawan & Hatzikiriakos, 2007; Tunick & Van Hekken, 2002). The results obtained from creep compliance curve, stress relaxation and deformation curve allow the determination of rheological characteristics of cheese such as firmness, elasticity, viscosity and shear moduli. These characteristics reflect the compositions, physico-chemical condition, microstructure and macrostructure of the cheese components. Rheological properties also demonstrate the gas retention, physical characteristics, eating quality and texture of the cheese samples (Guinee, 2011a). These qualities are very important for all level of marketing starting from manufacturer, distributor and consumer, thus, making rheological measurement a significant method for the cheese industry.

2.7 Particle size measurements

Particle size is one of the most important factors in determining the quality and performance of many food systems. In food materials, the knowledge of particle size can assists scientists to design and modify food products. For example, the size and shape of milk powders can influence the solubility, flow and milk viscosities. Smaller particles of milk powders tend to dissolves more quickly than the larger ones, thus creating a stable dispersion with higher
viscosity (Crowley, Gazi, Kelly, Huppertz, & O’Mahony, 2014). Besides that, particle size in cocoa powder also plays an important role in determining its flavour and colour (Ben Abdelaziz, Sahli, Bornaz, Scher, & Gaiani, 2014).

There are various methods to determine particle size distributions. According to Yoshida et al. (2014), using dynamic light scattering, laser diffraction and scattering method, the measurement time can be reduced and the results obtained have good repeatability. Analysing sample particle size is not easy due to its uneven shapes and sizes. If the particle size is spherical, it would be easy to measure since every dimension is identical. However, most particles have different shapes and sizes which create the complexity of determining the real particle size of a sample (Allen, 2013). Figure 2.18 shows the example of two different particle sizes and shapes.

![Figure 2.18 Example of particle sizes and shapes. A sphere shape (left) can be measured by its diameter (d). An uneven shape (right) has to be measured using various length and width.](image)

Different techniques and methods give different particle size values. For example, in light scattering method, results of the various particle size dimensions will be averaged.

### 2.7.1 General principles of particle size measurements by laser diffraction

Using laser diffraction method a light scattering technique, particle size of a particle can be determined by averaging all the dimensions. Regular shaped particles can be described based on a concept of equivalent spheres (Gregory, 2005). Figure 2.19 displays the concept of equivalent spheres for particle size determination. Figure 2.19 also shows the different possible particle size results for a certain particle for example.
Figure 2.19 Schematic diagram of the concept of equivalent spheres. Source: Rawle (2003).

Particle size measurements using laser diffraction result in a particle size distribution. In particle size distributions, there are three statistical properties involved as shown in Figure 2.20. The properties are:

- Mode: size with highest frequency
- Median: the size that divides the frequency distributions into two equal areas
- Mean: average size of a population

Figure 2.20 Schematic diagram of a typical particle size distribution. Source: Yang (2003).
Mean is usually used in determining the particle size in a sample. The most commonly used mean are $D_{[1,0]}$, $D_{[4,3]}$ and $D_{[3,2]}$. $D_{[1,0]}$ is known as number length mean (the mean dimensions based on length), $D_{[4,3]}$ is known as volume or mass moment mean (the mean sizes are based on volume) and $D_{[3,2]}$ is known as surface area moment mean (the mean sizes are based on surface area). Appropriate mean is chosen depending on the information required from the sample. For example, $D_{[4,3]}$ can be used to determine coarse particulate of a sample while $D_{[3,2]}$ may be used for determining the fines presence in the sample (Harker, Backhurst, & Richardson, 2013). $D_{[3,2]}$ will be selected as mean for particle size measurement in this study because the impact of the surface area of the oil droplets in model processed cheese on some physico-chemical properties will be investigated.

Laser diffraction analysis allows the measurement of particle size from 20 nm to 2 mm, when using the Malvern Mastersizer 2000, for instance. In this equipment, which is used in this thesis, there are two light sources: red (He-Ne) and blue (solid-state) of different wavelength that make up the laser beam. The red laser (632 nm) is used for measuring the larger particles, while the blue laser (405 nm) detects the smaller particles. First, the laser will pass through the sample which has been well dispersed in air or liquid media. This will cause particles diffraction to occur, thus, creating diffraction pattern which is measured by several detectors. Based on an optical model and statistical analysis, the signal is then transformed to a particle size distribution (McGarvey, McGregor, & McKay, 1997). Figure 2.21 describes the determination of particle size distribution using laser diffraction analysis.

There are many studies which have been conducted on particle size analysis related to dairy products. Fava, Serpa, Külkamp-Guerreiro, and Pinto (2013), studied the particle size distribution of fresh, chilled and frozen sheep milks. Their study uses laser diffraction method. According to their study, the particle diameter of the sheep milk was not affected by the storage method applied. Another study done by Jhanwar and Ward (2014), discovered that the particle size of skim milk lipid material contains at least three most abundant distributions of very small particles. In cream cheese, Sainani, Vyas and Tong (2004) investigated the effects of particle size on cheese texture. Textural defects such as grainy or gritty mouthfeel can affect consumer preference. According to their study, grittiness in cream cheese increased with higher amounts of large particles.
2.8 Melting properties

Melting properties also commonly known as meltability is a crucial test for cheese industry. The impact of process and formulations on cheese can be determined using meltability assessment. However, the real definition of meltability often changes according to product’s suitability, end-use and ingredients. Cheese shreds used as pizza toppings for example, will be glued together once heated. This definition will only suit food applications such as pizza but will not be suitable to become a measurement criterion to assess food quality (Gunasekaran & Ak, 2002; Sun, 2011). In general, meltability can be defined as “the ease and extent to which cheese will melt and spread upon heating” (Park, Rosenau, & Peleg, 1984). This means that, the determination of cheese meltability must depend on the solid cheese’s thermal phase change properties and the melt’s flow or rheological characteristics (Ustunol, Kawachi, & Steffe, 1994).

Over half a century, various quantitative methods have been introduced to measure cheese meltability. Empirical methods such as Arnott test, Olson and Price test and Schreiber test with several modifications have been used to assess melt quality of cheese (Arnott, Morris, & Combs, 1957; Kosikowski, 1977; Olson & Price, 1958). Besides empirical methods, objective methods such as steady shear viscometry, capillary rheometry and squeeze-flow
rheometry also have been developed to obtain melt characteristics of cheese (Gunasekaran & Ak, 2002). Another test which is known as computer vision method was developed to improve Schreiber test. This method has been claimed as more consistent, rapid and accurate to determine cheese melt quality and browning of cheese (Nollet & Toldra, 2009). Wang and Sun (2001) obtained interesting results on the efficiency of computer vision method on the functional characteristics of Cheddar cheese.

2.8.1 Arnott melt test

Arnott melt test was originally developed by D. R. Arnott in 1957. It is one of the earliest reported melt test to assess meltability in cheese. In his work, he observed the melting properties of processed cheese. He also observed a correlation between the melting properties and the chemical properties (pH, fat, moisture and free tyrosine contents) of Cheddar cheese (Arnott et al., 1957). The processed cheese samples were cut into a standard cylinder of 17 X 17 mm and placed into an oven at 100°C ± 2°C for 15 minutes. Then, the samples were allowed to stand at room temperature for 15 minutes before placing the samples into the refrigerator for 30 minutes. The measurements were done in triplicate. Sample height measurements were determined using the depth micrometer. Figure 2.22 demonstrates the melting of cheese samples using the Arnott test.

![Figure 2.22](image)

**Figure 2.22** Cheese samples positioned on a glass tray before heat treatment (A) and after heat treatment (B). **Source:** Arnott et al. (1957).
The melting quality is defined as:

\[
\% \text{Melt} = \frac{\text{Before melt (height)} - \text{After melt (height)}}{\text{Before melt (height)}}
\]

### 2.8.2 Olson and Price melt test

Olson and Price melt test, known as tube method (Figure 2.23) was introduced by Olson and Price in 1958. They came with this method after reporting problems with Arnott melt test. A film formed on the surface of cheese samples during heat treatment and the flow of the melted cheese after heat treatment was uneven (Olson & Price, 1958). In this method, 15g of cheese sample is inserted into a glass tube with both ends closed with rubber stoppers. However, one of the stoppers is vented with a 1-mm-diameter glass tube.

A reference line is drawn on the glass tube (27.5 mm from the opposite end). The tube is held horizontally and heated in an oven at 110°C for 6 minutes, then tilted to stop sample from flowing further. Distance of flow from reference line is measured. The melted sample is once again heated for 2 minutes and the distance of flow is measured again. The combination of these two distance measurements (sample in 6 + 2 minutes of heating) was called as “cheese flow” (Olson & Price, 1958).

![Figure 2.23](image)

**Figure 2.23** Cheese samples inserted into a glass tube before heat treatment (A) and melted cheese samples in the glass tube after heat treatment (B). *Source: Gunasekaran & Ak (2002).*
2.8.3 Schreiber melt test

In 1977, a method for meltability assessment was introduced by Kosikowski. This method is generally known as the Schreiber melt test (Figure 2.24). In this test, cheese samples were cut into 5 mm (thickness) X 41 mm (diameter) and placed into a petri dish. Then, the prepared samples were placed into the oven at 232ºC for 5 minutes.

![Figure 2.24](image)

**Figure 2.24** Cheese samples positioned on a petri dish before heat treatment (A) and melted cheese samples on petri dish after heat treatment (B). Concentrically numbered graph was placed under the petri dish for measurement purposes. *Source: Gunasekaran & Ak (2002).*

The melted samples were cooled at room temperature for 30 minutes. The cooled samples were then centred onto concentrically numbered graph and the largest diameter of spread is considered as its meltability (Kosikowski, 1977). In recent years, Schreiber melt test has been modified in various ways by researches to suit different type of cheese (Altan, Turhan, & Gunasekaran, 2005; Glenn, Daubert, Farkas, & Stefanski, 2003; Muthukumarappan, Wang, & Gunasekaran, 1999; Savello et al., 1989).

Based on the literature review done on melting properties, the Arnott method for meltability assessment has been selected in this thesis. Although it is one of the earliest methods
reported, still, Arnott method has gained popularity among the cheese industries due its simplicity and quick results. Although Arnott, Schreiber and tube methods have undergone many modifications, Arnott method was chosen for melting assessment in this study based on its simplicity, quick results and its suitability with the model processed cheese samples prepared in this thesis.

### 2.9 Protein content evaluation

Protein content evaluation play an important role in many field of studies since protein exist in major parts of life. For example, the knowledge of each specific protein contributes to pharmaceutical applications (e.g. hormone, blood and insulin), foods (e.g. dairy, meat and bakery products) and industrial applications (e.g. textiles, glue) (Hasan, Shah, & Hameed, 2006; Meisel, 1997). Protein content determination allows different protein to be analysed individually thus, provides information for protein purification and extraction techniques development. In dairy product, milk protein characterization during cheese-making and processing has been studied by various methods such as colorimetric method (e.g. Bradford), electrophoresis and chromatography (El Abboudi, Pandian, Trepanier, Simard, & Lee, 1991).

#### 2.9.1 Bradford Method

Bradford protein assay was founded by Marion Bradford in 1975 to determine the amount of protein in a sample (Bradford, 1976). This method has been claimed to be more sensitive than Lowry method which was introduced in 1951 (Kruger, 1994). Lowry method is a protein determination using colorimetric technique where proteins bind with copper and respond with Folin reagent which results in strong blue colour. Lowry method is most suitable for protein concentration ranging from 0.01 to 1.0 mg/mL (Waterborg & Matthews, 1994). In Bradford method, the protein binds to the acidic solution of Coomasie Brilliant Blue G-250 dye. The absorbance of the dye binding is determined based on the maximum absorbance of the dye cations (470 nm) and the maximum absorbance of the dye anions (595 nm) using a spectrophotometer. However, the ability of the dye to bind protein is limited and free amino acids do not bind the dye. Over the years, several modifications have been done on the Bradford method to solve this problem, and Bradford method has been widely used to determine various types of proteins (Appenroth, Augsten, Liebermann, & Feist, 1982;
Marchal, Seguin, & Maujean, 1997; Sharma & Tihon, 1988). Research also has been performed on dairy studies where total protein in milk powder or cheese samples was determined using Bradford method (Kamizake, Gonçalves, Zaia, & Zaia, 2003).

2.9.2 Electrophoresis

Electrophoresis is also a known method for protein determination. This method uses electric field to isolate macromolecules from the complex protein mixtures where each macromolecule moves at different speed. This movement depends on the gel nature and properties of the macromolecules (Corley, 2005). Over the years, electrophoresis techniques has evolved into different category such as polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate-PAGE (SDS-PAGE), isoelectric focusing (IEF) and two-dimensiononal (2D) gel electrophoresis.

2.9.2.1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most commonly used method for protein characterization (Smith, 1994). The ability of SDS-PAGE to separates protein according to their size has increased its popularity to determine protein purity, protein molecular mass and identifies thousands of protein with a single experiment run (Schagger, Cramer, & Vonjagow, 1994; Sheehan, 2009). In this method, the protein mixtures are denatured by boiling the mixtures in buffers containing SDS. The negatively charged SDS bind to the denatured protein forming negatively charged complexes. In general the ratio of SDS bound to protein is 7:5 (w/w) where 1.4 g of SDS can bind to 1.0 g of protein (Smith, 1994). Some suggest that SDS bind proportionally to the amount of aminoacids present in the protein (Corley, 2005).

The presence of electric field assists the negatively charged complexes to migrate between the chloride ions (Cl\(^-\)) and the glycinate ions which are present in the system and the buffer. Small protein molecules move faster compared to larger protein molecules, thus, protein molecules can easily be characterized (Smith, 1994). SDS-PAGE may be done under reducing or non-reducing conditions. In reducing conditions, the existence of 2-
mercaptopethanol (2-ME) in the SDS loading buffer helps to cut down the protein disulphide bonds. On the other hand, the proteins move abnormally on the gels using non-reducing conditions (Corley, 2005). In cheese studies, SDS-PAGE has been widely used for protein determination. Figure 2.25 shows an example of SDS-PAGE patterns of proteins characterized from goat milk cheeses.

Lau, Barbano, and Rasmussen (1991) studied the effect of milk pasteurization on Cheddar cheese protein breakdown. By using SDS-PAGE, the results of their study showed that β-casein disintegrates slowly in pasteurized milk cheese compared to cheese made with raw milk. Another study done by Kaminarides, Ilias-Dimopoulos, Zoidou, and Moatsou (2015), proved that SDS-PAGE method was successful to separate different serum proteins and whey proteins from Myzithra cheese.

![Figure 2.25 Example of SDS-PAGE patterns of proteins from different goat milk cheeses. Source: Park and Jin (1998). (Reproduced with permission from Park and Jin, 1998 © Journal of Food Science).](image)

2.9.3 Chromatography

Chromatography, another separating technique, provides knowledge on different protein using single experiment. The first few types of chromatography (ion-exchange, gel filtration and hydroxyapatite) were introduced in 1950s and 1960s (Boyer & Hsu, 1993). The basic concepts of chromatography are similar, having a mobile phase and a stationary phase. The
mobile phase is the protein mixture which moves at different speeds on the stationary phase. This allows protein separation to occur (Touchstone, 1992).

In cheese studies, various types of chromatography techniques are used for protein characterization. These include hydrophobic interaction chromatography (HIC), ion-exchange chromatography, and high-pressure liquid chromatography (HPLC) (Figure 2.26) (Bramanti, Sortino, Onor, Beni, & Raspi, 2003; Ferreira & Caçote, 2003; Francis, Regester, Webb, & Ballard, 1995; Pham & Nakai, 1984).

![Figure 2.26 Example of reversed-phase HPLC chromatograms of different bovine milks. (A) milk powder; (B) half skimmed UHT milk; (C) half skimmed pasteurised milk; (D) raw milk. Source: Bordin, Cordeiro, de la Calle, and Rodriguez (2001). (Reproduced with permission from Bordin et al., 2001 © Journal of Chromatography A).](image-url)
2.10 Microstructural evaluation

Microstructure can be defined as the structure of a material which can be seen by any microscopic technique. Microstructural evaluation is very important in food industry to determine, for example, the food functionality, shelf-life and effects of storage (Bhandari, 2012). Many studies have been done on cheese using various techniques such as fluorescence image analysis, light microscopy (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) are among the popular methods to investigate fat globules in cheese (El-Bakry & Sheehan, 2014).

Sutheerawattananonda, Fulcher, Martin, and Bastian (1997) studied the size and shape of fat globules of processed cheese made with Cheddar cheese, trisodium citrate and sodium chloride using fluorescence image analysis. Based on their study, the fat globules in processed cheese made with trisodium citrate were more spherical compared to processed cheese treated with sodium chloride (Figure 2.27).

![Figure 2.27 Example of fluorescence images of processed cheese treated with sodium chloride (left) and processed cheese made with trisodium citrate (right). F: fat globule; P: protein network. Source: Sutheerawattananonda et al. (1997). (Reproduced with permission from Sutheerawattananonda et al., 1997 © Journal of Dairy Science).](image)

2.10.1 Transmission electron microscopy (TEM)

TEM has been commercialized in 1939 and throughout the years, this technique has evolved to become one of the most important technique used in scientific research to obtain micro-scale images. In dairy and cheese studies, TEM is chosen by many researchers to visualize
casein micelle structure and its possible modifications during various treatments the impact of different parameters on cheese matrix and also the presence of bacterial exopolysaccharides (EPS) in hard cheese (Dabour, LaPointe, Benhamou, Fliss, & Kheadr, 2005; Geng, Van den Berg, Bager, & Ipsen, 2011; Dalgleish, 2014). Figure 2.28 displays an example of microstructural images of cheese obtained by TEM.

Figure 2.28 Example of TEM image of protein network in analogue cheese grain. Source: Geng et al. (2011). (Reproduced with permission from Geng et al., 2011 © International Dairy Journal).

2.10.2 Scanning electron microscopy (SEM)

The use of scanning electron microscopy (SEM) started in 1937 and has now evolved to several other names according to its application. This evolution includes cryo-scanning electron microscopy (cryo-SEM), field emission scanning electron microscopy (FESEM) and environmental scanning electron microscopy (ESEM) (El-Bakry & Sheehan, 2014).

In cheese studies, SEM has been proven to become a valuable tool and has been widely used for microstructural evaluation. SEM offers digital images with high resolution of fat particles, protein network, moisture voids and bacterial dispersion (Impoco, Tuminello, Fucà, Caccamo, & Licitra, 2011). Study done by Cunha, Dias, and Viotto (2010) demonstrated clear microstructural images of traditional cheese and analogue processed cheese using SEM. Figure 2.29 reports an example of SEM images of the two cheeses used in their study. Another study based on SEM and done by Sainani et al. (2004), proved that the protein
profile and composition of gritty cheese taken from pilot-scale was similar with the commercial gritty cheese.

![Figure 2.29 Example of SEM image of traditional cheese (left) and analogue processed cheese (right). Source: Cunha, Dias, & Viotto (2010). (Reproduced with permission from Cunha et al., 2010 © Food Research International).](image)

### 2.10.3 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) is one of the prominent microscopy techniques used in scientific research. CLSM provides high resolution optical images as small as 0.2 μm for lateral resolution and 0.5 μm for axial resolution. The main feature of CLSM that stands out from other microscopy techniques is its capability to acquire deep in-focus images known as optical sectioning without physically slicing the samples (Park & Kihm, 2006).

Although microstructural images obtained from SEM are better due to its high magnification, CLSM is preferred because this method preserves the internal structure of the sample (Joshi, Muthukumarappan, & Dave, 2004). Ong, Dagastine, Kentish, and Gras (2011) used CLSM and cryo-SEM to study the microstructure of cheese curd and milk gel. Figure 2.30 demonstrates an example of CLSM images obtained in their study. Based on their research, CLSM and cryo-SEM provided similar information on the influence of milk processing on cheese microstructure. In CLSM, sample is stained with specific dyes where chemical interactions among the proteins and fats with the dyes occur (Nollet & Toldra, 2009). Ong et al. (2011) and Soodam, Ong, Powell, Kentish, & Gras (2015) used Fast Green FCF for proteins identification and Nile Red for fat identification in their cheese samples. Hosseini-
Parvar, Matia-Merino, and Golding (2015) also used Fast Green FCF for the protein phase in their model processed cheese sample but used Nile Blue for the oil phase.

**Figure 2.30** Example of CLSM images of cheese made from unhomogenised milk (left) and cheese made from homogenised milk (right). NGF represents non-globular fat. Markers- red: fat; green: protein network. *Source: Ong et al. (2011).* (Reproduced with permission from Ong et al., 2011 © LWT- Food Science and Technology)

In this thesis, three microscopic techniques (CLSM, cryo-SEM and ESEM) will be used to visualise the structural properties of processed cheese.

### 2.11 Small angle X-ray scattering (SAXS)

X-ray was first discovered by Wilhem Conrad Röntgen in 1895 and has become a very powerful tool to study atomic structures (Lederman, 1981; Mould, 1995). One of techniques which exploits X-rays includes small-angle X-ray scattering (SAXS) which is used to study nanoscale structures in soft matter (Borsali & Pecora, 2008). Using SAXS, the internal structures of the samples ranged between 0.1 to <500nm can be evaluated (Stawski & Benning, 2013). As foods are very complex systems and mostly consist of carbohydrates, proteins and lipids, the range of scales for these soft materials can vary between nanometer to millimeter (Li, Rong, & Huang, 2012).

SAXS is a powerful technique that could monitor (i) the molecule changing shapes (Occhipinti et al., 2003), (ii) interaction between the internal structures of food systems, and (iii) 3-dimensional (3D) structures of complex soft matter samples. SAXS is also more
convenient to provide information with little or no sample preparation and is also suitable to monitor in-situ reactions (Li et al., 2012; Pauw, 2013).

2.11.1 General principles of SAXS

The production of the X-ray radiation experiment is done in a synchrotron which is a large structure that consists of a long circular tube to which electrons are accelerated to near the speed of light. These storage rings induces powerful focused beam of X-ray needed to determine structure in food system (Willmott, 2011). Figure 2.31 displayed a schematic diagram of a typical SAXS setup where the X-ray beam is subsequently cut by collimator into a parallel beam.

![Schematic diagram of typical SAXS experiment.](source)

When the beam hits the nano-structures in the sample, the electron-density differences of the sample causes a small fraction of the radiation to be scattered. The beam stop is designed to cut away the main beam to prevent the main beam from damaging the detector (usually a camera), while the scattered radiation falls onto the detector. Principally in SAXS, elastic collisions occur between the incoming wave (X-rays) and a particle, producing scattered reflected waves. These waves later overlapped with each other, generating scattering intensity peaks (Kirschbrown, 2007).
The scattered intensity increases with the decrease in the scattering angle (Saisho & Gohshi, 1996). The scattering vector, \( q \), can be calculated using this equation:

\[
q = \frac{4\pi}{\lambda} \sin \theta
\]

(1.7)

where \( \theta \) is the scattering angle and \( \lambda \) is the radiation wavelength (Cohaut & Tchoubar, 2013). Figure 2.32 represents the description of the scattering vector, \( q \).

![Figure 2.32 Schematic diagram of SAXS setup and the description of scattering vector \( (q) \). Source: Borsali and Pecora (2008).](image)

**2.11.2 Some SAXS applications**

There are wide ranges of SAXS applications. Figure 2.33 shows variety molecular systems investigated by SAXS (De Kruif, 2014; Li et al., 2012). For decades, SAXS has been used to determine micro and nano structures of particle systems and is a valuable tool to obtain quality results. Furthermore, the technique was simple and straightforward which helps to reduce the experiment time. Li et al. (2012) suggested that SAXS has the ability to assist food scientists to probe into the internal structures of dairy products where alterations of the protein-active compound can be easily detected. De Kruif (2014) studied casein micelles structure using SAXS and found that the structure of a casein micelle looks like a protein matrix with dispersed calcium phosphate clusters thus, ruling out the possibility of submicelle model or models with large voids and channels.

Another study done by Bouchoux et al. (2010) on casein micelle structure using SAXS showed that, the deformation of casein micelle is nonaffine under osmotic stress. This behaviour is suited for a model known as sponge model that has a triple hierarchical structure.
as shown in Figure 2.5f (Section 2.3.2 in this chapter). Gliguem et al. (2011) studied the influence of thermal history and storage conditions on the physical properties of fat phase and the viscoelastic properties in processed cheese using SAXS. They found that the storage conditions of processed cheese (4°C) and then its equilibration at room temperature (25°C) lead to the partial crystallization of milk fat. Besides, they also proved that polymorphism of the fat phase (triacylglycerols) occur in processed cheese.

![Schematic illustrations of SAXS applications.](image)

**Figure 2.33** Schematic illustrations of SAXS applications. *Source: Bernadó et al. (2010); Holt, De Kruif, Tuinier, and Timmins (2003); O’Kane et al. (1994)*.

One of the aims of this thesis is to use synchrotron SAXS method to investigate processed cheese made under different conditions. However, a model system is needed to simplify the structure of the system to ensure that results can be easily interpreted.

### 2.12 Concluding remarks

In this literature review, the topics related to cheese, processed cheese and analogue processed cheese were covered. A number of emulsifying salts and surface active materials (emulsifiers) were presented based on the published literature. In addition, the methods needed to carry out the experimental work required in this thesis are covered; these include, oscillatory rheology, particle sizing, melting measurements, protein determination, microscopy and small angle X-ray scattering (SAXS). Based on the literature, it is known that many studies have been done using various types of emulsifying salts on processed cheese.
However, there have been very few studies focusing on the variation of fat and protein concentration. Among the emulsifying salts, TSC is a unique emulsifying salt as it is not a phosphate group but has the capability to act as an emulsifying agent. Other than that, some studies reported that model processed cheese made with TSPP demonstrated higher firmness compared to model processed cheese made with other emulsifying salts. It is interesting to look at the comparison between TSC and TSPP on the model processed cheese made with different fat and protein concentrations. Apart from the variation in the major solids components (fat and protein), very few studies have been done to determine the interaction between surface active materials on the oil droplets especially on processed cheese. Finally, no studies have been done to see the effect of different types of emulsifying salts on the internal structure of the casein micelles using SAXS.
3
Materials & Methods
3.1 Materials

3.1.1 Chemicals for preparation of model processed cheese

Model processed cheese was made from calcium caseinate (Westland Co-Operative Dairy Company Limited, Hokitika, New Zealand) with chemical composition: protein, 92.7%; fat, 0.98%; moisture, 3.9%; and minerals, 1.4%; sunflower oil (Home Brand, Manukau, New Zealand), trisodium citrate dehydrate (TSC), sodium pyrophosphate (TSPP) and citric acid (all were analytical grade and purchased from Sigma-Aldrich NZ Ltd, Auckland, New Zealand).

3.1.2 Chemicals for preparation of emulsions

Other than calcium caseinate and sunflower oil mentioned in Section 3.1.1, emulsions also were made from BiPRO whey protein isolate (Davisco Foods International, Minnesota, USA) and liquid lecithin (Hawkin Watts Ltd, Auckland, New Zealand).

3.1.3 Chemicals for preparation of milk samples

Low heat skim milk powder was supplied by Westland Co-operative Dairy Company Limited (Hokitika, New Zealand). The chemical composition of the skim milk powder as provided by the manufacturer was: protein, 33.5%, moisture, 3.7%; and minerals, 7.8%. Other chemicals were sodium phosphate (SP), sodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP). Sodium azide was used as a preservative. All of the chemicals were analytical grade and purchased from Sigma-Aldrich NZ Ltd, Auckland, New Zealand.

3.1.4 Minor chemicals

Sodium chloride (NaCl) and SHMP (Sigma-Aldrich NZ Ltd, Auckland, New Zealand) were used for preliminary study on processed cheese. Soybean oil (Amco, Goodman Fielder NZ Ltd, Auckland, New Zealand) was used during rheological measurements to cover the sample
against evaporation. Sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) (both from Sigma-Aldrich NZ Ltd, Auckland, New Zealand) were used for oil droplet size measurements. Ethanol and chloroform (both from J.T Baker Chemical Co., PA, USA) were used for scanning electron microscopy (SEM) preparation. Acetonitrile and trifluoroacetic acid (TFA) (both were HPLC grade and supplied by ECP Labchem Ltd, Auckland, New Zealand).

3.2 Preliminary studies on model processed cheese

In the preliminary study, two methods to manufacture model processed cheese using casein were identified from the available literature. These two methods were selected based on the processed cheese manufacturing techniques which are the most repetitive and suitable for lab-bench scale.

3.2.1 Method A

The method of Cavalier-Salou and Cheftel (1991), where calcium or sodium caseinate were used to manufacture model processed cheese. The ingredients and type of cooker is summarised in Table 3.1.

Table 3.1: The ingredients, compositions of ingredients and type of cooker used to manufacture model processed cheese by Cavalier-Salou and Cheftel (1991).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Type of cooker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium caseinate or sodium caseinate</td>
<td>28.7</td>
<td>Stephan Cutter model S12.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Speed: 1500rpm-3000rpm.</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>18.3</td>
<td>Temperature: 75-85°C.</td>
</tr>
<tr>
<td>Emulsifying salt or NaCl</td>
<td>1-3</td>
<td>Cook time: 4.5 mins.</td>
</tr>
<tr>
<td>Water</td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> % corresponds to weight per total weight.

3.2.2 Method B

The method of Lee et al. (2004), where rennet casein was used to manufacture model processed cheese. The ingredients and type of cooker is summarised in Table 3.2.
Table 3.2 The ingredients, compositions of ingredients and type of cooker used to manufacture model processed cheese by Lee et al. (2004).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
<th>Type of cooker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rennet casein</td>
<td>12.6</td>
<td>Vorwerk Thermomix TM21</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>34.8</td>
<td>Speed: 100 rev/min-2000rev/min.</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>2.0</td>
<td>Temperature: 90-100°C.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.7</td>
<td>Cook time: 7 mins.</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>49-55</td>
<td></td>
</tr>
</tbody>
</table>

a. % corresponds to weight per total weight.

3.2.3 Method developed for this study

In this study, the combinations of these two methods were experimented to produce a new formulated model processed cheese. Sunflower oil was chosen instead of butter oil because it is easily accessible. Calcium caseinate was chosen instead of rennet casein due to reproducibility. Samples were prepared as described later in this chapter (Section 3.3.1) but with the addition of 1% NaCl.

![Temperature profile of model processed cheese which includes heat treatment phase and creaming phase. Note that model processed cheese made without the creaming phase is cooled just after the heat treatment phase.](image-url)

**Figure 3.1** Temperature profile of model processed cheese which includes heat treatment phase and creaming phase. Note that model processed cheese made without the creaming phase is cooled just after the heat treatment phase.
The temperature profile (Figure 3.1) during the manufacturing process and the need of sodium chloride as part of the ingredients used were analysed. These analyses were done using rheological measurements as described later in this chapter (Section 3.4.1) for validation purposes. Based on the temperature profile presented in Figure 3.1, the mixture of ingredients to make processed cheese was heated from 37ºC to 90ºC over 3 minutes, held at 90ºC for 2 minutes, then immediately cooled at a rate of 1ºC/min to 80ºC.

### 3.3 Sample preparations

#### 3.3.1 Manufacture of model processed cheese

The model processed cheese samples were prepared using a 2-L capacity Vorwerk Thermomix TM 31 blender cooker (Vorwerk & Co. Thermomix; GmbH, Wuppertal, Germany- shown in Figure 3.2) similar to the one used by Lee et al. (2004).

![Vorwerk Thermomix TM 31 blender cooker](image)

**Figure 3.2** Vorwerk Thermomix TM 31 blender cooker used for making model processed cheese.

The cooker has a four-blade chopper rotor (two blades facing upward and two blades facing downward) at the base of the cooker. The speed range is from 100 to 10,200 rev/min. There are twelve speed steps which are, the gentle stir (40 rev/min), the stirring steps; speeds 1-3 (100-500 rev/min), the mixing/blending steps; speeds 4-10 (1,100-10,200 rev/min) and a Turbo mixing step that runs at a single speed of 10,200 rev/min. The cooker is electrically heated around the base with heating scale varying from 37 to 100ºC.
Water (575.0g) was poured into the Thermomix and calcium caseinate powder (200.0g) was added using speed 3 for 3 minutes. Then, 200.0g of oil were poured slowly while the speed is increased from 3 to 4. After 2 minutes, the speed was increased to 5. At this step, the Thermomix was stopped to check if there is any remaining ingredients that did not mix or which sticks onto the wall of the Thermomix. A plastic spoon was used to take all the ingredients that did not mix and place in the middle of the mix. Then, the blend was mixed again at speed 5 for 2 minutes to ensure all the ingredients were mixed together. 20.0g of emulsifying salt (20.0g) for calcium chelation and 5.0g of citric acid for pH adjustment were poured into the mix using the same speed for 2 minutes. The speed was increased to speed 6 for one minute to ensure that all ingredients are well-mixed. Then, the cooking phase started: time, temperature and shear were programmed to heat from 37 to 90°C at speed 5 for 3 minutes and held at 90°C for 2 minutes.

### 3.3.2 Processed cheese with emulsions

Emulsions were prepared using water (59%), sunflower oil (40%) and 1% of calcium caseinate, whey protein isolate (WPI), or lecithin. The ingredients were homogenized using a laboratory homogenizer (Model L5T, Silverson, Massachusetts, USA). Then, the particle size of the mixture were further homogenised under high shear fluid processors using M-110L microfluidizer materials processor (Microfluidics, Massachusetts, USA). The emulsion was recycled twice with pressures of 750 bar and 206 bar, respectively. Figure 3.3 shows the steps to produce model processed cheese made with emulsions.

The model processed cheese samples were prepared as previously described in Section 3.3 but with slight modification. Briefly, water (298.5g) and calcium caseinate powder (201.5g) were poured into the Thermomix using speed 1 to 3. Then, the speed was reduced to 1 and an emulsion (500.0g) was poured into the mix. Later, emulsifying salt either sodium pyrophosphate (20.1g) or trisodium citrate (20.1g) and citric acid (5.2g) were added into the mix. The speed was maintained at speed 1 to avoid breaking further the reduced particle size obtained during the emulsion preparation. Finally, the mixture was heated from 37 to 90°C at speed 5 for 3 minutes and held at 90°C for 2 minutes.
Figure 3.3 Steps to produce model processed cheese made from emulsions.

### 3.3.3 Preparation of milk samples

Stock skim milk sample containing solid concentration of 20% (w/w) was prepared by mixing 100g low heat skim milk powder with 400g MilliQ water (18.2 MΩcm) and 0.02% (w/w) sodium azide (NaN₃). Sodium azide was added to inhibit microbial growth in the skim milk solution. The mixture was gently stirred for two hours using a magnetic stirrer (MR Hei-Standard, Germany) to ensure thorough dispersion and reconstitution of the milk powder.

All of the emulsifying salts were prepared separately in a 50-mL centrifuge tube (ThermoFisher Scientific, New Zealand) and stirred using vortex mixer (IKA MS1 Minishaker, IKA Works Asia, Malaysia) to obtain a clear solution. Each of the emulsifying salt solution was later added into the milk solution and left for an hour at room temperature. Then, under continual stirring and using a pH meter (Orion 320 PerpHecT LogR Meter, Thermo Scientific, USA), the pH of the emulsified milk samples were adjusted to 5.60 ± 0.01 using 1M NaOH solution or 1M HCl solution, followed by the addition of MilliQ water (18.2 MΩcm) into the sample until the milk sample reached 100ml. The final skim milk
concentration was 10% (w/w). All the milk samples were stored in the fridge (~4°C) for 2 to 3 days.

### 3.4 Physical methods

#### 3.4.1 Rheological measurements

Each batch of model processed cheese samples were characterized in terms of their rheological properties one or two days after manufacture.

![Controlled-stress rheometer](image)

**Figure 3.4** Controlled-stress rheometer (Paar Physica MCR 301), used for rheological measurements.

Rheological measurements were done using the method described by Lee et al. (2004) with slight modification, using a controlled-stress rheometer (Paar Physica MCR 301, Anton Paar GmbH, Graz, Austria-Europe; shown in Figure 3.4) with 50mm parallel plate geometry and 2.0mm gap. A small size of sample was held by a spatula, then the sample was gently pushed on to the bottom plate of the rheometer by a second spatula. The rotating plate was lowered slowly to the measuring position. Excess samples were trimmed off the edges of the parallel plate using a spatula. The temperature of the samples was maintained at 20°C on the bottom plate by a Peltier system. The outside edges of the sample were coated with soybean oil to prevent moisture loss.
Frequency sweeps were done on the samples by applying a constant strain of 10% over the frequency range from 0.01 to 10 Hz. These small amplitude oscillatory shear rheology (SAOS) were carried out on the model processed cheese samples to obtain the elastic ($G'$), loss ($G''$) and complex shear ($G^*$) moduli. Large deformation rheology was carried out by applying large strains from 0.1 to 10000% at a constant frequency of 1HZ, while measuring $G'$ and $G''$. This will allow determination of fracture characteristics of the model processed cheeses. All measurements were done in duplicate.

### 3.4.2 Particle size measurement

Oil droplet particle size distribution was measured by laser light scattering using a Malvern Mastersizer 2000 (Malvern Instruments Limited, Malvern, UK; shown in Figure 3.5).

![Malvern Mastersizer 2000](image)

Figure 3.5 Malvern Mastersizer 2000, used for particle size measurements.

The samples were prepared using the method described by Gassi, Famelart, and Lopez (2008). Processed cheese sample (0.2g) was dispersed in a solution containing 1 mL of 35mmol L$^{-1}$ EDTA and 1 mL of sodium dodecyl sulphate (SDS, 1% p/v). Sodium hydroxide was added to the solution for pH adjustment to pH 7. SDS was added to separate the oil droplets aggregates and EDTA to dissociate the milk proteins. The sample was left to stand overnight in a refrigerator at 5ºC. Before analyses, the mixtures were allowed to equilibrate at room temperature for 1 h. About 1 mL of suspension was loaded into the cell of the Mastersizer. The particle size measurements were carried out in triplicate.
Although the method from Gassi et al. (2008) was not designed for processed cheese and was designed for liquid systems, this method works well to determine the oil droplet particle size of the model processed cheese produced in this study. Using this method, homogenous solution is obtained after the sample was left to stand overnight. Other studies by Lee et al. (2004), Trivedi et al. (2008) and Chen & Liu (2012) used Tween 20 and EDTA to determine fat particle size of processed cheese. However, it was found in this thesis that precipitation occurred when using Tween 20 and EDTA after the sample was left to stand overnight.

### 3.4.3 Melting properties

Meltability of the manufactured cheeses was determined by the Arnott test (Arnott et al., 1957). The steps for meltability measurements are shown in Figure 3.6. Cheese samples were cut into cylinders (22 mm in diameter and 17 mm in height) with a corkborer and a knife. Each sample was placed in the center of a glass Petri dish. The Petri dish was heated in a laboratory convection oven (Thermo Scientific Heraeus) at 100°C for 15 min. After cooling to room temperature for 30 min, the height and diameter of the melted cheese samples were measured. The meltability of the cheese was expressed in terms of the percent decrease in cylinder height after heating. All measurements were done in triplicate.

The melting quality is defined as:

\[
\% \text{Melt} = \frac{\text{Before melt (height)} - \text{After melt (height)}}{\text{Before melt (height)}}
\]  

Arnott test was chosen to determine the meltability of the model processed cheese in this study because of its simplicity, reproducibility and quick results. The Schreiber test was also used in early works of this thesis. However, some model processed cheese samples (especially low protein concentration and processed cheese containing oil droplets stabilised by lecithin) were not suitable using Schreiber test as the samples melt extensively and touch the wall of the petri dish thus, making the meltability measurement difficult to replicate.
Figure 3.6 Steps for meltability measurements. The model processed cheese shown in the figure is made with 15% oil and 20% protein.

3.4.4 Microstructural evaluations

3.4.4.1 Scanning Electron Microscopy (SEM)

The microstructure of the model processed cheese samples were determined using scanning electron microscopy (SEM) as described by Kuo & Gunasekaran (2003). Approximately 1x1x10 (mm$^3$) of processed cheese sample were cut using a razor blade from the interior of the processed cheese and immediately fixed in 2.8% glutaraldehyde in a 0.05M sodium phosphate buffer (pH 6) for 48h at 7°C. The fixed samples were then dehydrated in a graded series of ethanol solutions which consist of 15 min in each of 25, 50, 70, 80, 95, 100, 100 and 100% (v/v) ethanol. The samples were then defatted three times with chloroform for 15 min.

The defatted samples were dehydrated three times with absolute ethanol for 15 min. Samples were then frozen in liquid nitrogen and fractured. The frozen and fractured samples were thawed in 100% ethanol and critical-point dried with liquid carbon dioxide using a Polaron
E3000 Critical-Point Dryer (Quorum Technologies, UK). The samples were then mounted on aluminium SEM stubs using a carbon-based tape and triple coated with platinum (Pt) in a Quorum Q150RS sputter coater (Quorum Technologies, UK). The samples were examined in a FEI Quanta 200 field emission scanning electron microscope (FEITM, QLD, Australia) operated at an accelerating voltage of 10 kV.

3.4.4.2 Cryo-Scanning Electron Microscopy (cryo-SEM)

Cryo-scanning electron microscopy (cryo-SEM) was done according to the method described by Ong, Dagastine, Kentish, and Gras (2011). The cryo-SEM sample preparation system (Alto 2500; Gatan, Abingdon, Oxon, U.K.) has three main components: a slushing station, a vacuum transfer device (VTD) and a sample preparation chamber. The processed cheese samples were prepared for cryo-SEM by mounting on a copper holder which was attached to the VTD that fits directly onto the slushing chamber. Fresh samples were rapidly immersed into liquid nitrogen slush (a mixture of solid and liquid nitrogen at its freezing point; -210 °C) for 15s. Then, the frozen specimens were immediately transferred using the VTD into an attached cryo preparation chamber. The sample was fractured using a chilled scalpel blade in the chamber which was maintained at -140 °C under a high vacuum condition (>10⁻⁴ Pa) with the aid of an externally fitted binocular microscope. The specimen was then etched at -95 °C for 15 min and coated using a cold magnetron sputter coater with 300 V, 10 mA of sputtered gold/palladium alloy (60/40) for 60 s (~3 nm). Then, the sample was transferred under vacuum onto a nitrogen gas cooled module, maintained at -140 °C and observed using a field emission gun SEM (FEITM, QLD, Australia) at 10 kV.

3.4.4.3 Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was done according to the method described by Trivedi et al. (2008). Processed cheese samples (approximately 5x 5x 5 (mm³)) were frozen to -20°C for 15 to 20 min and were sliced into approximately 30 µm sections using a cryo-cut machine (Leica Jung Frigocut 2800E Cryo-Microtome, Leica Instruments, Nussloch, Germany). Then, the sections were placed on a glass microscope slide and stained with a solution containing fluorescent dyes: Fast Green FCF (0.33% (w/w) solution in Citifluor, a glycerol / phosphate-buffered saline solution) and Nile Blue (0.33% (w/w) solution in
Citifluor), in equal proportion to stain protein and fat respectively. A cover glass was placed on top of the stained section. The samples were examined with a 40x objective lens or a 60x objective lens of a confocal laser microscope (Leica TCS SP2, Leica Microsystems, Heidelberg, Baden-Wurttemberg, Germany) powered by Ar/Kr and He/Ne lasers. Nile blue was excited at a wavelength of 488 nm and fast green FCF at 633 nm. The micrographs obtained were the combined images of fat/oil (shown in red) and protein (shown in green) phases.

### 3.4.5 Small angle X-ray Scattering (SAXS) measurements

SAXS measurements were done on milk solutions as described by Bouchoux et al. (2010) and Mata et al. (2011). The X-ray scattering measurements were performed at the Australian Synchrotron (Melbourne) on the SAXS/WAXS beamline which is shown in Figure 3.7.

All of the samples were measured in static mode. The measurement parameters were a wavelength of 0.995 Å, 7 m camera length and 12 keV photon energy. The total scattering vector \( q \) which is an essential parameter varies from 0.0015 Å to 0.09 Å. \( q \) is defined as:

\[
q = \frac{4\pi}{\lambda} \sin \theta
\]  

(3.2)

where \( \lambda \) is the wavelength and \( \theta \) is the scattering angle.

All the milk samples were inserted in a 96-well crystallization plates (MD11-58-20, CrystalQuick, USA) solution autoloader. All measurements were performed at room temperature (~24°C). The SAXS data were analyzed using scatterBrain (developed at the Australian Synchrotron). Relative intensity, \( I \), versus \( q \) was used to plot the scattering curves with water as blank. The combination between interfacial scattering intensity (\( I_{IF} \)) and internal structure scattering intensity (\( I_{IS} \)) will give total scattered intensity as described by the equation below:

\[
I_{Total} = I_{IF} + I_{IS}
\]  

(3.3)
3.5 Chemical methods

3.5.1 Reversed phase high-performance liquid chromatography (RP-HPLC)

The determination of whey protein denaturation in model processed cheese containing oil droplets stabilized with whey protein isolated was performed using reversed phase high performance liquid chromatography (RP-HPLC) as described by Parris & Baginski (1991).

Pre-treatment of samples

The samples were prepared using similar method as for particle size measurement (Section 3.4.2) but with slight modification using the method described by Gassi et al. (2008) and Jin & Park (1996). The model processed cheese samples (with or without heat treatment) (0.2g) was dispersed in a solution containing 1 mL of 35mmol L$^{-1}$ EDTA and 1 mL of sodium dodecyl sulphate (SDS, 1% p/v). Sodium hydroxide was added to the solution for pH adjustment to pH 7. The samples were left to stand overnight in a refrigerator at 5°C. The solution was centrifuged at 10,000 $\times$g (≈8310 rpm) at 4 °C for 30 min and then filtered through Whatman No. 1 filter paper to remove fat and other insoluble solids. The filtrate was diluted with an equal volume of 0.1% trifluoroacetic acid (TFA), and then passed through a 0.22 $\mu$M filter for RP-HPLC analysis.
Preparation of standards

The protein standards were prepared using the method described by Bobe, Beitz, Freeman, & Lindberg (1998) with slight modification. Individual protein standards: αs-casein (αs-CN), β-casein (β-CN), κ-casein (κ-CN), α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and bovine serum albumin (BSA) were diluted with water until the final concentration of standards were 4.0 mg/mL for αs-CN, 3.0 mg/mL for β-CN, 1.5 mg/mL for κ-CN, 0.5 mg/mL for α-Lac, 1.0 mg/mL for β-Lg and 1.0 mg/mL for BSA. About 100 µL of standard solutions were diluted with equal volume of 0.1% trifluoroacetic acid (TFA), then passed through a 0.22 µM filter for RP-HPLC analysis.

Protein determination

The analysis of whey protein denaturation was performed using RP-HPLC at room temperature using a HP series 1100 and a HP Interface 35900E (Agilent Technologies, CA, USA) (Figure 3.8).

The software package "OpenLAB CDS ChemStation Edition for LC and LC/MS systems" Rev.C.01.07 (Agilent Technologies, CA, USA), was used for data acquisition and peak integration. The column used was 0.46 X 25-cm, C-4 reversed phase (Jupiter C4 300A), 5-µ particle size equipped with guard column containing the same packing material (Phenomenex, CA, USA).

Elution buffers were: (A) 0.1% trifluoroacetic acid (TFA) and (B) acetonitrile, 0.1% TFA. The flow was 0.8 mL/min. The binary gradient was exponential from 30 to 45% B in 30 min. 20 µL of sample was injected into the column. The protein derivatives were detected at 280 nm absorbance. Percentage of denaturation was calculated from the sum of the standardized peak areas (SPA) or peak heights for the heated whey proteins BSA, α-LA, β-LG B and A variants and compared with those of control (model processed cheese stabilized with whey protein isolate without any heat treatment) using the equation below:

\[
\% \text{ Denaturation} = \frac{\sum \text{SPA}_c - \sum \text{SPA}_h}{\sum \text{SPA}_c} \quad (3.4)
\]

where \( \sum \text{SPA}_c \) is sum of SPA in control sample and \( \sum \text{SPA}_h \) is sum of SPA in heated sample.
Figure 3.8 Typical setup for reversed phase-HPLC (HP Series 1100), used for chromatography measurements.

3.6 Statistical analysis

The results were analysed statistically by one-way ANOVA using SPSS version 23 software (IBM SPSS Statistics, IBM, NY). Parameter estimates for the model were considered statistically significant at $P<0.05$. The results analysed were obtained in duplicate, unless otherwise stated.
Effect of processing shear and formulation on some physical properties of model processed cheese made with trisodium citrate (TSC)
4.1 Introduction

Emulsifying salts play an important role in processed cheese manufacture by chelating the calcium, disrupting the structural network and solubilizes the casein (Lee et al., 2004). Trisodium citrate (TSC) is one the earliest emulsifying salts found to create a smooth processed cheese product (Tamime, 2011). TSC which came from citric acid played an important role in preventing oiling-off and loss of moisture during processed cheese manufacture. Many studies have been done using TSC on processed cheese products (Gupta & Reuter, 1993; Mizuno & Lucey, 2005b; Purna et al., 2006; Savello et al., 1989; Shirashoji, Jaeggi, & Lucey, 2006; Sutheerawattananonda et al., 1997). Some studies used blends of TSC with other emulsifying salts such as disodium phosphate, sodium pyrophosphate, sodium hexametaphosphate and sodium tripolyphosphate (Abdel-Hamid, El-Shabrawy, Awad, & Singh, 2000; Awad et al., 2004; Gupta et al., 1984; Kaliappan & Lucey, 2011; Weiserová et al., 2011). However, there are only few studies that investigated the variations of fat and protein concentrations in processed cheese. The idea of this study is to improve the understanding of the relationship between emulsifying salt, protein matrix and oil droplets in model processed cheese structure. TSC was chosen because its function as an emulsifying agent in processed cheese has been supported by many studies. In this study, preliminary studies will be done to select the best formulation for processed cheese manufacture. Then, using the chosen formulation, model processed cheese will be manufactured and the product will be tested for rheological properties, oil droplet particle size, meltability and microstructural evaluation.

The objective of this study was to determine the impact of shear, different protein concentration and different oil concentration on the rheological properties, oil droplet particle size, meltability and microstructure on a model processed cheese spread system manufactured with citrate salts. Investigation of these parameters will allow the determination of optimal conditions (e.g. shear) for the preparation of model processed cheeses to be used in subsequent chapters. Furthermore, this will allow setting up the experimental methods to characterise these model processed cheeses.
4.2 Materials and Methods

4.2.1 Materials

The model processed cheese was made from calcium caseinate, sunflower oil, TSC, SHMP (2%), sodium chloride (NaCl) and citric acid. Note that SHMP and NaCl were only used in preliminary studies.

4.2.2 Preliminary studies on model processed cheese

The preliminary studies on model processed cheese were done as described in Chapter 3 (Section 3.2). Rheological measurements were done on these samples as described in Section 3.4.1 (Chapter 3). Microstructural evaluations were done on processed cheese samples using all the methods described in Section 3.4.4 (Chapter 3).

4.2.3 Sample preparation and experimental methods

The model processed cheese samples were prepared as previously described in Chapter 3 (Section 3.3.1). Briefly, water (575.0g), calcium caseinate powder (200.0g) and oil (200.0g) were poured into the thermomix using speed 3 to 5. Then, TSC (20.0g) and citric acid (5.0g) were added into the mix. The speed was increased to speed 6 to get a well-mixed mixture. Finally, the mixture was heated from 37 to 90ºC at speed 5 for 3 minutes and held at 90ºC for 2 minutes.

Model processed cheese samples were prepared in five different batches. The first batch contains model processed cheese with ten different mixing speeds (from 1 to 10). The second, third and fourth batches which were manufactured at speed 5 include model processed cheese with nine different protein concentrations (from 2.5 to 40%) with no oil, 20% oil and 30% oil respectively. The fifth batch which is the final batch of model processed cheese (Figure 4.1) had seven different oil concentrations (from 0 to 30%) with constant amount of protein (20%). For each batch, the ratio between protein and emulsifying salt remained constant (10:1) with pH around 5 to 6. The final composition, pH and moisture content of the model processed cheese samples in this chapter are shown in Appendix 2 and 3. Moisture content of
the model processed cheese samples was determined using the method as described in Appendix 1.

Each batch of model processed cheese samples were characterized in terms of their rheological properties, particle size measurements, melting properties and microstructure using the methods as previously described in Chapter 3 (Section 3.4). Among the microscopy methods, SEM was chosen to determine the microstructure of the model processed cheese samples.

![Processed cheese samples emulsified using TSC, with seven different oil concentrations ranging from 0 to 30\% (left to right) with constant amount of protein concentration (20\%).](image)

**Figure 4.1** Processed cheese samples emulsified using TSC, with seven different oil concentrations ranging from 0 to 30\% (left to right) with constant amount of protein concentration (20\%).
4.3 Results and discussion

4.3.1 Preliminary studies on model processed cheese

In this study, calcium caseinate was chosen as a protein source to manufacture processed cheese due to reproducibility. Furthermore, in order to create a new formulated processed cheese, a slight change of ingredients has to be made in comparison to Lee et al. (2004) and Cavalier-Salou & Cheftel (1991). Sunflower oil was chosen instead of butter oil to create a healthy model processed cheese. Processed cheese which originally has high sodium may need a reformulation to lower the salt content. Animal fat can be replaced with other fat from vegetable origins such as sunflower oil.

Figure 4.2 Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese between this study (left) and study from Lee et al. (2004) (right) (Reproduced with permission from Lee et al., 2004. © International Journal of Food Science and Technology). ($\rightarrow$) $G'$; ($\circlearrowleft$) $G''$. Arrow indicates $G'$ and $-G''$ cross-over of the two studies.

Figure 4.2 demonstrates the comparisons of elastic modulus ($G'$) and viscous modulus ($G''$) between model processed cheese manufactured in this study and those of a model processed cheese manufactured by Lee et al. (2004). This comparison was done to validate the
formulation and process used in this study. Based on result shown in Figure 4.2, the values of $G'$ and $G''$ and $G'$-$G''$ crossover (0.2 Hz, 2400 Pa) of the model processed cheese in this study closely represents the values of $G'$ and $G''$ and $G'$-$G''$ crossover (0.6 Hz, 2600 Pa) of model processed cheese with 49% moisture from Lee et al. (2004). The model processed cheese in this study has 41% moisture content. The variation between each cheese in this study is less than 14% (e.g. at low protein concentration, the difference is 13.6% and at high protein concentration, the difference is less than 2%) and the pH is within ±0.05 (see Appendix 2 and 3). This result proved that the formulation and process to manufacture model processed cheese in this study are adequate and can be used for further research.

### 4.3.1.1 Creaming phase during model processed cheese manufacture

![Graph showing the comparison of elastic modulus ($G'$) and loss modulus ($G''$) of model processed cheese with and without creaming phase and emulsified with TSC or SHMP.](image)

**Figure 4.3** Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) of model processed cheese with creaming phase and without creaming phase emulsified with TSC or SHMP. (△) $G'$ processed cheese emulsified with TSC with creaming phase; (△) $G''$ processed cheese emulsified with TSC with creaming phase; (△) $G'$ processed cheese emulsified with SHMP with creaming phase; (△) $G''$ processed cheese emulsified with SHMP with creaming phase; (△) $G'$ processed cheese emulsified with TSC without creaming phase; (△) $G''$ processed cheese emulsified with TSC without creaming phase; (△) $G'$ processed cheese emulsified with SHMP without creaming phase; (△) $G''$ processed cheese emulsified with SHMP without creaming phase. Protein concentration: 20%, oil concentration: 20%.
Besides that, the need of creaming phase during the manufacture of model processed cheese was also investigated. Creaming process involves alteration in viscosity of processed cheese. The viscosity modification occurs when processed cheese is manufactured with longer cooking time using certain types of emulsifying salt (Shirashoji et al., 2006). Study performed by Lee, Buwalda, Euston, Foegeding, and McKenna (2003) showed that, the alteration in viscosity of processed cheese during creaming phase can occur with or without the addition of fat. However, in this study, Figure 4.3 shows no significant difference ($P<0.05$) between the rheological behaviour ($G'$ and $G''$ moduli) for model processed cheese samples (emulsified with TSC or SHMP) with creaming phase and without creaming phase (manufacture is stopped just after the heat treatment as presented in Chapter 3; Figure 3.1).

This result showed that prolonged cooking would not affect the viscosity of model processed cheese. This could be due to the fact that calcium caseinate is used as the source of protein. A study done by Srinivasan, Singh and Munro (2001) showed that the creaming stability of emulsion with calcium caseinate increased gradually with the increased protein concentration. This is because calcium caseinate consists of large casein aggregates (20 to 1000 nm) which is too large to cause flocculation by depletion. Therefore, it is regarded that, in this study, the creaming phase can be eliminated from the cheesemaking process to reduce manufacturing time.

### 4.3.1.2 Addition of NaCl

Sodium chloride (NaCl) was added in cheese for various purposes such as preservation, final moisture control, enzyme activity control, providing better texture and flavour enhancement (Johnson, Kapoor, McMahon, McCoy, & Narasimmon, 2009). The reduction of NaCl concentration was investigated in this study due to high sodium levels in processed foods which may negatively affect human health (Kawasaki, Delea, Bartter, & Smith, 1978). With the existence of other preservation methods such as refrigeration, the need of NaCl in cheese is less important (Gould, 1995).

In processed cheese manufacture, the addition of NaCl in cheese affects the structure and increase the hardness of the cheese (Paulson, McMahon, & Oberg, 1998). In this study, rheological measurements of model processed cheese with added NaCl (1% w/w) either emulsified with TSC or SHMP showed no significant difference ($P<0.05$) in terms of
rheological behaviour with model processed cheese manufactured without NaCl either emulsified with TSC or SHMP (Figure 4.4). Therefore, it can be concluded that, the addition of NaCl (1%) in this study has very little or no effect on the structure of model processed cheese. Based on the results of the preliminary studies on model processed cheese, creaming phase is unnecessary for this study and the use of sodium chloride can be omitted as part of the ingredients of the model processed cheese considered in this thesis.

**Figure 4.4** Comparisons of elastic modulus ($G'$) and loss modulus ($G''$) of model processed cheese emulsified with TSC or SHMP and made with 1% NaCl or without NaCl. (●) $G'$ processed cheese emulsified with TSC made with 1% NaCl; (○) $G''$ processed cheese emulsified with TSC made with 1% NaCl; (▲) $G'$ processed cheese emulsified with TSC without NaCl; (◇) $G''$ processed cheese emulsified with TSC without NaCl; (■) $G'$ processed cheese emulsified with SHMP made with 1% NaCl; (□) $G''$ processed cheese emulsified with SHMP made with 1% NaCl; (◆) $G'$ processed cheese emulsified with SHMP without NaCl; (▼) $G''$ processed cheese emulsified with SHMP without NaCl. Protein concentration: 20%, oil concentration: 20%.

### 4.3.1.3 Comparison of microscopic images

As mentioned in the *Chapter 2* (Section 2.10), three microscopic techniques were used to obtain the best image of the model processed cheese protein network and oil droplets. Figure 4.5 displays the images of model processed cheese made with 20% protein and 20% oil using
CLSM, Cryo-SEM and SEM. SEM was found to offer the best image of the model processed cheese giving a clearer picture of how the oil droplets interact with the protein network. The fat globules and the protein strands can be seen clearly using SEM compared to CLSM and Cryo-SEM.

**Figure 4.5** Comparisons of microscopic images of model processed cheese made with 20% protein and 20% oil using CLSM (a), Cryo-SEM (b) and SEM (c).
4.3.2 Effect of shear

Complex modulus \( G^* = (G' + G'')^{1/2} \) takes into account both the contributions of the elastic modulus \( G' \) and the loss modulus \( G'' \) (Dimitreli & Thomareis, 2008). The impact of shear during processed cheese cooking on the evolution of \( G^* \) has been studied and presented in Figure 4.6.

![Figure 4.6](image.png)

**Figure 4.6** Evolution of the complex modulus \( (G^*) \) of model processed cheese samples (20% protein, 20% oil) manufactured in a lab cooker (Thermomix) with different speeds (1 to 8) (rheological parameters: 20°C, 1Hz, applied strain of 10%). Data represent means \((n = 2)\) and error bars correspond to standard deviations.

\( G^* \) values increased quasi-linearly with the increased of speed (value around 8 kPa at speed 1 to values around 16 kPa at speed 6). Processed cheese made with speed 1 had a softer texture compared to processed cheese made with speed 6 that present a higher resistance. However, when speed 8 was used during the cheese processing, the \( G^* \) value slightly decreased (dropped to \(~13,570 \) Pa). The optimal speed that affect the processed cheese firmness for the lab scale manufacture of processed cheese is 6 on a scale of 10. These results were consistent with those of Tamime (2011) that present an increase of firmness and elasticity \( (G' \) and \( G'' \) moduli) for unheated block processed cheese manufactured at processing speed of 300 to 2700 rpm. Increasing the speed will decrease the size of the oil droplets resulting in more efficient oil droplet emulsification. Lee et al. (2003) suggested that higher shear rates during
processed cheese manufacture caused higher amount of small, evenly distributed oil droplets in the protein network compared to lower shear rates upon constant cooking time and temperature.

![Figure 4.7](image_url)

**Figure 4.7** Mean average oil droplet particle sizes ($D(3, 2)$ in $\mu m$) of processed cheese manufactured in a lab cooker (Thermomix) with different speeds (1 to 10). Data represent means ($n = 2$) and error bars correspond to standard deviations.

The results shown in Figure 4.7 demonstrates an agreement with this theory. Indeed, the speed applied during processed cheese manufacture significantly influenced the oil droplet particle size of the samples. Cheese matrix made with higher speeds (> 6) presenting smaller oil droplet particles (~0.2 $\mu m$) than with lower shear (< speed 6, $D(3, 2)$ average: ~0.4 $\mu m$). By increasing the processing speed, homogenization of the processed cheese matrix occurs where the oil droplet size and size heterogeneity decreased. This enhances the inclusion of oil droplets into the protein matrix (Jost, Baechler, & Masson, 1986). Due to the complete disruption of the native heterogenous structure, homogenization also caused an increased in protein surface area. This would enhance water binding capacity of the protein matrix which leads to increased proportion of protein being adsorbed to oil-water interface (Glenn et al., 2003; Jost et al., 1986).

Gelation occurs when the gel matrix containing caseins and recombined protein-coated oil droplets act like large protein units forming a network (Aguilera & Kinsella, 1991). During
heat treatment process, these protein-coated oil droplets are able to bind more proteins, thus facilitate the gel matrix formation (Lee et al., 2004).

When the processing speed reached the maximum limit, oil-protein and protein-protein interactions were becoming stronger to an extent that the casein molecules coagulate into a “pudding-like” structure (Purna et al., 2006). This kind of occurrence is known as “overcreaming” where there is an increase in viscosity and reduction in meltability (Glenn et al., 2003). This phenomenon can be seen from Figure 4.6, where the highest viscosity occurs when speed 6 was applied during processed cheese processing.

As the speed increased higher than speed 6, the $G^*$ of the processed cheese decreased. This may have occurred because once the shear rate exceeded (speed 6), the protein-gel network structure may have collapsed when higher shear rate was applied (speed 8; ~7164 rpm). Study done by Lee et al. (2003) indicated that, increasing shearing time up to 40 minutes decreased their processed cheese apparent viscosity. Another study done by Swenson, Wendorff, & Lindsay (2000) is in agreement with Lee et al. (2003), where increasing shearing time up to 20 minutes decreased the firmness and increased the meltability of the processed cheese. This would confirm that the extent of high shear is detrimental to the final processed cheese elasticity. The excessive amount of shear eventually breaks the newly formed protein, thus breaking the gel network structure and forming weaker cheese matrix upon cooling.
4.3.3 Effect of protein concentration

The increase of protein concentration resulted in an increase in viscoelasticity as evidenced by increases in $G'$ and $G''$ (Figure 4.8). The increase in oil concentration (20% of oil) only has an impact on low protein product (5% of protein or less, i.e. 2.5%).

Figure 4.8 Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 5, 15 and 30% of protein concentration with (20%) and without oil addition. (–) $G'$ 5% protein, 0% oil; (––) $G''$ 5% protein, 0% oil; (–) $G'$ 15% protein, 0% oil; (– –) $G''$ 15% protein, 0% oil; (– – –) $G'$ 30% protein, 0% oil; (– – – –) $G''$ 30% protein, 0% oil; (– – – –) $G'$ 5% protein, 20% oil; (– – – – –) $G''$ 5% protein, 20% oil; (– – – – – –) $G'$ 15% protein, 20% oil; (– – – – – – –) $G''$ 15% protein, 20% oil; (– – – – – – – –) $G'$ 30% protein, 20% oil; (– – – – – – – – –) $G''$ 30% protein, 20% oil.

Indeed, the $G^*$ of processed cheese samples made with 2.5% protein increased with increasing oil concentration giving the lowest sets of $G^*$ values compared to other protein concentration (Figure 4.9). As the protein concentration increased to 15%, the difference between the processed cheese samples with 0, 20 and 30% oil (~3.5 kPa, ~4.6 kPa and ~6.5 kPa respectively) starts to get smaller which is an indication that the processed cheese samples started to show gel-like behaviour, and became more difficult to spread. All
processed cheese samples formed good emulsions except those at and below 5% of protein concentration, where oil separation was observed. White and homogenous emulsions were formed easily for the processed cheese samples with protein concentration of 10% and above.

Figure 4.9 Evolution of complex modulus ($G^*$) of model processed cheese samples manufactured with different protein concentrations (2.5 to 40%) and different oil concentrations (0 to 30%). (■) No oil; (○) 20% oil; (▲) 30% oil. Data represent means (n = 2) and error bars correspond to standard deviations.

Processed cheese samples with higher protein concentration appeared firmer than the samples with lower protein concentration (appeared as soft gel-like emulsions). Improper emulsion formation of the cooked processed cheese produced a defect known as oil separation (Kapoor & Metzger, 2008). There are many factors contributing to the improper emulsion including (i) the amount of emulsifying salts in the processed cheese, (ii) the low final pH of processed cheese, (iii) the low level of intact casein in the processed cheese or (iv) insufficient or very extensive processing time or temperature during processed cheese manufacture (Kapoor & Metzger, 2008). Intact casein is defined as the amount of casein that has not been hydrolysed (Bachmann, 2001).

In this study, it can be seen that improper emulsion might be caused by the low final pH of the model processed cheese.
McClements (2004) stated that all food emulsions are thermodynamically unstable, which can break down and show phase separation when they are left to a certain extent of time. Therefore, the addition of emulsifiers salts such as chelating salts during the homogenization process can enhance the casein solubility and the food’s kinetics stability. In this study, the addition of TSC to the model processed cheese system results in calcium-sodium ion exchange with the calcium present in the calcium-caseinate, resulting in the dissociation of the casein aggregates. In this study, when very low protein concentration (2.5%) was used, all the calcium (Ca) molecules have been chelated by TSC which results in high casein dispersion. However, the final amount of dispersed casein was not enough to react with excess oil and water in the protein network thus oil separation was formed.

Research done by Lee et al. (2004), showed that processed cheese with lower moisture contents were firmer than those with higher moisture contents. The $G'$ and $G''$ moduli also decreased with increasing moisture content. Besides that, the cross-over of $G'$ and $G''$ shifted to lower frequencies as the moisture content decreased. This study showed consistent result with Lee et al. (2004). In this study, when the protein concentration increased (from 2.5 to
$G^*$ also increased (from ~0.7 Pa to ~68 kPa for 0% oil concentration; from ~12 Pa to ~49 kPa for 20% oil concentration; from ~165 Pa to ~44 kPa for 30% oil concentration) (Figure 4.9) and the cross-over of $G'$ and $G''$ shifted to lower frequencies (Figure 4.8). Processed cheese manufactured with 30% oil has the highest $G^*$ values due to highest resistance followed by the processed cheese manufactured with 20% oil then without any oil (lowest resistance). This trend is similar for protein concentration starting from 25% to 2.5%. Furthermore, in this study, the measured $G^*$, $G'$ and $G''$ moduli and the shear stress increased as the protein concentration increased (Figures 4.8 to 4.11). This indicates greater fluidity due to greater protein-protein interactions, enhanced by protein-oil interactions.

Protein-protein interactions occur via their exposed nonpolar groups during cooking (Lee et al., 2003). In the presence of mechanical and heat energies, the emulsifying salts (strong polyvalent anions) break the intramolecular interactions (i.e., electrostatic bonds) causing the charged groups on casein molecules to expose and develop new bonds or interactions with other casein molecules. A new network between casein molecules is formed via hydrophobic and, electrostatic interactions, but also hydrogen bonds upon cooling. This process which is known as caseins reassociation or repolymerization may produce a stiffer and less meltable matrix due to the greater dispersion or exposure of groups on casein (Lee et al., 2003; Shirashoji et al., 2006). Yield stress is observed for processed cheese to monitor weak gel formation (Norton, Spyropoulos, & Cox, 2010).

For a formulation with 30% protein concentration or more, the addition of oil (0-30%) has no impact on the $G^*$ for processed cheese (superimposed values) (Figure 4.9); no significant difference can be seen in model processed cheese made with 30% protein with different oil concentration ($P<0.05$). Figure 4.9 also presented that oil content seems to play a role only in processed cheese emulsions made with low protein concentration ($\leq5\%$). At high protein concentrations, protein content seems dominating the rheological behaviour of the processed cheeses.

Protein-protein interactions and protein-oil interactions are reported to highly affect the viscosity of processed cheese at the end of manufacture (Lee et al., 2003; Purna et al., 2006). Lee et al. (2004) suggested that greater oil phase surface was formed when the protein hydration increased. Increasing protein hydration enhances the interaction between protein molecules with the oil droplet surface which produce better stabilization (Lee et al., 2004).
But in this range of low protein concentration, emulsion remained liquid. Protein network as well as oil droplet size and moisture have the ability to affect the viscoelastic properties of processed cheese (Norton et al., 2010). The elastic response ($G'$) was mostly influenced by protein–protein bonds while the viscous response ($G''$) was affected by the ability of the liquid present in the matrix to flow through the matrix network and the movement of other structural elements relative to each other.

**Figure 4.11** The evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (●) of model processed cheese manufactured with different protein concentrations (2.5 to 40%) (oil concentration: 30%). Data represent means ($n = 2$) and error bars correspond to standard deviations.

In Figure 4.10, processed cheese with different protein concentrations showed different large deformation behaviour. The $G'-G''$ crossover of processed cheese made with 5% of protein occur at higher strain but lower measured moduli compared to processed cheese made with 10% protein. The $G'-G''$ crossover of processed cheese made with 20% of protein showed the highest strain but occurred at lower measured moduli compared to processed cheese made with 10% of protein. Strain is defined as the deformation of solid due to stress (Kazimi, 2001). Comparison between the different processed cheeses critical strain $\gamma_c$ and the corresponding critical shear stress $\sigma_c$ at which the $G'-G''$ crossover occurs are plotted in Figure 4.11. It can be seen that increasing protein concentrations from 2.5 to 40%, increased the critical shear stress $\sigma_c$ (from $\sim$38 Pa to $\sim$7 kPa), which indicates processed cheese became firmer (Figure
On the other hand, the critical strain $\gamma_c$ of model processed cheese decreased from ~89 to ~29% when the protein content increased from 2.5 to 10% protein, then increased from ~142 to ~225% when the protein increased again from 15 to 40%. As discussed earlier, model processed cheese made with very low protein concentration ($\leq 5\%$) possessed low stability which makes the measurement of their rheological properties difficult. A more reliable result can be seen when the model processed was made of $\geq 10\%$ protein concentration. The critical strain $\gamma_c$ of model processed increased relatively in a similar fashion as the increase in the critical shear stress $\sigma_c$. This indicates that, as protein concentration increased, the gel network of model processed cheese becomes stronger, thus require higher stress to break the firmer internal matrix, as shown in Figure 4.9, where model processed cheese becomes firmer (more elastic) as the protein concentration increases.

![Figure 4.12](image)

**Figure 4.12** Oil droplet particle size distribution for model processed cheese manufactured with 5, 20 and 40% of protein concentration (oil concentration: 20%). (■) 5% protein, 20% oil; (▲) 20% protein, 20% oil; (●) 40% protein, 20% oil.

Processed cheese made with 5% of protein showed the widest oil droplet particle size distribution followed by processed cheese made with 40 and 20% of protein (Figure 4.12). Both model processed cheese made with 5 and 40% of protein concentration displayed 2 peaks (bimodal distribution) compared to model processed cheese made with 20% of protein concentration. Model processed cheese made with 5% protein concentration demonstrated
product instability by having a wider range of particle size and having 3 peaks in Figure 4.12.

Figure 4.13 shows a decrease in oil droplet particle size (~6.2 to ~0.4 µm) as the protein concentration increased from 2.5% up to 20% of protein. As the protein concentration increased (from 25 to 40%), the oil droplet particle size also increased (~0.5 to ~2.4 µm). Model processed cheese made with 2.5 and 5% of protein presented the highest oil droplet particle size (~6.2 and ~4.2 µm, respectively) and a liquid-like emulsion structure. As the protein concentration increased to 10%, a significant drop of oil droplet particle size can be seen (~0.5 µm) corresponding to the initiation of a solid-like emulsion.

![Figure 4.13](image)

**Figure 4.13** Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%). Data represent means ($n = 3$) and error bars correspond to standard deviations.

The lowest particle size was obtained for 20% of protein (~0.4 µm) while for more concentrated samples, the oil droplet particle size increased again. This indicated that TSC (2%) showed its best emulsifying ability when processed cheese made with 20% protein and 20% oil concentrations. This is also supported by the oil droplet particle size distribution result in Figure 4.12 where model processed cheese made with 20% of protein concentration demonstrated a single peak compared with other model processed cheeses. Processed cheese with 40% of protein displayed higher oil droplet particle size (~2.4 µm). As the protein
concentration increased, the viscosity of the continuous phase increased. Since the amount of shear is constant, theoretically the particle size will decrease. However, in this study, as the protein concentration increased up to 40%, the oil droplet particle size increased. There has been no studies done to discuss this issue and this phenomenon (at higher protein concentration) could be interesting to be further studied in the future.

Oil droplet particle size is also associated with melting percentage, properties extensively relevant to analogue processed cheese that consist to transform a ‘solid-like’ into a ‘liquid-like’ state under heat treatment. Based on Figure 4.14, the protein concentration influenced the melting percentage of the processed cheese. Melt test was unable to be done on processed cheese manufactured with 2.5 and 5% protein due to its liquid form.

![Figure 4.14](image)

**Figure 4.14** The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations.

Processed cheese made with 15% of protein presented the highest melting percentage. When protein concentration increased from 15 to 40%, the melting percentage of the processed cheese decreased rapidly from approximately 88 to approximately 31%. These results might indicate that the optimal protein concentration (20%) consistent with the presence of small
particles size ($D_{3,2} < 1 \mu m$) after manufacture and optimal melting properties. This might be because protein to oil ratio (POR) in processed cheese made with 20% protein concentration was the most optimum for the interaction between emulsified oil droplet with the casein matrix. In this sample, the adsorbed hydrophobic protein parts are buried within the oil droplet since the oil droplet surface was hydrophilic resulting in a smaller amount of hydrophobic interaction (Everett & Olson, 2003). This causes the processed cheese sample (20% protein) to become less cohesive (lower gel strength or $G^*$). Processed cheese with 40% of protein concentration has the highest POR compared to other processed cheese samples. According to Prentice (1992), once the amount of casein reached certain extent (maximum), extra casein will aid in providing a stronger matrix and chain junctions. This statement corresponds to the processed cheese with 40% of protein concentration which demonstrated the lowest melting percentage.

### 4.3.4 Effect of oil concentration

Figure 4.15 shows that the measured moduli ($G'$ and $G''$) of model processed cheese increased with the increase in oil concentration. Processed cheese made with 20% and 10% of oil concentration showed higher $G'$ and $G''$ moduli compared to processed cheese made without oil (Figure 4.15). This indicates that model processed cheese made with 20% oil concentration is firmer compared to model processed cheese made with less oil content. The presence of oil in the protein network system during the manufacture of model processed cheese has induced protein-oil droplets interactions along with protein-protein interaction (Purna et al., 2006). These interactions assist the formation of a stronger gel network within the processed cheese matrix (Cunha et al., 2010).

Besides that, the cross-over of $G'$ and $G''$ shifted to lower frequencies as the oil concentration increased (Figure 4.15). This indicates that the molecular interactions were stronger in the processed cheese with 10% and 20% oil concentration compared to processed cheese without oil. $G^*$ of processed cheese with fixed amount of protein (20% of protein) increased linearly (~7.8 to ~13.5 kPa) with the increasing amount of oil concentration (0 to 15%) (Figure 4.16). However, when the oil concentration reached 15% and increased to 30%, $G^*$ values reached a plateau (~13.5 kPa).
Figure 4.15 Evolution of elastic modulus \((G')\) and loss modulus \((G'')\) as a function of frequency of model processed cheese manufactured with 0, 10 and 20% of oil concentration at 20% protein concentration. \((\square)\) \(G'\) 0% oil; \((\square)\) \(G''\) 0% oil; \((\triangle)\) \(G'\) 10% oil; \((\triangle)\) \(G''\) 10% oil; \((\circ)\) \(G'\) 20% oil; \((\circ)\) \(G''\) 20% oil.

Cheng, Augustin, McKinnon, and Sutherland (1997) stated that, when high level (0.2 mol/kg of milk solids non-fat (MSNF)) of phosphate or Ca was added to cheese milk, the stretchability and meltability of the cheese milk decreased. The caseins were cross-linked by the formation of a new caseinate-Ca phosphate complex. This caused the formation of more casein-casein interactions thus reducing the meltability (Cheng et al., 1997; Mizuno & Lucey, 2005b).

Figure 4.16 seems to confirm this hypothesis (optimal value of \(G^*\) obtained for a specific ratio between protein and oil (15% oil and 20% protein). As discussed earlier in Section 4.3.3 of this chapter, TSC (2%) demonstrated its best emulsifying capability when model processed cheese was manufactured with 20% protein and 20% oil. This could be the reason for the insignificant changes when oil concentration was increased from 15 to 30%.
Figure 4.16 Complex modulus ($G^*$) of processed cheese samples as a function of oil concentration (protein concentration: 20%). Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 4.17 presents critical strain $\gamma_c$ and critical shear stress $\sigma_c$ of model processed cheese made with different oil concentration. Based on Figure 4.17, the critical shear stress $\sigma_c$ decreased from ~3.4 to ~1.5 kPa with the increasing of oil concentrations from 0 to 30%.

Figure 4.17 Evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (●) of model processed cheese manufactured with 20% of protein and different oil concentration. Data represent means ($n = 2$) and error bars correspond to standard deviations.
This occurred due to the increase in oil concentration in the processed cheese system where it decreases the critical shear stress $\sigma_c$ required to induce a flow of the processed cheese. On the other hand, the critical strain $\gamma_c$ of model processed cheese increased from ~126 to ~158% when the oil concentration increased from 0 to 15%, then plateaued at ~158%, when the oil concentration was further increased from from 15 to 30%. Since the model processed cheese made with $\geq$15% oil concentration are firmer compared to model processed cheese made with lower oil concentration, these high oil processed cheese showed higher $\gamma_c$.

Figure 4.18 Oil droplet particle size distribution for model processed cheese manufactured with (––) 5% oil; (–•–) 20% oil and (––) 30% oil concentration (protein concentration: 20%).

Figures 4.18 and 4.19 presented the oil droplet particle size distributions and oil droplet particle size of model processed cheese made with 5 to 30% of oil concentration. Based on Figure 4.18, model processed cheese manufactured with 30% oil concentration demonstrated the widest oil droplet particle size distributions compared to model processed cheese manufactured with 5% oil and 20% oil. Model processed cheese made with 20% oil displayed a more stable oil droplet particle size distribution (having one peak) compared to model processed cheese made with 5% and 30% oil concentrations (having 2 peaks). Oil droplet particle size ($D(3, 2)$) of model processed cheese increased from ~0.2 to ~0.6 $\mu$m with
the increasing amount of oil concentration from 5 to 30% (Figure 4.19). This will be discussed further along with the results of SEM images and melting properties of the model processed cheese.

**Figure 4.19** Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese manufactured with 20% of protein as a function of oil concentration (5 to 30%). Data represent means (n = 3) and error bars correspond to standard deviations.

SEM images obtained from model processed cheese made with 20% oil concentration also supported this statement which showed smaller oil droplets and a more homogenously distribution of oil droplets compared to model processed cheese made from 30% oil concentration (Figure 4.20). Increasing the oil concentration in processed cheese may decrease the surface area of the oil-water interface that could be stabilised due to the low levels of protein solubilisation, thus, forming large oil droplets and wider particle size distributions (Figures 4.18 and 4.19) (Cavalier-Salou & Cheftel, 1991; Williams & Phillips, 2000). In this study, the oil to water interface was calculated using an equation giving the surface area as a function of $D(3, 2)$ of the oil concentration. Based on the calculation, the oil to water interface decreased (from 258 to 68 mg/m²) with the increased of oil concentration (0 to 30%). Figure 4.20 showed the images of model processed cheese with 5 to 30% oil concentration taken from scanning electron micrographs (SEM).
Figure 4.20 Scanning electron (SEM) micrographs of model processed cheese made with 20% of protein and different concentrations of oil: a) 5%, b) 10%, c) 15%, d) 20%, e) 30%. Scale bar = 2µm.
Based on the SEM images, as the oil concentration increased (15 to 30%), the number of oil droplets decrease and the diameters of the oil droplets increased (by visual observation, from 0.6 to 1.3 µm). This result is consistent with Cunha, Dias, and Viotto (2010) who compared SEM images of traditional processed cheese with milk fat and SEM images obtained from model processed cheese with increasing amount of vegetable fat. According to their study, high amounts of small fat particles were dispersed in a uniform protein network in traditional processed cheese.

The model processed cheese with increasing amount of vegetable fats on the other hand, demonstrated less uniformly distributed fat globules within the protein matrix with greater diameter. Cunha et al. (2010) relates the difference in fat globules diameter and distributions to the nature of the oil and the poor emulsification process in model processed cheese made with vegetable fat. Poorly emulsified model processed cheese showed large oil droplets size (Williams & Phillips, 2000). The greater the diameter of oil droplets created a greater protein mass per unit area of oil which is connected to higher hardness values and greater resistance to deformation (Cunha et al., 2010). This statement is consistent with the result of the melting percentage of model processed cheese.

![Figure 4.21](image)

**Figure 4.21** The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%). Protein concentration: 20%. Data represent means (n = 3) and error bars correspond to standard deviations.
Based on Figure 4.21 and 4.22, the melting percentage of model processed cheese made with 0 to 20% oil concentration was independent of oil concentration (~80.3%). However, as the oil concentration increased from 25 to 30%, the height melting percentage of the processed cheese decreased from ~71 to ~69%. When the oil concentration increased to 25% and 30%, the oil phases of these processed cheese samples were poorly emulsified. This can be seen as processed cheese made with 20 and 30% of oil concentration produced wider oil droplet particle size distribution compared to processed cheese made with 5% of oil concentration (Figure 4.18). A simple explanation is that at low oil concentration there are a lot of proteins available for full coverage of the oil droplets. When the concentration increase markedly, the amount of protein to oil decreases and poor emulsification is expected to occur. According to the study done by Srinivasan, Singh and Munro (1999), the adsorption behaviour of calcium caseinate on the oil droplet surface increased with the increased of protein concentration (e.g. at 1% protein concentration, the surface protein load is 1.2 mg/m²). In this study, the surface protein load can be calculated using $D(3,2)$, and it is estimated that at 1% protein concentration, the surface protein load is ~1.29 mg/m² which is higher to the value reported by Srinivasan et al. (1999). Therefore, it can be concluded that the amount of protein used is high enough to stabilise the oil droplets.

**Figure 4.22** Melting pictures of model processed cheese manufactured with different oil concentration (0 to 30%). Protein concentration: 20%.

Melting of processed cheese can be influenced by the degree of oil droplet emulsification and the degree of casein dissociation (Cavalier-Salou & Cheftel, 1991). In this study, ESEM image of processed cheese with 5% oil concentration displayed loose protein matrix compared to processed cheese made with higher oil concentration (Figure 4.20). The melting percentage also demonstrated that processed cheese with 5% of oil concentration was higher (less firmness, ie. smaller $G^*$) compared to processed cheese with 30% oil concentration (high firmness). This was due to higher degree of casein dissociation and higher degree of oil droplet emulsification which results in higher melting properties in processed cheese made
with 5% oil concentration. In processed cheese made with 30% oil concentration, close interactions between oil and hydrophobic zones of proteins with excessive amount of calcium bridging may inhibit melting properties (Cavalier-Salou & Cheftel, 1991; Hokes et al., 1982).

4.4 Summary to chapter

The preliminary studies on model processed cheese in this study proved that, creaming phase is unnecessary and the use of sodium chloride can be omitted as part of the ingredients during the manufacture of model processed cheese. Increasing shear rates and varying protein and oil concentrations affected the rheological properties, oil droplet particle size, meltability and microstructure of model processed cheese spread system. Increasing the shear during processing decreased the size of the oil droplets and promoted oil droplet emulsification. Processed cheese samples made with higher shear presented a more homogenous distribution of oil droplet particle size which clearly indicates that increasing the speeds promotes better oil droplet emulsification. The $G^*$ of the processed cheese with no oil demonstrated the highest value due to higher protein-protein interactions in the gel matrix. Processed cheese made with 20% of protein concentration and 20% oil concentration presented optimal results for particle size, rheology ($G^*$) and melting properties due to optimal interaction between emulsified oil droplet with the casein matrix. Increasing oil concentrations on the other hand, would decrease the surface area of the oil-water interface thus forming large oil droplets and wider particle size distributions. Higher amount of oil droplets can enhance the oil-protein and protein-water interactions thus forming a stronger gel network within the processed cheese matrix. This study demonstrated that it is possible to tailor the rheological and melting properties of the processed cheese by manipulating the different compositional parameters.
Effect of formulation on the physical properties of model processed cheese made with sodium pyrophosphate (TSPP)
5.1 Introduction

Processed cheese in comparison to natural cheese is a product which was developed about a century ago (Lucey, Maurer-Rothmann, & Kaliappan, 2011). During the manufacturing process of processed cheese, some important elements of physicochemical properties (rheological, textural, meltability and microstructure) to improve stability and shelf life were taken into account (Lu et al., 2008). Emulsifying salts in processed cheese are not known as real emulsifiers as they are not surface active compounds but rather act as melting salts (Lee, Klostermeyer, Schrader, & Buchheim, 1996; Lucey et al., 2011). The use of emulsifying salts in processed cheese is important as it assists the product to melt smoothly and uniformly when heated. This process includes calcium chelation from the para-casein network and protein solubilisation, thus creating stable emulsions (Purna et al., 2006).

In 2003, the Code of Federal Regulations in the United States of America came out with 13 different emulsifying salts which can be part of the ingredients to make processed cheese (CFR, 2012; Lucey et al., 2011; Purna et al., 2006). The emulsifying salts can be used alone or in combination with other emulsifying salts. These emulsifying salts can be added up to 3% during the manufacture of the processed cheese. Weiserová et al. (2011) combined phosphate emulsifying salts (disodium hydrogen phosphate, tetrasodium diphosphate, pentasodium triphosphate and sodium polyphosphate) to a certain ratios to modify the hardness, cohesiveness and adhesiveness of model processed cheese. The outcome of their study proves that combination of monophosphates created softer cheese samples whereas involving polyphosphates gave the processed cheese samples a more rigid form.

Sodium pyrophosphate (TSPP) is one of the emulsifying salts that are commonly used in the cheese-making process. The selection of emulsifying salts for cheese-making process usually depends on its chelating ability (Chen & Liu, 2012). Guinee et al. (2004) made a summary of the chelating ability of emulsifying salts as follows: sodium polyphosphates > TSPP > sodium orthophosphate > trisodium citrate (TSC). Chen and Liu (2012) made analogue processed cheese using natural cheese (Mozarella cheese), butter, whey protein concentrate, skim milk powder and different types of emulsifying salts (citrate, orthophosphate, pyrophosphate and polyphosphate). The results obtained from their study showed
contradicting result as the hardness of processed cheese was as follows: polyphosphate (sodium hexametaphosphate) < orthophosphate < citrate < pyrophosphate. Their result indicated that the use of pyrophosphate as emulsifying salt provides better emulsification of the oil droplets during cheese-making process compared to other emulsifying salts. Another study done by Lu et al. (2008) also described that model processed cheese made with citrate and pyrophosphate displayed higher hardness compared to model processed cheese made with other emulsifying salts. These contradicting results may be due to variation of natural cheese used, the quality of milk, experimental conditions, processing conditions and the composition of the final product (Chen & Liu, 2012; Henning, Baer, Hassan, & Dave, 2006).

In this chapter, TSPP was chosen as an emulsifying salt for model processed cheese because based on the reports, processed cheese made with TSPP demonstrated higher firmness compared to processed cheese made with other emulsifying salts. Furthermore, we would like to develop better understanding of the interaction between casein and emulsifying salts. In Chapter 4, it was found that it is possible to prepare model processed cheese using calcium caseinate and TSC as the emulsifying salt with and without oil. The rheological properties, oil droplet particle size, meltability and microstructure of model processed cheese made with TSC were highly affected by the increase of the amount of processing shear and changes of the oil and protein concentrations. Therefore, in this chapter, we would like to compare the difference between using trisodium citrate and sodium pyrophosphate as the emulsifying salts for the model processed cheese.

The objective of this study was to determine the influence of different protein concentration and different oil concentration on the rheological properties, oil droplet particle size, meltability and microstructure on a model processed cheese spread system manufactured with TSPP. The outcome of this chapter will be compared with the previous chapter on citrate salts.
5.2 Materials and Methods

5.2.1 Materials

The model processed cheese was made from calcium caseinate, sunflower oil, TSPP and citric acid.

5.2.2 Sample preparation and experimental methods

The model processed cheese samples were prepared as previously described in Chapter 3 (Section 3.3.1). Briefly, water (575.0g), calcium caseinate powder (200.0g) and oil (200.0g) were poured into the thermomix using speed 3 to 5. Then, TSPP (20.0g) and citric acid (5.0g) were added into the mix. The speed was increased to speed 6 to get a well-mixed mixture. Finally, the mixture was heated from 37 to 90°C at speed 5 for 3 minutes and held at 90°C for 2 minutes.

Model processed cheese samples were prepared in seven different batches. The first batch contains model processed cheese with seven different oil concentrations (from 0 to 30%) with constant amount of protein (20%) as shown in Figure 5.1. The second, third, fourth, fifth, sixth and seventh batches which were manufactured at speed 5 include model processed cheese with nine different protein concentrations (from 2.5 to 40%) with no oil, 5% oil, 10% oil, 15% oil, 20% oil and 30% oil respectively. For each batch, the ratio between protein and emulsifying salt remained constant (10:1) with pH around 5 to 6. The final composition and pH of the model processed cheese samples in this chapter are shown in Appendix 2 and 3.

![Figure 5.1 Processed cheese samples made with TSPP, with seven different oil concentrations ranging from 0 to 30% (left to right) with constant amount of protein concentration (20%).]
Each batch of model processed cheese samples were characterized in terms of their rheological properties, particle size measurements, melting properties and microstructure using the methods as previously described in Chapter 3 (Section 3.4). Among the microscopy methods, SEM was chosen to determine the microstructure of the model processed cheese samples.

5.3 Results and discussion

5.3.1 Effect of protein concentration

Protein concentration plays an important role in this study. The results of frequency sweep tests on model processed cheese emulsified with sodium pyrophosphate (TSPP) at different protein concentration is presented in Figure 5.2.

![Figure 5.2](image)

**Figure 5.2** Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 5, 15 and 30% of protein concentration with (20%) and without oil addition. (---) $G'$ 5% protein, 0% oil; (---) $G''$ 5% protein, 0% oil; (---) $G'$ 15% protein, 0% oil; (---) $G''$ 15% protein, 0% oil; (---) $G'$ 30% protein, 0% oil; (---) $G''$ 30% protein, 0% oil; (---) $G'$ 5% protein, 20% oil; (---) $G''$ 5% protein, 20% oil; (---) $G'$ 15% protein, 20% oil; (---) $G''$ 15% protein, 20% oil; (---) $G'$ 30% protein, 20% oil; (---) $G''$ 30% protein, 20% oil.
Both $G'$ and $G''$ of the model processed cheese samples were dependent on frequency which indicates the viscoelastic nature of the samples. The viscoelasticity of processed cheese ($G'$ and $G''$) increased with the increase of protein concentration (Figure 5.2). This result showed that the elasticity of model processed cheese increased with the increase of protein concentration. In Figure 5.2, processed cheese made with 5 and 15% protein with 20% oil concentration displayed higher values of $G'$ and $G''$ compared to processed cheese made with 5 and 15% protein without oil. This indicates that oil concentration also contributes to the overall viscoelasticity of the model processed cheeses.

Marshall (1990) studied the influence of moisture and amount of fat on the rheological, structural and sensory properties of model processed cheese. From his literature, fat content plays an important role in determining the effects of processing temperature during processed cheese manufacture. The processing temperature has less effect on the processed cheese with the least amount of fat. The result of his study showed that, higher amount of fat and protein increased the elastic modulus ($G'$) of model processed cheese. The result of his study is in agreement with our study. However, processed cheese made with 30% protein without oil was ~7% higher (e.g. $G'$ value at 1 Hz) compared to processed cheese made with similar protein concentration but with 20% oil concentration. This phenomenon occurs due to the presence of excess oil which lowers the protein/oil ratio creating softer cheese. Poorly emulsified oil droplets disrupt the casein matrix producing model processed cheese with low resistance towards shear (Pereira et al., 2001). Our result was also supported by Swenson et al. (2000) who compared their fat-free model processed cheese with full-fat processed cheese. Their result showed that, full-fat processed cheese was low in firmness and melted slightly less than the model processed cheese without oil emulsified with TSC. Based on our result in Figure 5.2, TSPP also showed similar trend as TSC when comparing the presence of oil in model processed cheese.

Figure 5.3 demonstrated an increase pattern of complex modulus ($G^*$) with the increase of protein concentration. Similar with Chapter 4 (Section 4.3.3), processed cheese samples made with low protein concentration (2.5 and 5% protein) presented low $G^*$ values compared to other protein concentration. Low level of protein concentration would cause fewer protein association which creates poor emulsification and oil separation in the model processed cheese matrix (Awad et al., 2004). At low protein concentration (<10%), oil dominates the rheological behaviour of processed cheese system (Figure 5.3). As the protein concentration
increased to ≥10%, the difference of $G^*$ values observed between processed cheese samples made with 0% to 30% oil starts to get smaller as an indication that the processed cheese samples have formed stable emulsions (solid-like behaviour). The effect of different oil concentration on processed cheese made with different protein concentration can be obviously seen on the processed cheese with 20 and 25% protein concentration (please note that the y-axis is plotted in a log scale which minimises the difference between the data points). At these oil concentration, the values of $G^*$ increased with the increasing level of oil concentration.

![Graph](image)

**Figure 5.3** Evolution of complex modulus ($G^*$) of model processed cheese samples manufactured with different protein concentrations (2.5 to 40%) and different oil concentrations (0 to 30%). (■) No oil; (●) 5% oil; (▲) 10% oil; (▼) 15% oil; (▲) 20% oil; (▼) 30% oil. Data represent means ($n = 2$) and error bars correspond to standard deviations.

The addition of TSPP during processed cheese manufacture is known to affect the model processed cheese structure (Hashimoto & Sunada, 1976; Tsumura & Hashimoto, 1978). TSPP removes the calcium from the casein system, which solubilises, hydrate and disperse the protein (Fennema, 1996; Kilcast & Angus, 2007). Furthermore, TSPP stabilised the emulsion by oil droplet emulsification, controls the cheese pH and forms uniform structure upon cooling (Awad et al., 2002). In this study similar results with Chapter 4 (Section 4.3.3), where the $G'$ and $G''$ cross-over shifted to lower frequencies as the protein concentration
increased (Figure 5.2) are also observed indicating that the emulsification trend of TSPP is similar to that of TSC. As the protein concentration increased from 30 to 40%, the $G^*$ values also increased, however the effect of the oil concentration is lesser. In other words, at these range of protein concentration (30 to 40% protein), the variation in oil concentration seems to give less effect towards the elasticity of the model processed cheese because the protein matrix is dominating the processed cheese system rheological behaviour.

In Chapter 4 (Section 4.3.3, Figure 4.9), model processed cheese manufactured with 30% protein and emulsified with TSC displayed no significant difference ($P<0.05$) between different oil concentration. In this chapter, it can be seen from Figure 5.3 that model processed cheese manufactured with 30% and 35% protein and emulsified with TSPP showed no significant difference ($P<0.05$) between different oil concentrations. This might indicate that TSPP has better emulsifying capability when compared to TSC at high protein concentration. Mizuno and Lucey (2005b) also supported this claim based on their result on the calcium-phosphate interactions in milk protein concentrate (MPC). Their result demonstrated that, milk with added TSPP has higher numbers of casein-bound calcium and inorganic phosphorus compared to TSC.

Furthermore, a research done by Rayan, Ernstrom, and Kalab (1980) showed that TSPP was the quickest to emulsify fat compared to sodium aluminium phosphate. Gao (2010), estimated the logarithm of intrinsic ion association constant ($pK_{ass}$) of sodium citrate based on experimental results and model calculation. Then, he compared with the $pK_{ass}$ of TSPP obtained from the literature (Smith & Martell, 1981). The $pK_{ass}$ of sodium citrate was lower ($pK_{ass} \approx 1.9$) compared to TSPP ($pK_{ass} \approx 2.3$) which supported the statements that TSPP has better emulsifying ability compared to sodium citrate.

Figure 5.4 displays large deformation rheology of model processed cheese with different protein concentration at 30% oil concentration. Based on this figure, the $G'-G''$ crossover of model processed cheese made with 20% protein occurs at higher % strain and higher measured moduli compared to model processed cheese made with 10% and 5% protein concentrations. This rheological measurement also showed that the viscous modulus ($G''$) of model processed cheese made with 10% protein was higher compared to the elastic modulus ($G'$). This showed that at 10% protein, the model processed cheese exhibits no transition from liquid-like behaviour to solid-like behaviour.
Figure 5.4 Large deformation ($G'$ and $G''$) as a function of strain of model processed cheese containing 30% of oil manufactured with 5, 10 and 20% of protein concentration. ($-$) $G'$ 5% protein, 30% oil; ($-$) $G''$ 5% protein, 30% oil; ($-$) $G'$ 10% protein, 30% oil; ($-$) $G''$ 10% protein, 30% oil; ($-$) $G'$ 20% protein, 30% oil; ($-$) $G''$ 20% protein, 30% oil.

Based on Figure 5.5, increasing protein concentration from 2.5 to 40% in model processed cheese resulted in an increased of critical shear stress $\sigma_c$ from ~2.4 to ~5,615 Pa; that is the stress at which the $G'$ and $G''$ cross-over is observed when a strain sweep is applied. The $\sigma_c$ increment gave an indication of the transition from liquid-like behaviour to solid-like behaviour in processed cheese samples. Model processed cheese made from 2.5 to 10% protein concentration displayed the lowest $\sigma_c$ values (between ~2.4 and ~6.5 Pa) due to low amount of intact casein which further affected the protein association and emulsion stability (Awad et al., 2004).

The results of this study are also supported by Wedholm, Larsen, Lindmark-Månsson, Karlsson, and Andrén (2006) who studied the influence of protein composition in milk on cheese-making characteristics. Based on their study, low concentration of casein will result in poorly coagulated samples. Processed cheese made with 40% protein displayed the highest $\sigma_c$ (~5.6 kPa) as an indication of highest firmness compared to processed cheese made with lower concentration of protein.
Figure 5.5 The evolution of critical strain $\gamma_c$ (■) and critical shear stress $\sigma_c$ (●) of model processed cheese as a function of protein concentrations (2.5 to 40%) (oil concentration: 30%). Data represent means (n = 2) and error bars correspond to standard deviations.

The critical strain $\gamma_c$ also increased from ~1 to ~251% with the increase of protein concentration from 2.5 to 40%. This suggests that model processed cheese made with higher protein concentration are more elastic and deform more compared to model processed cheese made with lower protein concentration. The frequency sweep moduli ($G'$ and $G''$), complex modulus ($G^*$), critical strain ($\gamma_c$) and critical shear stress ($\sigma_c$) of model processed cheese can be related to the moisture content. In this study, the addition of protein concentration up to 40% decreases the moisture content in model processed cheese. The decreasing of moisture content will increase the viscoelasticity of the processed cheese formulations. Since the same mixing speed was used to blend all model processed cheese with different formulations (varying oil and protein concentrations), thus a more viscoelastic formulation will result in an increased in mechanical work (shear stress) during the processed cheese manufacture (Pereira et al., 2001).
Figure 5.6 Oil droplet particle size distribution for model processed cheese manufactured with 5, 20 and 40% of protein concentration (oil concentration: 20%). (■) 5% protein, 20% oil; (■) 20% protein, 20% oil; (■) 40% protein, 20% oil.

In addition, the decreasing of moisture content also created model processed cheese with smaller oil droplets which yield a more compact and stable structure which increases the firmness of the processed cheese (Pereira et al., 2001). Our study also demonstrated similar results with Pereira et al. (2001) which were shown in Figure 5.2 and 5.3 for higher firmness, higher critical shear stress (Figure 5.5) smaller oil droplets (Figure 5.7) and low meltability (Figure 5.8) with the increase of protein concentration. These occurrences suggested that, as the protein concentration of model processed cheese increased, the moisture content of the processed cheese decreased, and smaller oil droplets are homogenously distributed within the processed cheese matrix.

In Figure 5.6, the widest oil droplet particle size distribution was presented by model processed cheese made with 5% protein followed by processed cheese samples made with 40 and 20% protein. The overall oil droplet particle size for processed cheese samples made with different protein concentrations can be observed further in Figure 5.7 where the values of D(3,2) the surface mean diameter are reported. Based on Figure 5.7, the oil droplet particle size of processed cheese samples decrease from ~8.6 to ~0.5 μm when the protein concentration is increased from 2.5 to 20% of protein. Then, a slight increment of oil droplet
particle size can be seen for the model processed cheese made with 25 to 40% of protein concentration, typically D(3,2) increases from ~0.6 to ~2.2 μm when the protein concentration is increased from 25% to 40% protein.

![Figure 5.7](image)

**Figure 5.7** Mean average oil droplet particle sizes (D(3, 2) in μm) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations.

The result of oil droplet particle size of model processed cheese made with different protein concentration in this section showed similar result with *Chapter 4* (Section 4.3.3) where model processed cheese made with low protein concentration (2.5 and 5%) demonstrated the highest oil droplet particle size (~8.6 μm and ~6.4 μm, respectively) and a liquid-like emulsion behaviour.

The comparison between model processed cheese made with TSPP and TSC will be discussed further later in this chapter. Based on Figure 5.6 and Figure 5.7, model processed cheese made with 20% protein and emulsified with TSPP demonstrated smallest particle diameter (~0.5 μm) compared to processed cheese samples made with other protein concentrations. As discussed earlier for Figure 5.3, TSPP seems to work best as an emulsifying agent when 20 and 25% of protein concentration were used to make processed cheese.
Figure 5.8 The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations.

During processed cheese manufacture, the anions from TSPP interact with the calcium ions from calcium caseinate (Mizuno & Lucey, 2005b). As more protein was added into the system, the dispersed casein molecules and extra casein interact with each other forming stronger protein-protein interactions, thus creating a firmer processed cheese matrix (Mizuno & Lucey, 2005b; Prentice, 1992). This statement also supported the results shown in Figure 5.8 which reports that the meltability of processed cheese samples decreased (from ~76 to ~24%) with increasing protein concentration (from 10 to 40%). Model processed cheese made with 40% concentration demonstrated the lowest % melt (~24%), approximately 4 times lower than the processed cheese made with 15% having the highest meltability (~83%). Note that in Figure 5.8 the melting percentage of processed cheese made with 2.5 and 5% are not reported since these cheeses have a liquid-like consistency.
5.3.2 Effect of oil concentration

The model processed cheese samples were also manufactured with TSPP at different oil concentration (0 to 30%). Figure 5.9 presents examples of the viscoelasticity ($G'$ and $G''$ as a function of frequency) of some processed cheese.

![Graph showing evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency](image)

**Figure 5.9** Evolution of elastic modulus ($G'$) and loss modulus ($G''$) as a function of frequency of model processed cheese manufactured with 0, 10 and 20% of oil concentration and 20% protein concentration. (---) $G'$ 0% oil; (----) $G''$ 0% oil; (-----) $G'$ 10% oil; (----) $G''$ 10% oil; (----) $G'$ 20% oil; (----) $G''$ 20% oil.

In Figure 5.9, processed cheese made with 20% oil concentration demonstrated higher values of $G'$ and $G''$ compared to processed cheese made with 10% oil concentration or without oil. This showed that model processed cheese with higher oil content was firmer compared to model processed cheese with less oil or without oil. The addition of oil in the protein system during the cheese-making process has created an emulsion which is finely dispersed. This phenomenon increases the viscosity of model processed cheese manufactured with the addition of oil (Jost et al., 1986; Pal, 1996). The $G'$-$G''$ crossover also shifted to lower frequencies as the oil concentration increased which might indicate a stronger molecular interaction in the protein network of the model processed cheese made with 20% oil concentration.
Figure 5.10 Complex modulus ($G^*$) of processed cheese samples as a function of oil concentration (protein concentration: 20%). Data represent means (n = 2) and error bars correspond to standard deviations.

Model processed cheese with fixed amount of protein concentration (20% of protein) displayed an increase in the complex modulus ($G^*$) with an increase of oil concentration. This means that, the rigidity of the processed cheese samples increase with the increase of oil concentration. Model processed cheese made with 30% oil concentration (20% of protein concentration) demonstrated the highest firmness ($G^*$ = ~12.3 kPa) compared to processed cheese samples made with lower oil concentration ($G^*$ = ~6.7 kPa for processed cheese made with 0% oil). Research done by Marshall (1990) supports this study where the firmness of model processed cheese increased with higher amount of oil and protein incorporated into the cheese matrix. However, other research works claimed contradicting results with the findings of this study. Stampanoni and Noble (1991) investigated the effect of increasing fat concentration on the firmness of model cheese. Their results showed that increasing fat concentration from 10 to 25% decreased the firmness of model cheese samples. Another study done by Emmons et al. (1980) also showed that less-fat Cheddar cheese was firmer and more elastic compared to full-fat Cheddar cheese. These results were contradicting with the present work because of the difference in the two types of cheeses (Cheddar vs processed cheese), their processing methods (e.g. their cook time is 30 minutes, the cook time in this study is 5 minutes; the process involved for their study were blending, cutting and draining...
the whey while the cheese products in this study were obtained directly after cooking) and the ingredients they contain (e.g. their study used fat from animal origin, while this study used fat from vegetable origin). In the study by Emmons et al. (1980), the reduced fat cheese was firmer and elastic compared to full fat Cheddar cheese due to more protein matrix were cut or deformed in the reduced fat cheese.

Figure 5.11 Evolution of critical strain \( \gamma_c \) (■) and critical shear stress \( \sigma_c \) (○) of analogue processed cheese as a function of oil concentration. Protein concentration is 20%. Data represent means (n = 2) and error bars correspond to standard deviations.

Processed cheese samples made with TSPP showed similar result with Chapter 4 (Section 4.3.4) for critical strain \( \gamma_c \) and critical shear stress \( \sigma_c \) experiment (Figure 5.11). Based on the result presented in Figure 5.11, the values of critical shear stress \( \sigma_c \) decreased (~2.2 to ~0.7 kPa) with the increase of oil concentration (0 to 30%). Theoretically, the decreasing critical shear stress indicates that processed cheese become softer. However, this was not the case because as seen from Figure 5.9 and 5.10, increasing oil concentration in processed cheese system increased the firmness of processed cheese. The decreasing critical shear stress \( \sigma_c \) occurred due to the increase of oil concentration in the system. This might indicate that the critical stress \( \sigma_c \) required to induce a flow of the processed cheese decreases with the increase in oil concentration.
On the other hand, the critical strain $\gamma_c$ of model processed cheese increased from ~113 to ~179% with the increase in oil concentration from 0 to 30%, respectively. This indicates that model processed cheese made with higher oil concentrations deform more easily compared to model processed cheeses with lower oil concentration.

The size of oil droplets plays an important role in model processed cheese structure because the existence of oil droplets themselves can increase gel strength in the processed cheese matrix (Pereira et al., 2001). In Figure 5.12, the widest oil droplet particle size distribution was found for the model processed cheese made with 5% oil followed by processed cheese samples made with 30% and 20% oil. To compare between the different processed cheeses, the mean surface diameter $D(3,2)$ is plotted as a function of oil concentration in Figure 5.13. Based on Figure 5.13, the oil droplet particle size of processed cheese samples increase from ~0.25 to ~0.66 $\mu$m when the oil concentration is increased from 5 to 30%. In this study, the amount of shear applied during model processed cheese manufacture was maintained at the same rate, and for this experiment, the amount of protein concentration also was maintained at 20%. Thus, it can be speculated that the increase in size with the oil concentration can be due to the ratio of protein to oil (the highest the ratio, the more effective the oil droplet.

**Figure 5.12** Oil droplet particle size distribution for model processed cheese manufactured with (-■-) 5% oil; (-○-) 20% oil and (-△-) 30% oil concentration (protein concentration: 20%).
emulsification) and also possibly due to oil droplet coalescence (the highest the oil, the higher
the number of oil droplets and more coalescence are expected to occur).

![Graph showing the evolution of mean average oil droplet sizes (D(3, 2) in μm) of processed cheese manufactured with 20% of protein as a function of oil concentration (5 to 30%). Data represent means (n = 3) and error bars correspond to standard deviations.]

**Figure 5.13** Evolution of mean average oil droplet sizes ($D(3, 2)$ in μm) of processed cheese manufactured with 20% of protein as a function of oil concentration (5 to 30%). Data represent means ($n = 3$) and error bars correspond to standard deviations.

Figure 5.14 showed the images of model processed cheese made with 5 to 30% oil concentration using scanning electron micrographs (SEM). Based on Figure 5.14, model processed cheese made with 5% oil concentration presented loose protein matrix with open spaces or voids. The oil droplets were dispersed throughout the casein network and were too small to be detected at the scale use during SEM observations. As the oil concentration increased, the number of oil droplets and the diameters of the oil droplets increased (Figure 5.14; by visual observation, the diameter of the oil droplets increased from 0.8 to 1.3μm as the oil concentrations increased from 15 to 30%) confirming the particle size measurements. Furthermore, as the oil concentration increased, the microstructure of model processed cheese presented a more compact protein network with voids and oil droplets which are homogenously distributed.
Figure 5.14 Scanning electron (SEM) micrographs of model processed cheese made with 20% of protein and different concentrations of oil: a) 5%, b) 10%, c) 15%, d) 20%, e) 30%. Scale bar = 2 to 5µm.
This would confirm the creation of a stronger gel network within the processed cheese matrix. Study done by Marshall (1990) described that model processed cheese observed by light microscopy displayed numerous fat globules when higher amount of fat used in model processed cheese compared to processed cheese with less fat.

Figure 5.15 presented the height melting percentage of model processed cheese manufactured with different oil concentration (0 to 30%). Based on Figure 5.15, as the oil concentration increased from 0 to 30%, the height melting percentage of processed cheese samples decreased. For instance, the melting height is ~84% at 0% oil and ~70% at 30% oil. This follows the rheological measurements which showed that the model processed cheese made without oil has the softest structure while model processed cheese made with 30% oil concentration has the highest firmness (Figure 5.10).

**Figure 5.15** The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%). Data represent means (n = 3) and error bars correspond to standard deviations.

As discussed previously in **Chapter 4** (Section 4.3.4), model processed cheese manufactured with high oil concentration (e.g. 30%) exhibit low melting properties due to lower degree of oil droplet emulsification compared to model processed cheese manufactured with low oil concentration (e.g. 5%). This statement is in agreement with Lee, Klostermeyer and Anema...
(2015), where the microstructural images of processed cheese samples with high fat concentration in their study suggested the presence of poorly emulsified fat. Besides, the presence of higher oil concentration may increase the interactions between oil and hydrophobic zones of proteins along with protein-protein interactions creating a stronger processed cheese network.

5.3.3 Comparison with TSC

![Graph](image)

**Figure 5.16** Evolution of complex modulus (\(G^*\)) of model processed cheese samples as a function of protein concentrations (2.5 to 40%) at different oil concentrations (0 to 30%) using TSC and TSPP. (■) TSC no oil; (□) TSPP no oil; (▲) TSC 20% oil; (△) TSPP 20% oil; (●) TSC 30% oil; (◇) TSPP 30% oil. Data represent means (n = 2) and error bars correspond to standard deviations.

In this section, the results obtained from model processed cheese emulsified with TSC will be compared with the results obtained from model processed cheese emulsified with TSPP on an equivalent mass basis. Figure 5.16 presents the complex modulus (\(G^*\)) of model processed cheese made with different protein concentration emulsified with TSC or TSPP. In both cases, \(G^*\) values increased with the increased protein concentration. The model processed cheese made with lower protein concentration (2.5 to 5%) demonstrated low sets of \(G^*\) values forming poor emulsions and oil separation due to the low protein amount and the final
pH of the processed cheeses.

\( G^* \) values of model processed cheese emulsified with TSC presented higher values (~165 to ~21,600 Pa) compared to model processed cheese emulsified with TSPP (~5.7 to ~19,500 Pa) when the processed cheeses were manufactured with 2.5 to 25% protein concentration and 30% oil concentration. At this stage, it can be hypothesized that, in low protein concentration and high oil concentration systems, TSC provided better emulsifying capability compared to TSPP. However, as the protein concentration increased from 35 to 40%, the \( G^* \) values of model processed cheese emulsified with TSPP were not significantly different \((P<0.05)\) to that of model processed cheese emulsified with TSC.

![Graph showing mean average oil droplet particle sizes (\( D(3, 2) \) in \( \mu \text{m} \)) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%) using TSC (●) and TSPP (◆). Data represent means \((n = 3)\) and error bars correspond to standard deviations.](image)

**Figure 5.17** Mean average oil droplet particle sizes \((D(3, 2) \text{ in } \mu \text{m})\) of processed cheese manufactured with different protein concentrations from 2.5 to 40% (oil concentration: 20%) using TSC (●) and TSPP (◆). Data represent means \((n = 3)\) and error bars correspond to standard deviations.

Mizuno and Lucey (2005a) studied the influence of different emulsifying salts (TSC, disodium orthophosphate (DSP), TSPP and sodium hexametaphosphate) in casein micelles using milk protein concentrate (MPC). Based on their results, TSC and TSPP formed different interaction in MPC cheese model system. TSPP may act as a cross-linking agent by bridging the casein molecules. Casein-calcium phosphate complex was formed when the
negatively charged pyrophosphate anions aggregate with casein. This enhances casein charge repulsion and cause higher casein dispersion. In the case of TSC which contains no phosphate ions, TSC does not cross-linked with caseins. Instead, TSC formed soluble complexes after calcium chelation from colloidal calcium phosphate (CCP) and increases the casein dispersion (Mizuno & Lucey, 2005a).

**Figure 5.18** The height melting percentage of model processed cheese as a function of protein concentrations (10 to 40%) using TSC (●) and TSPP (◆). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1), (oil concentration: 20%). Data represent means (n = 3) and error bars correspond to standard deviations.

The results obtained from rheological study ($G^*$ values) was also supported by the results obtained from oil droplet particle size shown in Figure 5.17. In Figure 5.17, although the results of the oil droplet particle size for both model processed cheese emulsified with either TSC or TSPP demonstrated similar trend, it can be seen that model processed cheese made with low protein concentration (≤20%) emulsified with TSPP displayed bigger oil droplet particle size (~0.5 µm at 20% protein concentration) compared to model processed cheese emulsified with TSC (~0.4 µm at 20% protein concentration). However, at higher protein concentration (35 to 40%), model processed cheese emulsified with TSPP showed smaller oil droplet particle size (~1.0 to ~2.2 µm) compared to model processed cheese emulsified with TSC (~1.6 to ~2.4 µm). At 25% protein concentration, model processed cheese emulsified
with TSPP had the same size of oil droplets with model processed emulsified with TSC (~0.6 μm). This phenomenon suggested that, at high protein concentration (20% oil concentration), TSPP and TSC have more capability to emulsify (stabilise through the casein protein) oil droplets by performing calcium chelation.

Figure 5.18 displays the height melting percentage of model processed cheese made with different protein concentration emulsified with either TSC or TSPP. Based on Figure 5.18, as the protein concentration increased from 15 to 40%, model processed cheese emulsified with TSC had higher meltability (~88 to ~31%) compared to model processed cheese emulsified with TSPP (~83 to ~24%).

Swenson et al. (2000) performed a research work on the effect of different emulsifying salts on the functionality of processed cheese without oil. By applying 3% of emulsifying salts during the processed cheese manufacture, processed cheese made with TSC was softer and had a higher meltability compared to processed cheese made with disodium phosphate and polyphosphate. Increasing the percentage of emulsifying salts during the processed cheese manufacture from 0.5% to 3.0% resulted in firmer cheese, lower meltability and a decrease in spreadability. In this study, increasing the amount of protein concentration seems to show a similar effect with Swenson and co-workers’ study of increasing the amount of emulsifying salts. This is due to the higher amount of protein-protein interaction from the increasing amount of casein introduced which further decreased the meltability of model processed cheese. Kaliappan and Lucey (2011) showed that the existence of TSC in calcium-chelating salts mixtures added into milk has lowered the number of colloidal calcium phosphate (CCP) centres while the addition of TSPP in calcium-chelating salt mixtures increased the number of CCP in the milk system. Model processed cheese made at a high protein concentration and emulsified with TSPP demonstrated higher firmness (Figure 5.16), smaller oil droplets (Figure 5.17) and low meltability (Figure 5.18) compared to model processed cheese emulsified with TSC at the same protein concentration.

Figure 5.19 presented the complex modulus ($G^*$) of model processed cheese made with different oil concentration emulsified with TSC or TSPP. In both cases, $G^*$ values increased with the increased of oil concentration.
Model processed cheese emulsified with TSC showed a linear increase in $G^*$ values from 0 to 15% oil ($R^2 = 0.9933$) followed by a plateau in $G^*$ values (~13.5 kPa) as the oil concentration increased from 15 to 30%. Based on the result of our study, the capability of TSC to perform its best emulsifying ability is limited to 20% oil, under the conditions of protein and emulsifier concentrations as well as the process used in this study. However, based on Figure 5.19, model processed cheese made with different oil concentration and emulsified with TSC always displayed higher $G^*$ values compared to model processed cheese emulsified with TSPP.

**Figure 5.19** Evolution of the complex modulus ($G^*$) of processed cheese samples as a function of oil concentration (protein concentration: 20%) using TSC (●) and TSPP (■) as emulsifiers. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Although TSC has better emulsifying capability compared to TSPP for model processed cheese made with different oil concentration, the $G^*$ values of model processed cheese emulsified with TSPP seem to increase linearly ($R^2 = 0.9916$) which gave an indication that further addition of oil concentration (>35%) may results in $G^*$ values of model processed cheese emulsified with TSPP higher than model processed cheese emulsified with TSC. This hypothesis was later supported by oil droplet particle size (Figure 5.20). Figure 5.20 presents oil droplet particle size of model processed cheese manufactured with different oil concentration and emulsified with TSC or TSPP. Based on this, as the oil concentration increased from 5 to 30%, the oil droplet particle size of both batches of model processed...
cheese also increased (TSC: ~0.19 to ~0.61 μm; TSPP: ~0.25 to ~0.66 μm).

Processed cheese samples made with different oil concentration and emulsified with TSPP showed bigger oil droplet particle size compared to model processed cheese emulsified with TSC. Pereira et al. (2001) suggested that, smaller oil droplets that are homogeneously distributed within the processed cheese matrix can be seen in a good emulsified system. The protein-protein and protein-oil interactions became stronger, thus provide better resistance towards shear. The result from our study (Figure 5.19) clearly showed that in the case of model processed cheese emulsified with TSC, increasing oil concentration would increase the complex modulus ($G^*$) of the processed cheese. This result agrees with Pereira et al. (2001) suggestion as mentioned above that model processed cheese treated with TSC experienced better oil droplet emulsification compared to model processed cheese treated with TSPP as the model processed cheese appeared firmer at higher oil concentrations (5 to 30%) and presented smaller oil droplets.

![Graph](image)

**Figure 5.20** Mean average oil droplet particle sizes ($D(3, 2)$ in μm) as a function of oil concentration (0 to 30%) for processed cheese manufactured with 20% protein using TSC (●) and TSPP (◆). Data represent means (n = 3) and error bars correspond to standard deviations.

On the other hand, the height of melting percentage of model processed cheese emulsified TSPP demonstrated no significant difference ($P<0.05$) with the meltability of model
processed cheese emulsified with TSC (Figure 5.21). This is despite the fact that TSC promotes better oil droplet emulsification compared to TSPP. Therefore, we have calculated the concentration of both TSPP and TSC added into the processed cheese system for different oil concentrations. Based on the calculations, due to the large molecular weight of TSPP, the concentration of TSPP added into the processed cheese system for different oil concentrations were smaller (0.0780M) compared to the concentration of TSC (0.1183M) added into the processed cheese system.

![Figure 5.21](image.png)

**Figure 5.21** The height melting percentage of model processed cheese as a function of oil concentration (0 to 30%) using TSC (○) and TSPP (◇). Data represent means (n = 3) and error bars correspond to standard deviations.

Most of the studies on processed cheese showed TSPP as having better emulsifying capability compared to TSC (Awad et al., 2002; Lu et al., 2008; Mizuno & Lucey, 2005b). However, in this study, based on the rheological result (Figure 5.19), oil droplet particle size (Figure 5.20) and melting properties (Figure 5.21), binding of calcium from casein by TSC was stronger compared to TSPP for oil concentration from 5 to 30%, on an equivalent mass basis. As suggested earlier, TSPP may act as strong emulsifying agent if the oil concentration was further increased (>35%) because the effectiveness of TSC as emulsifying agent seem to slow down at >20% oil concentration (Figure 5.19) while the effectiveness of TSPP as emulsifying agent seem to stay active (increasing $G^*$ values in Figure 5.19) even when the
highest oil concentration (30%) is used. Further work is needed to demonstrate if this is the case.

5.4 Summary to chapter

Overall, the results obtained on processed cheese samples made with TSPP demonstrated similar results to the results obtained from Chapter 4 (processed cheese samples manufactured with TSC). The firmness ($G'$, $G''$ and $G^*$) increased and the meltability of processed cheese decreased with the increase of protein concentration. Model processed cheese made with 20% protein (oil concentration: 20%) and emulsified with TSPP demonstrated smallest particle diameter (~0.5 μm) compared to processed cheese samples made with other protein concentrations due to highest oil droplet emulsification. Model processed cheese made with higher protein concentration (30% to 40%) showed larger particle size. Model processed cheese with fixed amount of protein concentration (20%) displayed an increase of hardness ($G^*$), oil droplet particle size and homogenous microstructure with an increase of oil concentration. TSC and TSPP demonstrate its best ability to emulsify oil droplets by performing calcium chelation at 20% protein concentration. TSC binds more calcium than TSPP on an equivalent mass basis. This might have resulted in better emulsification when TSC was used. In conclusion, the ability of each emulsifying salts (TSC or TSPP) to perform calcium chelation, and thus emulsification of the oil depends greatly on the amount of protein and oil concentrations.
Effect of trisodium citrate (TSC) and sodium pyrophosphate (TSPP) on model processed cheese made with emulsions stabilised by different emulsifiers
6.1 Introduction

Processed cheese consists of phases such as fat and water besides proteins. The proteins in processed cheese can adsorb at the oil-water interface via its surface-active sites, creating an emulsion (McClements, 2004). A typical food emulsion may consist of three different regions which are the interfacial region, the internal part of the droplets and the continuous phase (surrounding liquid) (Friberg, Larsson, & Sjoblom, 2003). The interfacial region is only a few nanometers thick but this region is responsible in the organoleptic and physicochemical characteristics of food emulsions (McClements, 2004).

Lobato-Calleros et al. (2008), investigated the effect of canola oil stabilised with carboxymethylcelulose (CMC), low-methoxyl pectin (LMP) and gum Arabic (GA) either individually or as blends on the textural characteristics of analogue processed cheese. They found that the textural characteristics of reduced-fat cheese stabilised with CMC is similar with full-fat cheese. The hardness and chewiness also increased when the reduced-fat cheese was stabilised with GA and LMP. Another study done by Everett & Olson (2003), explored the influence of fat globules stabilised with different types of proteins on the physical properties of Cheddar cheese and they found that cheese containing fat globules stabilised with $\alpha_s2$-casein fractured at a lower strain and stress compared to other cheeses. Lee, Klostermeyer, Schrader, & Buchheim (1996) also studied on the effect of fat globules stabilised with low molecular weight emulsifiers on the physical properties of model processed cheese and reported that processed cheese containing fat globules stabilised with cationic cetyltrimethylammonium bromide (CTAB) was the hardest and the most elastic while processed cheese containing fat globules stabilised with sodium dodecyl sulphate (SDS) was the softest and the least elastic.

In Chapter 4 and Chapter 5 (model processed cheese emulsified with trisodium citrate (TSC) and sodium pyrophosphate (TSPP)), calcium caseinate was not just part of the continuous-phase but also contributed to the stabilisation of the oil droplets. The amount of protein used was found to have an effect on the stability and rheological behaviour of the model processed cheese, particularly at low protein concentration, where emulsification was not optimal (e.g. large oil droplets, lower $G^*$ etc). The type of interaction that occurs between the oil droplets
and the protein matrix can be determined by the nature of the surface active material on the oil droplets. Therefore, in this chapter, the effect of different types of food emulsifiers on the rheological properties of model processed cheese obtained using TSC or TSPP will be investigated. The rheological properties, oil droplet particle size, melting and microstructural properties of the emulsified model processed cheese stabilised by different types of oil droplet surface materials will be determined.

6.2 Materials and Methods

6.2.1 Materials

Model processed cheese was made from calcium caseinate, sunflower oil, TSC, TSPP, whey protein isolate (WPI), liquid lecithin and citric acid.

6.2.2 Sample preparation

Emulsions and model processed cheese samples were prepared as previously described in Chapter 3 (Section 3.3.2). Briefly, emulsions were prepared using water (59%), sunflower oil (40%) and 1% of calcium caseinate or WPI or lecithin. The ingredients were homogenized using a laboratory homogenizer and high pressure homogenizer (microfluidizer; recycled twice with the pressure 750 bar and 206 bar).

Briefly, model processed cheese samples were prepared using water (298.5g), calcium caseinate powder (201.5g), emulsion (500.0g), TSC or TSPP (20.1g) and citric acid (5.2g). The speed was maintained at speed 1 and the mixture was heated from 37 to 90\(^\circ\)C for 3 minutes and held at 90\(^\circ\)C for 2 minutes.

Model processed cheese samples were prepared in six different batches. The first, second and third batches contains model processed cheese with seven different oil concentrations (from 0% to 30%) made with calcium caseinate (first batch), WPI (second batch) or lecithin (third batch) with constant amount of protein (20%) and emulsified with TSC. The fourth, fifth and sixth batches include model processed cheese with seven different oil concentrations (from 0% to 30%) made with calcium caseinate (fourth batch), WPI (fifth batch) or lecithin (sixth
batch; shown in Figure 6.1) with constant amount of protein (20%) and emulsified with TSPP. Non-heated processed cheese samples (20% protein and 20% oil concentrations) containing oil droplets stabilised by WPI were prepared (in duplicate) as previously described in this section (6.2.2) at speed 1, with the absence of heat treatment. For all the processed cheese samples, the ratio between protein and emulsifying salt remained constant (10:1) with pH around 5 to 6. The final composition and pH of the model processed cheese samples in this chapter are shown in Appendix 4, 5 and 6.

![Processed cheese samples made with emulsions (lecithin) and emulsified using TSPP, with six different oil concentrations ranging from 5 to 30% (left to right) with constant amount of protein concentration (20%).](image)

**Figure 6.1** Processed cheese samples made with emulsions (lecithin) and emulsified using TSPP, with six different oil concentrations ranging from 5 to 30% (left to right) with constant amount of protein concentration (20%).

### 6.2.3 Experimental methods

Each batch of model processed cheese samples were characterized in terms of their rheological properties, particle size measurements, melting properties and microstructure using the methods as previously described in Chapter 3 (Section 3.4). Among the microscopy methods, SEM was chosen to determine the microstructure of the model processed cheese samples. Reversed phase high performance liquid chromatography (RP-HPLC) method as previously described in Chapter 3 (Section 3.5.1) was also done on model processed cheese samples containing oil droplets stabilised with WPI.
6.3 Results and Discussion

6.3.1 Effect of trisodium citrate (TSC) on model processed cheese made with emulsions stabilised by different emulsifiers

This section discusses the influence of model processed cheese (PC) samples emulsified with trisodium citrate (TSC) and stabilised by calcium caseinate or whey protein isolate (WPI) or lecithin.

Figure 6.2 Evolution of elastic ($G'$) and loss ($G''$) modulus as a function of frequency for model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). ($\square$) $G'$ of PC made with emulsion stabilised by calcium caseinate; ($\triangle$) $G''$ of PC made with emulsion stabilised by calcium caseinate; ($\uparrow$) $G'$ of PC made with emulsion stabilised by WPI; ($\downarrow$) $G''$ of PC made with emulsion stabilised by WPI; ($\blacktriangle$) $G'$ of PC made with emulsion stabilised by lecithin; ($\blacklozenge$) $G''$ of PC made with emulsion stabilised by lecithin.

Figure 6.2 shows the elastic and loss moduli ($G'$ and $G''$) of model processed cheese made with TSC and containing oil droplets stabilised by different emulsifiers. Based on the result shown in Figure 6.2, model processed cheese stabilised by WPI presented the highest $G'$ and $G''$ moduli (highest firmness) compared to model processed cheese stabilised by calcium
caseinate. Model processed cheese stabilised by lecithin demonstrated the lowest measured moduli ($G'$ and $G''$).

Cho, Lucey, and Singh (1999) studied the influence of different fat globule membrane materials on acid milk gels. In their study, the fat globules were stabilised with skim milk powder (low to high heat), whey protein concentrate (WPC) (heated or non-heated) or Tween 60. Based on the results obtained by Cho et al. (1999), acid milk gels containing fat globules stabilised by WPC and Tween 60 presented the lowest $G'$ values while acid milk gels containing fat globules stabilised by heated WPC demonstrated the highest $G'$ values. The results obtained in this study is partly in agreement with Cho et al. (1999) results. Since the manufacturing process to make model processed cheese in this study involved heat treatment, therefore WPI was heat treated during the manufacture. This statement will be discussed further in Figure 6.4.

Figure 6.3 exhibited the complex modulus ($G^*$) of model processed cheese samples containing oil droplets stabilised by different emulsifiers. Based on Figure 6.3, model processed cheese samples presented almost similar values of $G^*$ up to 5% oil. As the oil concentration increased from 10 to 30%, $G^*$ values of model processed cheese samples stabilised with WPI and calcium caseinate increased indicating that the samples became firmer. Model processed cheese stabilised with WPI demonstrated the highest firmness (from ~4.2 to ~8.2 kPa) with increasing oil concentration (from 0 to 30%) compared to model processed cheese stabilised with calcium caseinate (from ~4.3 to ~6.7 kPa). However, the $G^*$ values of model processed cheese stabilised with lecithin decreased (from ~4.2 to ~2.5 kPa) with increased oil concentration (0 to 30%), indicating that the samples became softer.

During the manufacture of model processed cheese containing oil droplets stabilised with WPI, the mixture was heated from 37 to 90°C for 3 minutes and held at 90°C for 2 minutes at speed 1 (total cook time: 5 minutes). Theoretically, WPI gelatinizes at around 74°C (Aguilera & Stanley, 1999). It is expected that the whey proteins have denatured after this process. This will lead to their aggregation and interaction with caseins present in the calcium caseinate (Anema & Li, 2003). This could be the main reason for the high $G'$ and $G''$ moduli and the $G^*$ values of model processed cheese containing oil droplets stabilised by WPI.
Figure 6.3 Complex modulus ($G^*$) of model processed cheese samples manufactured with different types of emulsions with added TSC as a function of oil concentration (protein concentration: 20%). (■) $G^*$ of PC made with emulsion stabilised by calcium caseinate; (▲) $G^*$ of PC made with emulsion stabilised by WPI; (◆) $G^*$ of PC made with emulsion stabilised by lecithin. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Another factor which contribute to the high $G^*$ values of model processed cheese containing oil droplets stabilised by WPI or calcium caseinate is the type of interaction that occur between the material adsorbed at the surface of the oil droplet with the protein matrix (Everett & Olson, 2003). WPI and calcium caseinate are known as active fillers or interacting materials (Cho et al., 1999; Lucey, Munro, & Singh, 1998). Both WPI and calcium caseinate adsorbed on the oil droplet surface interact positively with the protein network forming a strong gel matrix. On the other hand, oil droplets stabilised by lecithin may act as an inert filler or non-interacting material where the oil droplet membrane is intact and does not interact or cross-links with the casein particles (Cho et al., 1999; Lucey, Munro, et al., 1998). Therefore, model processed cheese containing oil droplet stabilised by lecithin showed the lowest measured moduli ($G'$ and $G''$) and $G^*$ values.

Figure 6.4 presented the elution profile of whey protein in model processed cheese containing oil droplet stabilised by WPI obtained by reversed-phase HPLC. The heated model processed cheese samples (b), were compared with a non-heated model processed cheese containing oil
droplets stabilised by WPI (a). The percentage of whey protein denaturation for α-LA and β-LG was calculated based on equation 3.4 (Chapter 3; Section 3.5.1).

![Figure 6.4](image)

**Figure 6.4** Reversed-phase HPLC elution profiles of model processed cheese containing oil droplets stabilised by WPI manufactured without heat treatment (a) and with heat treatment (b). Gradient 30% to 45%B, 30 min. $A_{280} =$ Absorbance at 280 nm, α-LA = α-lactalbumin, β-LG = β-lactoglobulin, κ-CN = κ-casein.

Based on the result shown in Figure 6.4, the whey protein denaturation in our study is 25.32%. According to Parris & Baginski (1991), almost 95% of whey proteins have been denatured at 85ºC. Millqvist-Fureby, Elofsson, & Bergenståhl (2001) suggested that, time and temperature are very important in determining the degree of whey protein denaturation. The percentage of whey protein denaturation in this study was not as high as expected may be due to the short heat treatment time used to manufacture the processed cheese. In this study, the processed cheese mixture was heated only for 5 minutes. The gel samples from other studies such as Parris & Baginski (1991), Cho et al. (1999) and Mantovani, Cavallieri, Netto, & Cunha (2013) were heated for 30 minutes which is sufficient to denature the majority of the whey proteins. However, achieving 25% of whey protein denaturation already proved that the whey protein isolate on the oil droplet surface in the processed cheese samples have been
partially denatured. SDS-PAGE was also done on these samples (heated and non-heated model processed cheese containing oil droplets stabilised by WPI), but the result was not convincing because the bands obtained can’t be seen clearly. According to Lucey, Tamehana, Singh, and Munro (1998), milk samples heated at >70°C may cause denatured whey proteins (β-lactoglobulin) to form complexes with κ-casein via hydrophobic interactions and intermolecular disulphide bonds. The complexes of κ-casein formed can be seen by the RP-HPLC elution profile presented in Figure 6.4.

**Figure 6.5** Evolution of critical shear stress $\sigma_c$ of model processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (0 to 30%) (protein concentration: 20%). ■ $\sigma_c$ (shear stress) of PC made with emulsion stabilised by calcium caseinate; ▲ $\sigma_c$ (shear stress) of PC made with emulsion stabilised by WPI; ● $\sigma_c$ (shear stress) of PC made with emulsion stabilised by lecithin. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 6.5 demonstrates the evolution of critical shear stress $\sigma_c$ of model processed cheese containing oil droplets stabilised by different surface active materials. Based on Figure 6.5, the critical shear stress $\sigma_c$ of model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin decreased with the increasing oil concentration. Model processed cheese containing oil droplets stabilised by calcium caseinate exhibited the highest critical shear stress $\sigma_c$ of ~2.4 kPa and ~2.0 kPa, for 5 and 10% of oil concentration respectively. As the oil concentration increased from 15 to 30%, model processed cheese
containing oil droplets stabilised by WPI presented the highest critical shear stress $\sigma_c$ (~1.8 kPa at 15% oil and ~1.0 kPa at 30% oil) followed by model processed cheese containing oil droplets stabilised with calcium caseinate (~1.8 kPa at 15% oil and ~0.6 kPa at 30% oil) and lecithin (~1.5 kPa at 15% oil and ~0.5 kPa at 30% oil). Higher shear stress is related to higher firmness of the cheese matrix (Hort & Le Grys, 2001; Lucey et al., 2003). This result is consistent with the results obtained in Figure 6.2 and 6.3 where the firmness of the model processed cheese samples were in the following order: WPI > calcium caseinate > lecithin.

**Figure 6.6** Evolution of critical strain $\gamma_c$ of model processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (0 to 30%) (protein concentration: 20%). (■) $\gamma_c$ (% strain) of PC made with emulsion stabilised by calcium caseinate; (▲) $\gamma_c$ (% strain) of PC made with emulsion stabilised by WPI; (◆) $\gamma_c$ (% strain) of PC made with emulsion stabilised by lecithin. Data represent means ($n = 2$) and error bars correspond to standard deviations.

The evolution of critical shear stress $\sigma_c$ is also related to the critical strain as shown in Figure 6.6. Based on Figure 6.6, the critical strain $\gamma_c$ of model processed cheese containing oil droplets stabilised by different surface active materials increased with the increased of oil concentration. Model processed cheese containing oil droplets stabilised by lecithin demonstrated the highest critical strain $\gamma_c$ (from ~126% at 0% oil to ~200% at 30% oil) followed by model processed cheese containing oil droplets stabilised with calcium caseinate.
(from ~126% at 0% oil to ~178% at 30% oil) and WPI (from ~126% at 0% oil to ~158% at 30% oil).

As discussed earlier in Chapter 4 (Section 4.3.3), strain is how much the cheese samples deform due to stress. Processed cheese is a viscoelastic material and stretch properties are important in cheese studies (Lucey et al., 2003). Elastic processed cheese samples can deform more than viscous processed cheese samples before breaking, even though they might be firmer than the viscous samples. In this study, model processed cheese containing oil droplets stabilised with lecithin are less elastic (Figure 6.3) and easily stretched before breaking but these samples can’t withstand as much stress. On the other hand, model processed cheese containing oil droplets stabilised with WPI and calcium caseinate are more elastic, do not stretch much and easily break (as shown by lower critical shear strain $\gamma_c$ which indicate less deformation at breaking-Figure 6.6) compared to model processed cheese containing oil droplets stabilised by lecithin. Overall, based on the results obtained from Figure 6.5 and 6.6, increasing oil concentration provide a more elastic model processed cheese samples and better stretchability.

Figure 6.7 and 6.8 presented the oil droplet particle size distributions and oil droplet particle size of emulsion only (at 0% oil concentration for Figure 6.8) and model processed cheese containing oil droplets stabilised with calcium caseinate or WPI or lecithin. In Figure 6.7, WPI emulsion (before being added into processed cheese) demonstrated the widest oil droplet particle size distribution. Model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin showed almost similar oil droplet particle size distribution. All of the processed cheese samples displayed high stability by having monomodal (one) peak in the oil droplet particle size distribution. A more detailed result can be seen from Figure 6.8 where model processed cheese containing oil droplets stabilised with WPI demonstrated the smallest oil droplet particle size ($D_{3,2}$) (~1.2 µm at 30% oil) compared to model processed cheese containing oil droplets stabilised with calcium caseinate (~1.6 µm at 30% oil) or lecithin (~2.3 µm at 30% oil).
Figure 6.7 Oil droplet particle size distribution for model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). (■) PC made with emulsion stabilised by calcium caseinate; (▲) PC made with emulsion stabilised by WPI; (●) PC made with emulsion stabilised by lecithin; (○) WPI emulsion- before being added into PC.

Based on Figure 6.8, increasing oil concentration does not affect markedly the size of particle size for model processed cheese containing oil droplets stabilised with WPI or calcium caseinate except from 0 to 10% oil (WPI: from ~1.0 µm at 0% oil concentration to ~1.3 µm at 10% oil; calcium caseinate: from ~1.5 µm at 0% oil to ~1.9 µm at 10% oil). However, increasing oil concentration from 10 to 15% increased the oil droplet particle size of model processed cheese containing oil droplets stabilised with lecithin (from ~2.0 µm to ~2.2 µm). Model processed cheese containing oil droplets stabilised with lecithin also demonstrated the largest oil droplet particle size compared to model processed cheese containing oil droplets stabilised with calcium caseinate and WPI.

Partial coalescence may occur in model processed cheese samples containing oil droplets stabilised by lecithin. As discussed in Chapter 4 and Chapter 5, the presence of emulsifying salts (TSC) enhances casein dispersion by exposing the hydophilic and hydrophobic regions thus, promotes emulsification between oil droplets and protein network (Lee et al., 1996). However, with the presence of lecithin surrounding the oil droplet surface, the oil droplets
may not be attracted to casein.

**Figure 6.8** Mean average oil droplet particle sizes \(D(3, 2)\) in \(\mu m\) of processed cheese manufactured with different types of emulsions with added TSC as a function of oil concentration (oil concentration: 0 to 30%) (protein concentration: 20%). (■) \(D_{3,2}\) of PC made with emulsion stabilised by calcium caseinate; (▲) \(D_{3,2}\) of PC made with emulsion stabilised by WPI; (◆) \(D_{3,2}\) of PC made with emulsion stabilised by lecithin. Data represent means \((n = 3)\) and error bars correspond to standard deviations.

The results obtained in Figure 6.7 and 6.8 were in agreement with the SEM images of the processed cheese samples. Figure 6.9 exhibited the images of model processed cheese containing oil droplets stabilised with calcium caseinate, WPI or lecithin taken from scanning electron micrographs (SEM). Model processed cheese containing oil droplets stabilised with WPI (Figure 6.9b) or calcium caseinate (Figure 6.9a) showed small sizes of oil droplets being distributed within the protein matrix compared to the processed cheese made with emulsion stabilised by lecithin (Figure 6.9c).
Figure 6.9 Scanning electron (SEM) micrographs of model processed cheese made with different types of emulsions with added TSC (oil concentration: 20%) (protein concentration: 20%). a) PC made with emulsion stabilised by calcium caseinate, b) PC made with emulsion stabilised by WPI, c) PC made with emulsion stabilised by lecithin. Scale bar = 20µm.

The microstructure of the model processed cheese containing oil droplets stabilised by WPI seems to show a more compact protein network compared to the microstructure of model processed cheese containing oil droplets stabilised by calcium caseinate or lecithin. This result is consistent with Everett and Olson (2003) who compared microstructural images of Cheddar cheese containing fat globules stabilised by caseins or whey proteins with Cheddar cheese containing native fat globules. According to their study, Cheddar cheese containing fat globules stabilised by α-lactalbumin (a protein present in WPI) demonstrated small fat particles which were dispersed in a uniform protein network whereas Cheddar cheese containing native fat globules displayed larger fat globule size. On the other hand, the microstructure images of the model processed cheese containing oil droplets stabilised with
lecithin displayed loose protein matrix with open spaces with large oil droplets as inclusions.

**Figure 6.10** The height melting percentage of model processed cheese manufactured with different types of emulsions with added TSC (oil concentration: 0 to 30%) (protein concentration: 20%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (■) % Melt of PC made with emulsion stabilised by calcium caseinate; (▲) % Melt of PC made with emulsion stabilised by WPI; (◆) % Melt of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations.

Figure 6.10 presented the height melting percentage of model processed cheese containing oil droplets stabilised with calcium caseinate, WPI or lecithin. Based on Figure 6.10, model processed cheese containing oil droplets stabilised by WPI presented the lowest melting percentage followed by model processed cheese containing oil droplets stabilised with calcium caseinate or lecithin. Besides, the height melting percentage of processed cheese samples containing oil droplets stabilised with calcium caseinate and WPI decreased (from ~84 to ~75% and from ~83 to ~65%, respectively) with the increasing amount of oil concentration (5 to 30%). In the case of processed cheese samples containing oil droplets stabilised with lecithin, the height melting percentage slightly increased from ~90 to ~94%, when the oil concentration increased from 5 to 30% respectively indicating that the model processed cheese becomes softer.
Cho et al. (1999) suggested that, denatured whey proteins-coated oil droplets have the ability to cross-link with the casein network extensively. The increasing degree of cross-linking between the denatured whey proteins and the casein network created a strong protein-oil interactions which further form a very firm structure with low meltability. This statement is consistent with the results obtained in this study when model processed cheese containing oil droplets stabilised with WPI showed the highest $G^*$ values (Figure 6.3), higher critical shear stress $\sigma_c$ (Figure 6.5), lower critical strain $\gamma_c$ (Figure 6.6), smallest oil droplet particle size (Figure 6.8), a more compact protein network structure (Figure 6.9) and lowest meltability (Figure 6.10) compared to model processed cheese containing oil droplets stabilised by calcium caseinate or lecithin.

In model processed cheese containing oil droplets stabilised by calcium caseinate, the calcium caseinate coated around the oil droplets interact positively with the casein-based gel network via casein-casein interactions and increased the total surface area of the particles in the protein network (Cho et al., 1999; Everett & Olson, 2003). This enhances the development of stiffer protein gel network as seen from higher $G^*$ values (Figure 6.3), smaller oil droplet particle size (Figure 6.8) and low meltability (Figure 6.10) of processed cheese samples (Everett & Olson, 2003). However in the case of lecithin it is expected that the oil droplets will not interact with the protein matrix, which would explain the low elasticity and enhanced melting, compared to WPI or calcium caseinate.

### 6.3.2 Effect of sodium pyrophosphate (TSPP) on model processed cheese made with emulsions stabilised by different emulsifiers

This section will be discussing on model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin with added sodium pyrophosphate (TSPP). Figure 6.11 showed the viscoelasticity ($G'$ and $G''$) of model processed cheese emulsified with TSPP and stabilised by different types of oil droplet surface materials. Based on the result shown in Figure 6.11, model processed cheese stabilised by WPI presented the highest $G'$ and $G''$ moduli (highest firmness) followed by model processed cheese stabilised with calcium caseinate and lecithin (lowest firmness).
$G^*$ values of model processed cheese samples containing oil droplets stabilised with WPI (from ~4.6 to ~8.0 kPa) and calcium caseinate (from ~3.9 to ~7.1 kPa) increased with the increasing amount of oil concentration (Figure 6.12). This result gives an indication that the processed cheese samples became firmer as the oil concentration increased from 0 to 30%. In general, the model processed cheese samples emulsified with TSPP demonstrated similar trend with the model processed cheese samples emulsified with TSC. Model processed cheese containing oil droplets stabilised by WPI displayed the highest $G^*$ values (from ~4.6 to ~8.0 kPa) and model processed cheese containing oil droplets stabilised by lecithin presented the lowest $G^*$ values (became softer) (from ~3.9 to ~3.3 kPa) with the increased of oil concentration (from 0 to 30%).

![Graph](image)

Figure 6.11 Evolution of elastic ($G'$) and loss ($G''$) modulus of model processed cheese manufactured with different types of emulsions with added TSPP as a function of frequency (oil concentration: 20%) (protein concentration: 20%). (●) $G'$ of PC made with emulsion stabilised by calcium caseinate; (□) $G''$ of PC made with emulsion stabilised by calcium caseinate; (▲) $G'$ of PC made with emulsion stabilised by WPI; (●) $G''$ of PC made with emulsion stabilised by WPI; (★) $G'$ of PC made with emulsion stabilised by lecithin; (★) $G''$ of PC made with emulsion stabilised by lecithin.

Millqvist-Fureby et al. (2001) suggested that heat treatment at 60-80°C can unfold major components of whey proteins such as α-lactalbumin and β-lactoglobulin. These unfolding causes covalent and non-covalent bonds to break thus, producing aggregations of the whey
proteins. During model processed cheese manufacture, the aggregated whey proteins then associated with the dispersed casein (caused by the presence of TSPP) and form bridges between the caseins. Extensive association occur between the denatured whey proteins and the caseins particles increased the numbers and strength of the bonds produced resulting in the formation of a very firm gel network within the processed cheese matrix (Lucey, Munro, et al., 1998). This increases the $G'$ and $G''$ moduli and the $G^*$ values of model processed cheese containing oil droplets stabilised by WPI.

![Figure 6.12](image_url) Complex modulus ($G^*$) of model processed cheese samples manufactured with different types of emulsions with added TSPP as a function of oil concentration (protein concentration: 20%). (●) $G^*$ of PC made with emulsion stabilised by calcium caseinate; (▼) $G^*$ of PC made with emulsion stabilised by WPI; (★) $G^*$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations.

Besides, oil droplets coated with WPI or calcium caseinate are both active fillers which interact positively with the casein particles (Cho et al., 1999). During the manufacture of model processed cheese, samples containing oil droplets stabilised by calcium caseinate associated with the casein particles via casein-casein interactions (Cunha & Viotto, 2010; Dickinson, 2006; Ye & Singh, 2001). The increased protein-protein interactions then produced stronger gel network which increased the $G'$ and $G''$ moduli and the $G^*$ values of model processed cheese containing oil droplets stabilised by calcium caseinate. However, the
casein-casein interactions were not as strong as the interactions between denatured whey proteins and the casein particles (Lucey, Tamehana, et al., 1998). For model processed cheese containing oil droplets stabilised by WPI or calcium caseinate, the increasing amount of surface active oil droplets further increased the $G'$ and $G''$ moduli and the $G^*$ values of the processed cheese samples with the increasing amount of oil concentrations.

![Figure 6.13](image)

**Figure 6.13** Evolution of critical shear stress $\sigma_c$ of model processed cheese manufactured with different types of emulsions with added TSPP as a function of oil concentration (0 to 30%) (protein concentration: 20%). (■) $\sigma_c$ of PC made with emulsion stabilised by calcium caseinate; (△) $\sigma_c$ of PC made with emulsion stabilised by WPI; (☆) $\sigma_c$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations.

As previously explained in the Section 6.3.1, oil droplets stabilised by lecithin acted as inert filler because the presence of lecithin weaken the mechanical properties of the oil droplets interface thus, may induce partial coalescence between the oil droplets (Cho et al., 1999; Dalgleish, Srinivasan, & Singh, 1995). In model processed cheese containing oil droplets stabilised by lecithin, charge repulsion between the negatively charged lecithin layer and the negatively charged casein particles weaken the gel network structure formed (Lee et al., 1996). This lead to the decreased of the $G'$ and $G''$ moduli and the $G^*$ values of the processed cheese samples containing oil droplets stabilised by lecithin.
The critical shear stress $\sigma_c$ of model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin decreased with the increasing amount of oil concentration (Figure 6.13). Model processed cheese containing oil droplets stabilised by WPI presented the highest critical shear stress $\sigma_c \approx 2.5$ kPa at 5% oil, followed by model processed cheese samples containing oil droplets stabilised with lecithin ($\sigma_c \approx 2.3$ kPa) and calcium caseinate ($\sigma_c \approx 2.0$ kPa). With the increasing amount of oil concentration from 10 to 30%, $\sigma_c$ decreased linearly for all the emulsions stabilised with different emulsifiers. At these oil concentrations (>10%) the values of $\sigma_c$, within experimental errors, are also similar for the different emulsions.

**Figure 6.14** Evolution of critical strain $\gamma_c$ of model processed cheese manufactured with different types of emulsions with added TSPP as a function of oil concentration (0 to 30%) (protein concentration: 20%). (●) $\gamma_c$ of PC made with emulsion stabilised by calcium caseinate; (▲) $\gamma_c$ of PC made with emulsion stabilised by WPI; (★) $\gamma_c$ of PC made with emulsion stabilised by lecithin. Data represent means (n = 2) and error bars correspond to standard deviations.

The result for critical strain $\gamma_c$ of model processed cheese containing oil droplets stabilised by different surface active materials with added TSPP shown in Figure 6.14 also demonstrated similar behaviour with the critical strain $\gamma_c$ of model processed cheese samples emulsified with TSC. Within experimental errors, the critical strain $\gamma_c$ increases from ~79.4 to ~158 Pa.
when the oil concentration increases from 5 to 20% (for processed cheese with WPI). When
the oil concentration is higher than 20%, the critical strain $\gamma_c$ seems to plateau at values of
approximately of ~158%, ~179% and ~200% for the processed cheeses emulsified with WPI,
calcium caseinate and lecithin, respectively. Note that while small deformation rheology (e.g.
Figure 6.12) showed clear differences between the different processed cheese, the
measurements of the critical strain $\gamma_c$ and the critical shear stress $\sigma_c$ showed similar behaviour
and values for the different processed cheese. This is not contradictory as the measurements
of $\gamma_c$ and $\sigma_c$ are obtained using large deformations. The results in Figure 6.14 shows that
model processed cheese containing oil droplets stabilised by lecithin deform more. On the
contrary, model processed cheese containing oil droplets stabilised by WPI or calcium
caseinate deform less.

Figure 6.15 Oil droplet particle size distribution for model processed cheese manufactured
with different types of emulsions with added TSPP (oil concentration: 20%) (protein
concentration: 20%). (■) PC made with emulsion stabilised by calcium caseinate; (●) PC made with emulsion stabilised by WPI; (★) PC made with emulsion stabilised by lecithin; (■) WPI emulsion- before being added into PC.

The oil droplet particle size distributions of WPI emulsion (before incorporated into
processed cheese mixture) and model processed cheese containing oil droplets stabilised with
different oil droplet surface materials are presented in Figure 6.15. Based on the result shown
in Figure 6.15, the largest oil droplet particle size distribution is shown by WPI emulsion
(before being added to processed cheese). Similar with the result of oil droplet particle size
distribution shown in Figure 6.7 (model processed cheese containing oil droplets stabilised by
different surface active materials and emulsified with TSC), the model processed cheese
samples emulsified with TSPP in this section demonstrated almost similar oil droplet particle
size distribution.

![Figure 6.16](image)

**Figure 6.16** Mean average oil droplet particle sizes ($D(3, 2)$ in µm) of processed cheese
manufactured with different types of emulsions with added TSPP as a function of oil
concentration (oil concentration: 0 to 30%) (protein concentration: 20%). (●) $D_{3,2}$ of PC
made with emulsion stabilised by calcium caseinate; (▲) $D_{3,2}$ of PC made with emulsion
stabilised by WPI; (★) $D_{3,2}$ of PC made with emulsion stabilised by lecithin. Data represent
means (n = 3) and error bars correspond to standard deviations.

All of the processed cheese samples also exhibited monomodal peak in the oil droplet particle
size distribution. To compare between the oil droplet particle size of model processed cheese
samples corresponding to different oil concentration, a more detailed result is plotted in
Figure 6.16. Based on Figure 6.16, model processed cheese containing oil droplets stabilised
with WPI demonstrated the smallest oil droplet particle size ($D_{3,2}$) compared to model
processed cheese containing oil droplets stabilised by calcium caseinate or lecithin. Figure
6.16 also demonstrated that model processed cheese containing oil droplets stabilised by
lecithin exhibited the largest oil droplet particle size.
Increasing the oil concentration from 5 to 30%, increased the oil droplet particle size of model processed cheese containing oil droplets stabilised by calcium caseinate from ~1.6 to ~1.7 μm and lecithin ~2.0 to ~2.4 μm. For model processed cheese containing oil droplets stabilised by WPI, increasing oil concentration from 5 to 15%, increased the oil droplet particle size from ~1.2 to ~1.3 μm, respectively. However, further increase in oil concentration from 20 to 30% in processed cheese made with WPI emulsions, decreased the oil droplet particle size from ~1.3 to ~1.0 μm. The presence of TSPP in cheese samples may enhance protein solubilization and dispersion during manufacture. However, with the existence of surface active materials adsorbed on the oil droplet surface, the role of TSPP was not as vital as having the native oil droplets alone. In this case, after TSPP open up (dispersed) the casein particles, it solely depends on the surface active material on the oil droplet surface to cross-link or not to cross-link with the dispersed caseins. This may explain the changes of the oil droplet size with the increasing amount of oil concentration.

As explained in the Section 6.3.1, partial coalescence may have occurred for model processed cheese samples containing oil droplets stabilised with lecithin resulting in an increased of oil droplet size. The results of oil droplet particle size distributions and oil droplet particle size of model processed cheese samples in this study also correspond to the result of microstructural images obtained from SEM. Figure 6.17 showed the microstructural images of model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin. Based on Figure 6.17, both model processed cheese containing oil droplets stabilised with calcium caseinate or WPI displayed compact protein network with small sizes of oil droplets being spread within the protein matrix. Through visual observation of the SEM images of these processed cheese samples, it is not easy to determine which one between the two has a more compact or firm gel matrix.

However, the same phenomenon does not occur to model processed cheese containing oil droplets stabilised by lecithin. Figure 6.17 demonstrated that model processed cheese containing oil droplets stabilised by lecithin possess a smooth texture, loose protein matrix surrounded by large oil droplets. Research done by Cho et al. (1999) also presented similar result where milk gels containing fat globules stabilised by Tween 80 demonstrated loose gel matrix. The result of their study proved that Tween 80 acted as inert filler which is similar
with lecithin in our study. Lecithin do not cross-link with the dispersed casein and more attracted towards each other causing large oil droplet size and weaken the protein gel network.

**Figure 6.17** Scanning electron (SEM) micrographs of model processed cheese made with different types of emulsions with added TSPP (oil concentration: 20%) (protein concentration: 20%). a) PC made with emulsion stabilised by calcium caseinate, b) PC made with emulsion stabilised by WPI, c) PC made with emulsion stabilised by lecithin. Scale bar = 20µm.

The result of the melting properties of the processed cheese samples shown in Figure 6.18 is also in agreement with the results of $G^*$ values, oil droplet particle size and microstructural images. Based on Figure 6.18, model processed cheese containing oil droplets stabilised by
WPI presented the lowest melting percentage (~67% at 30% oil) (highest firmness) and the model processed cheese containing oil droplets stabilised by lecithin displayed the highest melting percentage (~88% at 30% oil) (softer texture).

**Figure 6.18** The height melting percentage of model processed cheese manufactured with different types of emulsions with added TSPP (oil concentration: 0 to 30%) (protein concentration: 20%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (●) % Melt of PC made with emulsion stabilised by calcium caseinate; (▲) % Melt of PC made with emulsion stabilised by WPI; (★) % Melt of PC made with emulsion stabilised by lecithin. Data represent means (n = 3) and error bars correspond to standard deviations.

The excessive amount of disulphide bridging between denatured whey proteins and casein network may inhibit melting properties in model processed cheese samples containing oil droplets stabilised by WPI. The casein-casein interaction in model processed cheese containing oil droplets stabilised by calcium caseinate increased the interconnectivity of the gel matrix thus, inhibit the melting properties. The presence of surface materials with lack cohesiveness (lecithin) coated around the oil droplet surface has prevented the interaction between the oil droplets and the casein particles causing an increase in the melting percentage of the processed cheese samples (Cho et al., 1999).
Overall, model processed cheese containing oil droplets stabilised by calcium caseinate, WPI or lecithin and made with TSPP demonstrated similar trend of $G^*$ values, oil droplet particle size, microstructure and melting properties with model processed cheese samples made by different types of emulsions and emulsified with TSC. The next section will discuss in detail if there is a difference in rheological properties, oil droplet particle size and melting properties of these samples using either TSC or TSPP as the emulsifying salts.

6.3.3 Comparison between processed cheese made by TSC or TSPP

Figure 6.19 Complex modulus ($G^*$) of model processed cheese samples manufactured with different types of emulsions with added TSC or TSPP as a function of oil concentration (protein concentration: 20%). (■) $G^*$ of PC made with emulsion stabilised by calcium caseinate with added TSC; (▲) $G^*$ of PC made with emulsion stabilised by WPI with added TSC; (♦) $G^*$ of PC made with emulsion stabilised by lecithin with added TSC; (□) $G^*$ of PC made with emulsion stabilised by calcium caseinate with added TSPP; (△) $G^*$ of PC made with emulsion stabilised by WPI with added TSPP; (◇) $G^*$ of PC made with emulsion stabilised by lecithin with added TSPP. Data represent means ($n = 2$) and error bars correspond to standard deviations.

Figure 6.19 shows the evolution of complex modulus ($G^*$) of model processed cheese samples stabilised by calcium caseinate, WPI or lecithin and emulsified with TSC or TSPP at
different oil concentration. Based on Figure 6.19, the results of processed cheese samples emulsified with TSC demonstrated similar trends with the results of processed cheese samples emulsified with TSPP.

![Graph showing average oil droplet particle sizes](image)

**Figure 6.20** Mean average oil droplet particle sizes ($D(3,2)$ in μm) of processed cheese manufactured with different types of emulsions (oil concentration: 5 to 30%) (protein concentration: 20%). (■) $D_{3,2}$ of PC made with emulsion stabilised by calcium caseinate with added TSC; (▲) $D_{3,2}$ of PC made with emulsion stabilised by WPI with added TSC; (◆) $D_{3,2}$ of PC made with emulsion stabilised by lecithin with added TSC; (□) $D_{3,2}$ of PC made with emulsion stabilised by calcium caseinate with added TSPP; (△) $D_{3,2}$ of PC made with emulsion stabilised by WPI with added TSPP; (◇) $D_{3,2}$ of PC made with emulsion stabilised by lecithin with added TSPP. Data represent means ($n = 3$) and error bars correspond to standard deviations.

The $G^*$ values of model processed cheese containing oil droplets stabilised by calcium caseinate or lecithin and emulsified with TSC showed no significant difference ($P<0.05$) with the results of model processed cheese containing oil droplets stabilised by calcium caseinate or lecithin and emulsified with TSPP as the oil concentration increased from 5 to 30%. In the case of model processed cheese containing oil droplets stabilised by WPI, no significant difference ($P<0.05$) can be observed for model processed cheese emulsified with TSC or TSPP when the oil concentration increased from 10 to 30%.
As discussed in the previous sections, in both cases, $G^*$ values increased with increasing oil concentration for model processed cheese samples containing oil droplets stabilised by WPI or calcium caseinate. This phenomenon occurs because oil droplets stabilised with WPI or calcium caseinate are active fillers which can interact positively with the casein network producing stiffer gel network (Cho et al., 1999; Everett & Olson, 2003; Lucey, Tamehana, et al., 1998). The $G^*$ values decreased with the increasing amount of oil concentration for model processed cheese samples containing oil droplets stabilised by lecithin either emulsified by TSC or TSPP. Oil droplets stabilised by lecithin may act as inert filler which does not interact with the casein network thus, weakening the gel matrix (Cho et al., 1999; Dalgleish et al., 1995; Lucey, Munro, et al., 1998).

Figure 6.20 demonstrates the oil droplet particle size of model processed cheese containing oil droplets stabilised by different surface materials and made with TSC or TSPP. The oil droplets particle size of these processed cheese samples either made with TSC or TSPP showed no significant difference ($P<0.05$) with each other at certain amount of oil concentrations (e.g. processed cheese made with emulsion stabilised by calcium caseinate, from 15 to 30% oil; processed cheese made with emulsion stabilised by WPI, at 5, 15 and 20% oil; processed cheese made with emulsion stabilised by lecithin, at 5% and from 15 to 30% oil). Significant differences ($P<0.05$) can be observed for few emulsions, e.g. at 10% oil concentration of emulsion stabilised by lecithin. These differences in particle size also can be observed for model processed cheese containing oil droplets stabilised by calcium caseinate and made with TSPP as the oil concentration increased from 5 to 10% where these samples showed smaller oil droplets compared to model processed cheese samples made with TSC.

The height melting percentage of model processed cheese samples containing oil droplets stabilised by different surface active materials and emulsified with TSC or TSPP is shown in Figure 6.21. Based on Figure 6.21, the results of the height melting percentage between processed cheese samples made either with TSC or TSPP demonstrated similar trends. Small changes can be seen for certain oil concentrations between the samples, but the changes was not significant ($P<0.05$) except for model processed cheese containing oil droplets stabilised by lecithin and emulsified with TSPP which showed lower melting percentage compared to model processed cheese samples containing oil droplets stabilised by lecithin and emulsified with TSC at 25 to 30% oil concentration. This result is consistent with the result shown in Figure 6.19 where model processed cheese containing oil droplets stabilised by lecithin and
made with TSPP demonstrated higher $G^*$ values (higher firmness) at 30% oil compared to model processed cheese samples emulsified with TSC.

**Figure 6.21** The height melting percentage of model processed cheese manufactured with different types of emulsions (oil concentration: 0 to 30%) (protein concentration: 20%). The ratio of protein concentration and emulsifying salt were the same for all samples (10:1). (■) % Melt of PC made with emulsion stabilised by calcium caseinate with added TSC; (▲) % Melt of PC made with emulsion stabilised by WPI with added TSC; (◆) % Melt of PC made with emulsion stabilised by lecithin with added TSC; (□) % Melt of PC made with emulsion stabilised by calcium caseinate with added TSPP; (△) % Melt of PC made with emulsion stabilised by WPI with added TSPP; (◇) % Melt of PC made with emulsion stabilised by lecithin with added TSPP. Data represent means (n = 3) and error bars correspond to standard deviations.

As discussed in **Chapter 5** (Section 5.3.3), TSPP demonstrated more ability to emulsify oil and perform calcium chelation at higher oil concentration. In the case of processed cheese samples containing oil droplets stabilised by lecithin at higher oil concentration (25 to 30%), the presence of TSPP may chelate calcium (thus solubilising the casein aggregates) more extensively compared to TSC. Due to the existence of the lecithin layer surrounding the oil droplet surface, the oil droplets does not interact with the casein particles and left the dispersed caseins (chelated by TSPP) to interact with each other forming stronger casein-
casein interaction compared to processed cheese samples emulsified by TSC. Therefore the melting percentage was lower in TSPP cheese (oil droplets stabilised by lecithin) samples.

6.4 Summary to chapter

In conclusion, the $G^*$ values increased with the increase in oil concentration when calcium caseinate or WPI were used as emulsions in model processed cheese with model processed cheese made from WPI emulsion displayed higher values. $G^*$ values of model processed cheese containing oil droplets stabilised by different surface materials were in the following order: lecithin < calcium caseinate < WPI. In this study, WPI coated around the oil droplet surface may have denatured during cheese-making process thus, enhance the association of casein and participate in the gel matrix which increased the $G^*$ values. Model processed cheese containing oil droplets stabilised by lecithin showed the lowest $G^*$ values because the adsorbed material may not interact with the protein matrix. The microstructure of model processed cheese made from lecithin emulsion displayed larger oil droplets surrounded by the protein structure which made the whole processed cheese matrix weaker compared to model processed cheese containing oil droplets stabilised by WPI or calcium caseinate. The comparison between the results of different emulsifying salts (TSC or TSPP) on model processed cheese samples containing different oil droplet surface materials provide similar trends. This study demonstrated that the type of adsorbed layer around the oil droplets strongly influences the rheological properties and melting properties of model processed cheese.
Small angle X-ray scattering (SAXS) investigation on the effect of different emulsifying salts on reconstituted milk
7.1 Introduction

Milk consists of proteins, fats, carbohydrates, enzymes, vitamins, minerals and salts (Talwar & Srivastava, 2002). About 80% of proteins in milk are casein. There are four main types of caseins which are $\alpha_s1$, $\alpha_s2$, $\beta$- and $\kappa$-caseins (CN). These caseins co-exist with colloidal calcium phosphate (CCP) in the form of aggregates known as casein micelles (Fox, 2004). Due to the high concentration of casein micelles in milk, casein play a vital role in determining the quality of milk, cheese and most dairy products during processing (Jenness, Wong, Marth, & Keeney, 1988). The structural and physical properties of casein have been widely studied over the past decades (Shukla, Narayanan, & Zanchi, 2009). However, the real structure of casein is still a debate among scientists due to the casein’s complex nature, which cannot be completely reproduced in vitro (Bouchoux et al., 2010). There were several acceptable models which have been proposed by previous researchers for the internal structure of the casein micelle. These models fall into two broad categories which are the sub-micelle model and the nanocluster model (Mata et al., 2011). The sub-micelle model refers to submicelles and consists of hard region made of proteins surrounding calcium-phosphate (Ca-P) nanoclusters. The nanocluster model on the other hand, refers to the colloidal calcium phosphate (CCP) nanoparticles which are randomly distributed within the casein micelle and surrounded by protein molecules (Mata et al., 2011).

The use of emulsifying salt plays an important role in determining the functional characteristics of dairy products such as processed cheese (Cunha & Viotto, 2010). The addition of emulsifying salts into the milk disrupts casein by reducing the calcium and CCP content which causes casein micelle dissociation (McSweeney & Fox, 2009). Different types of emulsifying salts gave distinctive emulsifying capacity and it is interesting to know the effects of different emulsifying salts on the internal structure of casein micelles. Small angle x-ray scattering (SAXS) is a powerful technique where structural features of different components can be distinguished (Lopez-Rubio & Gilbert, 2009). Quantitative information about the extent and correlation of structural inhomogeneities in the electron density distribution of a material can be obtained using this method (Diat, Narayanan, Abernathy, & Grübel, 1998). Calcium phosphate and casein components have different scattering length densities which enable the separation of the components to the overall scattering which allow
the proposed models to be made possible (Lopez-Rubio & Gilbert, 2009). Previous studies have demonstrated several casein micelle models and structures using SAXS (Holt et al., 2003; Kumosinski, Pessen, Farrell Jr, & Brumberger, 1988; Marchin, Putaux, Pignon, & Léonil, 2007). However, no study has been done to investigate the internal structures of the casein micelles when milk is reacted with emulsifying salts using SAXS. Previous study on emulsified milk has been done using other methods such as several microscopy techniques, rheological measurements and particle size (Cunha et al., 2010; El-Bakry & Sheehan, 2014; Mizuno & Lucey, 2005a; Ozcan et al., 2008). Based on Chapter 4, Chapter 5 and Chapter 6, we have developed a good understanding on the relationship between emulsifying salt, protein matrix and oil droplets. The emulsifying ability of each emulsifying salt either trisodium citrate (TSC) or sodium pyrophosphate (TSPP) was highly influenced by the amount of protein and oil concentrations present in the model processed cheese systems. Besides, changing the type of the adsorbed layer on the oil droplet surface can affect the model processed cheese properties. Therefore, in this study we would like to investigate the emulsifying ability of four different types of emulsifying salts on internal structure of the casein micelle using SAXS. The emulsifying salts chosen were sodium phosphate (SP), TSPP, sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP). These emulsifying salts were chosen based on the most commonly used emulsifying salts in processed cheese and also were supported by previous research (Cavalier-Salou & Cheftel, 1991; Cunha & Viotto, 2010; Dybing et al., 1982; Sádlíková et al., 2010; Shirashoji, Jaeggi, & Lucey, 2010; Wong, LaCroix, Mattingly, Vestal, & Alford, 1976). Although Chapter 4, Chapter 5 and Chapter 6 used calcium caseinates as the protein in the manufacture of processed cheese, in this chapter milk was used as it is a good model to study the chelation of calcium by different emulsifying salts.

Please note that the experiments were performed in the Australian Synchrotron in Melbourne by my PhD supervisors (Sylvie Marchesseau and Yacine Hemar) and the samples were prepared by a master student, Wei-Ping Pai and the author of this thesis.

The objective of this study is to determine the internal structures of milk system with added emulsifying salts using small angle X-ray scattering (SAXS).
7.2 Materials and Methods

7.2.1 Materials

The milk samples in this research were made from low heat skim milk powder, sodium phosphate (SP), sodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP) and sodium azide.

7.2.2 Sample preparation and experimental methods

The milk samples were prepared as previously described in Chapter 3 (Section 3.3.3). Briefly, stock skim milk sample containing solid concentration of 20% (w/w) was prepared by mixing 100g of low heat skim milk powder with 400g MilliQ water and 0.02% (w/w) sodium azide (NaN$_3$). The mixture was gently stirred for two hours using a magnetic stirrer. Then, emulsifying salts were added into the milk solution.

Two batches of milk were prepared. The first batch consist of five 10% (w/w) milk solution, one as a control (no emulsifying salts added), and each of the remaining four 10% (w/w) milk solution was added with different types of emulsifying salts (SP, TSPP, STPP and SHMP). The concentration of the emulsifying salts in this batch was 0.5% (w/w). In the second batch, apart from the control milk sample, five different concentrations (0.1%, 0.25%, 0.5%, 1% and 2%) of emulsifying salts (SP, TSPP, STPP and SHMP) were added into 10% (w/w) milk solution. Milk samples with 1% and 2% of emulsifying salts were heated up to 120ºC for 10 minutes and were cooled to room temperature (~24ºC). This heating step was performed since in processed cheese heating is an important parameter.

The internal structures of the milk samples were determined using SAXS as described in Chapter 3 (Section 3.4.5).
7.3 Results and Discussion

7.3.1 Influence of different emulsifying salts

Figure 7.1 shows the effect of different emulsifying salts on 10% (w/w) milk samples with fixed emulsifying salts concentration (0.5%, w/w). The results obtained in this study shows that the casein micelle demonstrated comparable SAXS pattern with three distinguish features as described by Bochoux et al. (2010) and Mata et al. (2011) for native phosphocaseinate powder (NPC) and milk protein concentrate, respectively. These features are known as level 0, 1 and 2 (arrows in Figure 7.1):

Level 0: Consist of low $q$ values, $\leq 6 \times 10^{-3}$ Å$^{-1}$, with the distance of less than 100nm. This range is believed to indicate the shape of casein micelles and distance separating them.

Level 1: Assigned to sub-micelles model where small protein micelles are linked together through bridging CCP nanoclusters. The range of $q$ values in this signal is from $6 \times 10^{-3}$ to $2 \times 10^{-2}$ Å$^{-1}$. The size of sub-micelles in this signal is approximately 40 nm. This range can be clearly seen when data are plotted as Kratky plot; $I(q) \times q^2$ versus $q$.

Level 2: Refers to nanoclusters model where CCP nanoparticles (CPN) randomly distributed within casein micelle with each other surrounded protein molecules. This signal exhibit high $q$ values; $\geq 7 \times 10^{-2}$ Å$^{-1}$, which is equivalent to a CPN with 4-5 nm in size.

Based on the SAXS intensities as shown in Figure 7.1, the size of casein micelles decreased according to this following order: control $\approx$ SP $>$ TSPP $>$ STPP $>$ SHMP. This result demonstrated that, the size of casein micelles decrease with the increase of the phosphate chain length. According to De Kort, Minor, Snoeren, Van Hooijdonk, and Van der Linden (2009), longer phosphate chain length can enhance calcium binding capacity compared to phosphate with shorter chain. The amount of cations added (protein concentration) and the number of available binding sites on phosphate affect the total binding between phosphates with cations ($Ca^{2+}$) and amino acids in the casein micelle (De Kort et al., 2009; Mizuno & Lucey, 2007; Pitkowski, Nicolai, & Durand, 2008; Vujicic, Batra, & DeMan, 1967).
Figure 7.1 SAXS profile of different emulsifying salts on 10% (w/w) milk samples. Emulsifying salts concentration is 0.5% (w/w). The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to size of casein micelles, level 1 is assigned to sub-micelles, and level 2 is related to calcium phosphate nanoclusters. (▼) Control milk; (■) Milk with SP; (▲) Milk with TSPP; (○) Milk with STPP; (□) Milk with SHMP.

Note that the chemical structures of each emulsifying salt used in this chapter are presented in Chapter 2 (Section 2.4) of this thesis. Based on Figure 2.8 to Figure 2.11 (Chapter 2, Section 2.4), there are six homogenously distributed negative charges around SHMP molecules compared to SP (three negative charges), TSPP (four negative charges) and STPP (five negative charges) which allow SHMP to interact better with cations in the caseins (De Kort, Minor, Snoeren, Van Hooijdonk, & Van der Linden, 2011). This means that SHMP have a stronger effect on casein micelle dissociation, which is higher compared to STPP and TSPP (even if all calcium seems to be chelated for each emulsifying salt with chain length higher than 2; see peak corresponding to level 2). Without any addition of emulsifying salt (control), both peaks at level 1 and 2 can be seen from the SAXS profile. With the addition of SP, the
intensity of the peak in level 1 was slightly lower compared to the control (inset figure; Kratky plot) but the intensity of the peak at level 2 was similar with control milk sample. This showed that with the addition of SP, casein micelles were slightly dispersed and the size of casein micelle becomes smaller. The signal of the peak in level 1 shifted to higher $q$ when milk was treated with TSPP or STPP or SHMP which indicates that the sub-micelles undergo an alteration of structure. Furthermore, the addition of TSPP or STPP or SHMP leads to the disappearance of the peak in level 2 which indicates that the addition of these emulsifying salts induce the dissolution of the CPNs due to the chelations of all the calcium in the milk.

When emulsifying salts are added into milk samples, the calcium in milk would be replaced by the cation in the emulsifying salts (sodium). This is known as calcium sequestering process. Then, the large hydrophobic aggregates of casein micelles will break down into sub-micelles or smaller units, exposing the polar and non-polar segments (Lee et al., 1996) which is known as peptization process. The peptization process enhances the protein’s water binding capacity and improves the emulsifications capabilities (Cunha & Viotto, 2010). This result proves that, SHMP has the greatest calcium sequestering ability compared to STPP, TSPP and SP which enhanced the protein-protein interactions in the emulsified milk (Swenson et al., 2000). Previous research also showed that SHMP is a good emulsifier because the ratio of the calcium-phosphate binding capacity for SHMP is 3:1 (De Kort et al., 2009).

7.3.2 Influence of different emulsifying salts concentrations

Figure 7.2 to 7.5 present the individual effect of each of the emulsifying salts on 10% milk where the emulsifying salts concentrations were varied from 0.1% to 2%. As discussed earlier in this study and from previous research (Guinee et al., 2004; Kaliappan & Lucey, 2011; Lu et al., 2008), SP was not a good chelator compared to other emulsifying salts. Therefore, in this section, this study would like to investigate how much concentration of each emulsifying salt is needed to affect fully the internal structure of the milk samples (full casein micelles dissociation).

In Figure 7.2, with the increasing of SP concentrations (0.1% to 2%), the size of casein micelles slowly decreased (peak in level 0). In the inset figure of Figure 7.2 (Kratky plot)
both signals of level 1 and 2 can be clearly seen when SP concentration was increased from 0.1 to 0.5% which is similar to control milk sample. This result proves that, increasing SP concentration from 0.1% to 0.5% has no or very little effect on the structure of milk sample. However, as the concentration of SP was increased to 1% and 2%, the intensities of the peak in level 1 shifted towards higher $q$ values and the shoulder of the peak in level 2 disappears. Milk sample with 2% SP showed lower peaks for both level 0, 1 and 2 compared to milk sample with 1% SP, indicating the extent of dissociation with the increase in SP concentration to 2%.

**Figure 7.2** SAXS profile of different concentrations of sodium phosphate (SP) on 10% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▾) Control milk; (■) Milk with 0.1% SP; (●) Milk with 0.25% SP; (○) Milk with 0.5% SP; (□) Milk with 1% SP; (●) Milk with 2% SP.

From the result in Figure 7.2, casein dissociation started to occur only when ≥1% SP was added into milk samples. At the SP concentration of 1% and 2%, the casein micelles started
to disperse, creating smaller sub-micelles which shifted the peak of level 1 to higher $q$ values. The peak in level 2 diminishing is an indication that the addition of $\geq 1\%$ SP caused the dissolution of the CPNs due to the chelations of all the calcium in the milk. The casein micelle dispersed more in SP $2\%$ compared to SP $1\%$ creating smaller sizes of casein aggregates (level 0), the existence of more smaller sub-micelles (level 1) and the removal of the CPNs (level 2). Based on the results in Figure 6.2, SP can act as a good calcium chelating agent if the concentration is high ($\geq 1\%$).

Figure 7.3 SAXS profile of different concentrations of sodium pyrophosphate (TSPP) on $10\%$ (w/w) milk samples. The temperature for all samples was $20^\circ$C. Intensities are in arbitrary units (a.u.) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 corresponds to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 is assigned to calcium phosphate nanoclusters. (▼) Control milk; (■) Milk with 0.1% TSPP; (▲) Milk with 0.25% TSPP; (○) Milk with 0.5% TSPP; (□) Milk with 1% TSPP; (●) Milk with 2% TSPP.

A study done by (Mizuno & Lucey, 2005a), also proves that low concentrations of SP ($\leq 0.7\%$) did not have major influence on CCP and casein micelles. SP has the lowest phosphate chain length compared to other emulsifying salts. In order to achieve more calcium sequestration,
more amount of SP is needed to chelate all the calcium in milk to fully dissociate the casein micelles.

Figure 7.3 demonstrates that, with the increasing of TSPP concentrations (0.1% to 0.5%), the size of casein micelles also decreased. Then, the size of casein aggregates increased gradually when TSPP concentration was increased from 1% to 2%. Based on the inset of Figure 7.3 (Kratky plot), both peaks for signals of level 1 and 2 can be clearly seen when TSPP concentration was increased from 0.1 to 0.25%.

As the concentration of TSPP increased from 0.5% to 2%, the signal of level 1 shifted to higher $q$ values and created a new peak at $q=\sim0.017\AA^{-1}$ (inset Figure 7.3) which is a sign of new interactions and gelation. Furthermore, the shoulders for the peak in level 2 becomes less prominent by increasing TSPP concentrations from 0.5% to 2%. This is caused by the demineralisation of the submicelle and the nanoclusters. The milk samples with TSPP become dissociated at TSPP ≥0.5%. Mizuno and Lucey (2005a) reported that, increasing the amount of TSPP from 0.3 to 0.7%, increased the amount of casein dispersion in milk protein concentrate. This is also in agreement with Ozcan et al. (2008), where increasing TSPP from 0.15% to 0.2% resulted in gels with bigger pores and less interconnectivity compared to lower TSPP concentrations. This might explain the increasing size of casein micelles when TSPP concentrations were increased from 1% to 2%. The peak for the signal of level 1 shifted to higher $q$ and the disappearance of the peak for the signal in level 2 in ≥0.5% TSPP milk samples correspond to the total calcium dissociation.

Figure 7.4 shows the SAXS profile of the effect of different STPP concentrations on 10% milk. By increasing STPP concentrations (0.1% to 2%), the size of casein micelles decreased (Figure 7.4). Based on the inset of Figure 7.4 (Kratky plot), both peaks for level 1 and 2 can be clearly seen when 0.1% STPP concentration was used. As the STPP concentration was increased to 0.25%, the peak at level 1 shifted towards higher $q$ values but the peak for the signal in level 2 become less pronounced. As more concentration of STPP is added (0.5% to 2%), the peak for level 1 shifted further towards higher $q$ values as the shoulder for level 2 peak disappears.
Figure 7.4 SAXS profile of different concentrations of sodium tripolyphosphate (STPP) on 10% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▼) Control milk; ( ▼ ) Milk with 0.1% STPP; ( ▼ ) Milk with 0.25% STPP; ( ▼ ) Milk with 0.5% STPP; ( ▼ ) Milk with 1% STPP; ( ▼ ) Milk with 2% STPP.

Milk samples treated with SHMP also shows similar results with milk samples treated with STPP. Based on Figure 7.5, by increasing SHMP concentrations (0.1 to 2%), the size of casein micelles decreased. The inset of Figure 6.5 (Kratky plot) showed that both signals for level 1 and 2 peaks can be clearly seen when 0.1% SHMP concentration was used. As the SHMP concentration was increased to 0.25%, the peak for the signal of level 1 shifted towards higher q values, and as the shoulder at level 2 peak becomes less prominent. As the concentration of SHMP increases from 0.5 to 2%, the level 1 peak shifted further towards higher q values as the shoulder for the signal of level 2’s peak disappears. The limit for SHMP concentration is 1%, therefore, adding more SHMP (2%) does not change the SAXS profile (Kratky plot of Figure 7.5).
Figure 7.5 SAXS profile of different concentrations of sodium hexametaphosphate (SHMP) on 10% (w/w) milk samples. The temperature for all samples was 20°C. Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 relates to the size of casein micelles, Level 1 is assigned to sub-micelles, and Level 2 corresponds to calcium phosphate nanoclusters. (▼) Control milk; (■) Milk with 0.1% SHMP; (▲) Milk with 0.25% SHMP; (○) Milk with 0.5% SHMP; (□) Milk with 1% SHMP; (●) Milk with 2% SHMP.

STPP and SHMP are good emulsifiers. The casein micelles in milk were dissociated when ≥0.25% of STPP or SHMP were added into the milk system. This created sub-micelles and shifted the peak of level 1 to higher q values. When 0.5% of STPP or SHMP were added into milk samples, the peak of level 1 shifted further towards higher q due to more dissociation of the casein micelles which created a larger number of sub-micelles. The disappearance of the peak in level 2 for both STPP and SHMP when their concentrations were 0.25% or more was due to the dissolution of the CPNs. For SHMP and STPP, the concentrations of 0.25% are enough to chelate all the calcium in the milk system.

According to Dimitreli and Thomareis (2009); De Kort, Minor, Snoeren, Van Hooijdonk, and Van der Linden (2012); and, Mizuno and Lucey (2005a) polyphosphates (SHMP and STPP)
are good calcium binders compared to other emulsifying salts since the larger amount of phosphorous anions in STPP and SHMP chains contribute to better peptization coefficients (degree of casein dissociation), thus better emulsification. Panouillé, Nicolai, Benyahia and Durand (2005) also suggested that, increasing polyphosphate concentration may increase the dissociation of casein micelles into sub-micelles through the removal of calcium ions. They estimated that 0.07g of polyphosphate is necessary to dissociate 1g of casein. Their study is not an over estimate compared to our data because they used native phosphocaseinate powder which has ~3.2% calcium, whereas this study used skim milk powder which has ~1.23g calcium. The higher amount of calcium in their samples may cause a higher estimation of polyphosphate to dissociate casein. Pitkowski et al. (2008) reported that, polyphosphate concentration of 1 gL⁻¹ is sufficient to perform full casein micelle dissociation. Furthermore, research done by Cuhna and Viotto (2010), also showed that by using SHMP as the emulsifying salt for processed cheese spreads, the % soluble calcium to total calcium ratio was lower compared to using other types of emulsifying salts for the cheese samples.

### 7.3.3 Influence of heat treatment on emulsified milk

In this section, the effect of heating the milk samples with added emulsifying salts at certain concentrations (1% and 2%) on the structure of the emulsified milk samples is investigated. Heat treatment is important parameter as it also relates to the stability of milk and processed cheese during processing or shelf life. Casein complexes are known to be very stable to severe heat treatments associated with modern dairy processing (De Kort et al., 2012; Pitkowski et al., 2008). However, aggregation of casein may occur after heating at ≥120°C for several minutes and this effect is irreversible. Addition of emulsifying salts may improve the stability of milk or processed cheese during heat treatment since they successfully bind calcium and amino acids of the aggregated casein micelles (De Kort et al., 2012). Figure 7.6 to 7.9 displayed the SAXS profiles for the heated and non-heated emulsified milk samples.

Based on Figure 7.6, heating the milk samples affected the size of casein micelles (level 0) of the milk with 1% and 2% SP. By heating the milk sample with 1% and 2% SP, the size of casein micelle was bigger compared to the sample without heat treatment (in inset Figure 7.6; Kratky plot). Besides, the peaks at level 1 and 2 also presented higher intensities when 2% SP was heated compared to non-heated SP 2%.
Figure 7.6 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium phosphate (SP) in 10% (w/w) milk samples. The temperature for 1% and 2% SP samples was at 20°C while 1% and 2% SP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (~24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (●) Milk with 1% SP at 20°C; (□) Heat treated milk with 1% SP; (▲) Milk with 2% SP at 20°C; (△) Heat treated milk with 2% SP.

When milk samples with added emulsifier salt are heated, the casein micelles will be further dispersed into smaller units such as sub-micelles and re-arrangements between the smaller units occur to form a new network (Panouillé, Nicolai, & Durand, 2004). However, when emulsified milk samples were heated for too long, the protein re-arrangements will become too active which resulted in bigger protein structures (Glenn et al., 2003; Lee et al., 2003; Shirashoji et al., 2006).
Milk samples treated with 1% and 2% TSPP on the other hand showed no difference when heated (Figure 7.7). This means heat treatments do not affect the structure of the milk samples emulsified with high concentrations of TSPP. Based on the result of the previous section in this study (Figure 7.3), the best concentration for TSPP to demineralise all the submicelles and nanoclusters was 0.5%. Therefore, heating higher concentrations of TSPP samples (1% and 2%) has no effect on the structure of emulsified milk systems due to all of the calcium in the TSPP milk samples has been chelated.
Figure 7.8 SAXS profile of heat treated and non-heat treated of 2 concentrations of sodium tripolyphosphate (STPP) in 10% (w/w) milk samples. The temperature for 1% and 2% STPP samples was at 20°C while 1% and 2% STPP samples were heated up to 120°C for 10 minutes and were cooled to room temperature (~24°C). Intensities are in arbitrary units (a.u) and the data has been shifted along the y-axis for clarity. Inset figure represents Kratky plot in a log-log scale. Level 0 indicates the size of casein micelles, Level 1 is assigned to sub-micelles, while level 2 corresponds to calcium phosphate nanoclusters. (■) Milk with 1% STPP at 20°C; (□) Heat treated milk with 1% STPP; (▲) Milk with 2% STPP at 20°C; (▲) Heat treated milk with 2% STPP.

Milk samples emulsified with 1% STPP (Figure 7.8) also presented similar results as milk samples emulsified with 1% TSPP (Figure 7.7) where the structures of the milk with 1% STPP were not affected when heated. However, when the milk sample emulsified with 2% STPP was heated, the size of casein micelle of heat treated sample was smaller compared to the sample without heat treatment. Both peaks for signals of level 1 and 2 remain unchanged when the milk sample with 2% STPP was heated.
According to Lee et al. (2003), heat-treatment will cause the casein micelles to disintegrate more into sub-micelles. Shirashoji et al. (2006), also agrees that heat-treatment enhance the casein dispersion and decrease the contact between the casein micelles. With the aid of excess STPP in the emulsified milk system, any remaining calcium were chelated and the calcium phosphate (Ca-P) bridges were further broken down which therefore, reducing the size of the casein micelle of the heat-treated 2% STPP milk samples.

Figure 7.9 also demonstrated similar results with Figure 7.7 where heat treatment do not affect the structure of the milk samples emulsified with 1% and 2% SHMP. SHMP on the
other hand, has the highest ability as a chelating agent. Since all of the calcium in the SHMP milk samples has been chelated, heating both SHMP samples (1% and 2%) has no effect on the structure of emulsified milk systems. De Kort et al. (2009) suggested that, SHMP can improve the heat stability of dairy products because the addition of SHMP may reduce protein aggregation during processing or shelf life.

In a study done by Rulliere, Perenes, Senocq, Dodi, and Marchesseau (2012), when polyphosphate salts was heated at 120°C for 10 minutes, in the presence of calcium, hydrolytic degradation increased. The long phosphate chain was broken down to shorter phosphate chain such as pyrophosphate and trimetaphosphate which change the emulsifying salt’s sequestering ability. The present study seems to show contradicting results with Rulliere et al. (2012) probably because SHMP has already chelated all the calcium in the milk system and that degradation of the emulsifying of SHMP (if any) does not affect calcium chelation, and thus dissociation of the emulsifying of the milk proteins.

### 7.4 Summary to chapter

In conclusion, the size of casein micelles decreased according to this following order: control ≈ SP > TSPP > STPP > SHMP which demonstrated that, the size of casein micelles decrease with the increase of the phosphate chain length. The signal of the peak in level 1 shifted to higher q when milk was treated with TSPP or STPP or SHMP indicates that the sub-micelles undergo an alteration of structure. Furthermore, the addition of TSPP or STPP or SHMP leads to the disappearance of the peak in level 2 which indicates that the addition of these emulsifying salts induce the dissolution of the CPNs due to the chelations of all the calcium in the milk. SHMP has the greatest calcium sequestering ability compared to STPP, TSPP and SP which enhanced the protein-protein interactions in the emulsified milk. Gelation started to occur when ≥1% SP, ≥0.5% TSPP, ≥0.25% STPP or ≥0.25% SHMP were added into milk samples. The increasing of the size of casein micelles in heat-treated 1% and 2% SP samples might be caused by protein aggregation in these milk systems. Milk samples treated with 1% and 2% TSPP or SHMP showed no response when heated. Overall, the findings of this study not only provide the understanding of the internal structures in milk, but also can give an insight into dairy product formulations, such as processed cheese.
Conclusions and future work
8.1 Overall conclusions

The overall objective of this thesis was to analyse the influence of different emulsifying salts on the physical properties of model processed cheese and to expand the knowledge on the effect of the oil droplet interfacial stabiliser in model processed cheese.

Preliminary work was done at the beginning of this study to select the correct formulation to manufacture processed cheese samples on a lab-bench scale. The main ingredients and temperature profile were set up based on the literature review. The model processed cheese sample was then tested for rheological properties to determine the effectiveness of each step (mixing of ingredients and heating the mixture for 5 or 15 minutes) taken during the processed cheese manufacture. Calcium caseinate which was used as the main protein phase, and sunflower oil were chosen to simplify the formulation of the model processed cheese samples. Both are also chosen to ensure reproducibility of the cheeses, since milk proteins from conventional cheese and milk fats have large variations, depending of their source, seasonal variations etc. The rheological measurements showed that the model processed cheese made in this thesis was comparable to those reported in the literature. The creaming phase and the addition of calcium were omitted from the manufacturing process of model processed cheese in this research, without affecting the final structure of the processed cheese. This study also recognized the use of scanning electron microscopy (SEM) as the best technique to visualise the fat globules and protein network in processed cheese samples.

After model processed cheese were successfully manufactured, this study started to focus on the impact of shear, different protein concentration (2.5 to 40%) and oil concentration (0 to 30%) on the physical properties of model processed cheese emulsified with trisodium citrate (TSC) (Chapter 4). On a scale of 10 of the machine used to manufacture the processed cheese, speed 6 was observed to provide optimum result (rheology) for model processed cheese samples. This study also revealed that the size of the oil droplets decreased with increasing shear rate thus, enhances the inclusion of oil into the protein matrix since no oiling off was observed. However, based on the rheological result, excessive amount of shear (~7164 rpm) may break the gel network structure forming softer processed cheese samples. The presence of TSC in the model processed cheese system induces calcium-sodium ion
exchange with the protein matrix which enhances the dissociation of casein aggregates. Improper emulsions were observed on model processed cheese with low protein concentration due to the low protein content and the final pH of the processed cheese samples. Higher amount of protein concentration (≥25%) displayed larger oil droplet size, firmer processed cheese structure and lowest melting properties. The presence of oil in processed cheese is vital because it enhances the protein-oil interaction along with protein-protein interaction thus, forming a stronger processed cheese gel network. TSC (2%) demonstrated the best emulsifying ability when processed cheese was made with 20% protein and 20% oil concentration. Increasing oil concentration may increase the oil droplet size and reduce markedly the melting properties.

Then, similar experiments (impact of different protein concentration and oil concentration) were tested on the physical properties of model processed cheese emulsified with sodium pyrophosphate (TSPP) (Chapter 5). TSPP was chosen because some studies (e.g. Chen & Liu (2012); Lu et al. (2008); Guinee et al. (2004)) reported that processed cheese made with TSPP demonstrates higher firmness compared to processed cheese made with other emulsifying salts. The results obtained from this chapter were then compared with the results obtained from Chapter 4. In brief, the results obtained from model processed cheese emulsified with TSPP demonstrated similar trends with the results of model processed cheese emulsified with TSC. Model processed cheese made with higher protein concentration demonstrated higher firmness ($G'$, $G''$ and $G^*$), lower meltability and larger oil droplet particle size compared to model processed cheese with lower protein concentration. Model processed cheese formulated with 20% protein and 20% oil displayed smallest particle diameter (~0.5 μm) compared with other processed cheese samples due to highest oil droplet emulsification. The firmness ($G^*$) and oil droplet particle size of model processed cheese with fixed amount of protein concentration increased with the increasing oil concentration. At 20% protein concentration TSC and TSPP have more ability to emulsify oil droplets by performing calcium chelation. Binding of calcium from casein by TSC was stronger compared to TSPP for oil concentration from 5 to 30%, on an equivalent mass basis. This chapter found that, the amount of protein and oil concentrations highly influenced the ability of TSC or TSPP to perform calcium chelation and oil droplet emulsification. The nature of the surface active material stabilising the oil droplets can influence the type of interactions that occurs between the oil droplets and the protein matrix. Based on the
knowledge obtained from previous chapters (*Chapter 4 and 5*), *Chapter 6* focused on engineering interfacial properties by changing the oil droplets stabiliser using different types of oil droplets surface materials. Three different types of oil droplets surface materials (food grade) were selected for this study. This includes calcium caseinate (same as the matrix), whey protein isolate (WPI) and lecithin. The oil droplets (40%) were stabilised with 1% of oil droplets surface materials using laboratory homogenizer and high pressure homogenizer (microfluidizer) first, then incorporated into the other components of the processed cheese before processing.

In this chapter (*Chapter 6*), Model processed cheese containing oil droplets stabilised with WPI emulsified either by TSC or TSPP demonstrated the highest $G^*$ values (highest firmness), smallest oil droplets, a more compact structure and lowest melting properties. This is likely due to the association of denatured whey proteins with dispersed casein producing stiffer gels. Model processed cheese containing oil droplets stabilised with calcium caseinate and emulsified with TSC or TSPP also displayed higher firmness and lower melting properties because of the casein-casein interactions between the oil droplet surface and the casein matrix. Both oil droplets containing WPI or calcium caseinate are active fillers which can interact positively with the casein network producing stiffer processed cheese samples. Model processed cheese containing oil droplets stabilised by lecithin either emulsified by TSC or TSPP exhibited lowest $G^*$ values, larger oil droplet particle size, very loose structure and highest melting properties. This is because oil droplets stabilised by lecithin may act as inert filler which does not interact with the casein network. There is no difference between the results of processed cheese samples made with TSC or TSPP. The study in this chapter (*Chapter 6*) concludes that the type of adsorbed layer on the oil droplet surface strongly influences the rheological properties, oil droplet particle size, microstructure and melting properties of model processed cheese.

In *Chapter 7* a preliminary study was carried out to investigate the casein micelle structures of skim milk containing four different types of emulsifying salts which are sodium phosphate (SP), TSPP, sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) using a powerful technique known as small angle X-ray scattering (SAXS). Difficulty might arise when dealing with experiment processed cheese samples using SAXS due to the solid nature of processed cheese. Skim milk was a good alternative since it is a well-known model dairy system. The study in this chapter (*Chapter 7*) found that, the size of casein micelles
decreased with the increased of the phosphate chain length according to the following order: control ≈ SP > TSPP > STPP > SHMP. The addition of TSPP or STPP or SHMP leads to alteration of the sub-micelles and dissolution of the colloidal calcium phosphate nanoparticles (CPN). The dissociation of the casein micelle started to occur when ≥1% SP, ≥0.5% TSPP, ≥0.25% STPP or ≥0.25% SHMP were added into skim milk samples. Heat treatment only affected milk samples emulsified with SP (1% and 2%). The results found in this chapter (Chapter 7) may provide an insight on the effect similar emulsifying salt (TSPP) on model processed cheese systems.

Overall, this thesis provides further knowledge on the interaction between emulsifying salts, oil droplets and protein matrix in model processed cheese systems.

8.2 Future work

Following the work presented in this thesis, several experimental work could be considered in the future. These potential research areas are listed below:

*Properties of model processed cheese emulsified by TSPP with increasing oil concentration up to 50%.*

The results reported in Chapter 5 provide an insight that TSPP may act as strong emulsifying agent if the oil concentration was increased further (≥35%) because it seems to remain effective even when the highest oil concentration (30%) is used. Early hypothesis suggested that by increasing the oil concentration up to 50%, the firmness will probably plateau when the emulsifying capability of TSPP has reached maximum. This specific point related to the oil concentrations can be easily investigated using the same methods developed in this thesis.

*Addition of hydrocolloids in model processed cheese at lower protein concentration*

The results of this study demonstrated that model processed cheese emulsified either by TSC or TSPP at lower protein concentration (<15%) have low stability and low elasticity. The incorporation of various hydrocolloids to increase the viscosity of processed cheese has been reported (Hosseini-Parvar et al., 2015; Joyner & Damiano, 2015). It would be interesting to investigate the influence of hydrocolloids such as starch, carrageenan, gum Arabic or basil seed gum on the rheological properties, oil droplet particle size, microstructure and
meltability of model processed cheese at lower protein concentration as performed in this study. This is not an easy task as proteins and polysaccharides might show phase separation.

*Emulsion produced by ultrasound technique.*

The emulsions in this thesis were made using homogenizer and high-pressure homogenizer. However, these methods require high mechanical energy to create disruptive forces to break the liquid interfaces (Ramisetty, Pandit, & Gogate, 2015). The use of ultrasound technique has gained popularity throughout the years because stable emulsions with very small sizes can be produced using lower energy and more cost-effectively (Ramisetty et al., 2015). It would be interesting to produce emulsions using ultrasound technique and produce cheeses as achieved in Chapter 6. The oil droplet particle size of emulsions produced by high-pressure homogenizer can be compared with the emulsions produced by ultrasound. Then, the influence of incorporating these emulsions into processed cheese systems emulsified with TSC or TSPP (or other emulsifying salts) on the physicochemical properties of model processed cheese can be investigated.

*Model processed cheese containing oil droplets stabilised by different surface active materials at different protein concentration.*

This thesis only focused on model processed cheese samples containing oil droplets stabilised by calcium caseinate, WPI or lecithin at different oil concentration. It would be interesting to study further this topic by changing the protein concentration (2.5 to 40%) introduced during the manufacture of model processed cheese. It is hypothesized that increasing protein concentration may increase the firmness of model processed cheese containing oil droplets stabilised by calcium caseinate and WPI since they are active fillers. However, question arises if the properties of model processed cheese containing oil droplets stabilised by lecithin will display similar behaviour as the effect of increasing oil concentrations. Early hypothesis suggested that firmness of the processed cheese samples may increase with the increase in protein concentration due to the increase of casein-casein interaction in model processed cheese samples with higher protein concentration despite having oil droplets stabilised by lecithin.
Model processed cheese emulsified by other emulsifying salts containing oil droplets stabilised by different surface active materials.

The study in this thesis only focuses on two emulsifying salts: TSC and TSPP. It would be interesting to study model processed cheese containing oil droplets stabilised by calcium caseinate or WPI or lecithin and emulsified with emulsifying salts with longer phosphate chain length (e.g. STPP or SHMP). Studies have shown that cheese emulsified with SHMP has higher firmness and lower meltability (Caric et al., 1985; Shirashoji et al., 2010). There is a possibility that model processed cheese containing oil droplets stabilised with calcium caseinate or WPI and emulsified with SHMP display higher firmness and lower meltability. On the other hand, model processed cheese containing oil droplets stabilised by lecithin and emulsified with SHMP may exhibit softer texture and higher meltability. Further studies need to be done to prove these assumptions. Besides, further experiments also can be done to change the surface properties of the oil droplets using other surface active agents (including non-food grade surfactants) such as SDS or Tween 20 and compare with the surface active agents used in this study.

Development of small-angle X-ray scattering (SAXS) method for model processed cheese.

In this thesis, small-angle X-ray scattering method was done only in milk samples emulsified with different emulsifying salts. This is due to the state of model processed cheese which already forms firm gel after manufacturing and it was not easy to place the sample inside the quartz glass capillary for SAXS measurements. It will be first important to perform SAXS measurements on model processed cheese. The presence of oil droplets will give different spectra. However to do so, it is important to perform preliminary experiments since processed cheese is highly viscous, and thus might require special sample environment on the SAXS setup.

Sensory properties on model processed cheese.

Sensory work for the model processed cheese samples has not been covered in this thesis. Sensory properties may be done in the future because flavour is one of the most important parameters to the consumer’s preference and acceptance (Kongo & Malcata, 2016a). Over the years, classical descriptive analysis has been used extensively for sensory characterization of processed cheese using trained panellists. However, this method can be very expensive, time-consuming and the results produced may be biased because trained panellists are no longer
typical consumers (Hanaei, Cuvelier, & Sieffermann, 2015). Therefore, various studies have come up with new descriptive methods to gather food perceptions directly from consumer such as Projective mapping, Flash Profile, Free Choice Profiling, open ended questions, free comments, etc. (Bárcenas, Elortondo, & Albisu, 2003; Gkatzionis et al., 2013; Hanaei et al., 2015; Marcano, Ares, & Fiszman, 2015; Thomsen, Gourrat, Thomas-Danguin, & Guichard, 2014; Torri et al., 2016). Further studies are needed to choose the best method to test consumer acceptance for the model processed cheese samples made in this thesis.

*Investigating real processed cheeses*

All of the investigations (the impact of the nature of the emulsifying salts, % protein, % oil, nature and concentration of surface active materials, etc.) carried out in this thesis have been studied on a simplified model processed cheese in order to develop a fundamental knowledge on the impact of different ingredients, including their concentrations and characteristics, under different mechanical, emulsifiers and thermal processes. Cheese companies however, manufacture more complex processed cheese, usually using natural cheese as a base. Therefore, a more complex model could be studied in the future, using natural cheese or hydrolysed protein, or using anhydrous milk fat (AMF) instead of vegetable oil, to further our understanding. These more complex systems could be also more worthy testing for their consumer acceptance.
References


Varnam, A. H., & Sutherland, P. J. (2012). Dairy protein products Milk and milk products: technology, chemistry and microbiology (pp. 159-182). USA: Springer Science and Business Media.


APPENDIX 1

Determination of moisture content of processed cheese

The moisture content of the model processed cheese was determined using the method as described by Uaboi-Egbenni et al. (2010) following AOAC (1990). About 5 g of the processed cheese sample was weighed into pre-weighed aluminum dry dish and the sample was leveled carefully in the dish. The dish and its content was then transferred into the oven at a temperature of 105°C and was dried for 3 h. Then, the dish was transferred to the desiccator to cool and to be weighed. The dish was returned into the oven for another half hour and again cooled and re-weighed. The process was repeated until a constant weight was reached.

The moisture content is defined as:

\[
\text{Moisture (\%)} = \left( \frac{W_1 - W_2}{W_1} \right) \times 100
\]

where:

- \(W_1\) = weight (g) of sample before drying
- \(W_2\) = weight (g) of sample after drying
APPENDIX 2

Table A.1 Composition and pH of model processed cheese made with different protein concentrations.

<table>
<thead>
<tr>
<th>Protein concentration (%)</th>
<th>Protein (g)</th>
<th>Oil (g)</th>
<th>ES (g)</th>
<th>Water (g)</th>
<th>Citric acid (g)</th>
<th>pH</th>
<th>Moisture (%)</th>
<th>Protein: water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>25</td>
<td>200</td>
<td>2.5</td>
<td>575</td>
<td>4.6</td>
<td>5.35</td>
<td>5.24</td>
<td>60.20</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>200</td>
<td>5</td>
<td>575</td>
<td>4.7</td>
<td>5.33</td>
<td>5.30</td>
<td>55.30</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>200</td>
<td>10</td>
<td>575</td>
<td>4.8</td>
<td>5.48</td>
<td>5.35</td>
<td>50.15</td>
</tr>
<tr>
<td>15</td>
<td>150</td>
<td>200</td>
<td>15</td>
<td>575</td>
<td>4.9</td>
<td>5.60</td>
<td>5.72</td>
<td>46.55</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>200</td>
<td>20</td>
<td>575</td>
<td>5</td>
<td>5.70</td>
<td>5.65</td>
<td>41.07</td>
</tr>
<tr>
<td>25</td>
<td>250</td>
<td>200</td>
<td>25</td>
<td>575</td>
<td>5.1</td>
<td>5.75</td>
<td>5.80</td>
<td>37.60</td>
</tr>
<tr>
<td>30</td>
<td>300</td>
<td>200</td>
<td>30</td>
<td>575</td>
<td>5.2</td>
<td>5.73</td>
<td>5.85</td>
<td>33.50</td>
</tr>
<tr>
<td>35</td>
<td>350</td>
<td>200</td>
<td>35</td>
<td>575</td>
<td>5.3</td>
<td>5.82</td>
<td>5.79</td>
<td>30.10</td>
</tr>
<tr>
<td>40</td>
<td>400</td>
<td>200</td>
<td>40</td>
<td>575</td>
<td>5.4</td>
<td>5.77</td>
<td>5.92</td>
<td>26.30</td>
</tr>
</tbody>
</table>
APPENDIX 3

Table A.2 Composition and pH of model processed cheese made with different oil concentrations.

<table>
<thead>
<tr>
<th>Oil concentration (%)</th>
<th>Protein (g)</th>
<th>Oil (g)</th>
<th>ES (g)</th>
<th>Water (g)</th>
<th>Citric acid (g)</th>
<th>pH</th>
<th>Moisture (%)</th>
<th>Protein: water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSC</td>
<td>TSPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>200</td>
<td>0</td>
<td>20</td>
<td>575</td>
<td>6.2</td>
<td>5.50</td>
<td>5.52</td>
<td>49.83</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>50</td>
<td>20</td>
<td>575</td>
<td>5.9</td>
<td>5.80</td>
<td>5.77</td>
<td>47.21</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>100</td>
<td>20</td>
<td>575</td>
<td>5.6</td>
<td>5.72</td>
<td>5.80</td>
<td>45.71</td>
</tr>
<tr>
<td>15</td>
<td>200</td>
<td>150</td>
<td>20</td>
<td>575</td>
<td>5.3</td>
<td>5.75</td>
<td>5.70</td>
<td>43.64</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>200</td>
<td>20</td>
<td>575</td>
<td>5</td>
<td>5.70</td>
<td>5.70</td>
<td>41.07</td>
</tr>
<tr>
<td>25</td>
<td>200</td>
<td>250</td>
<td>20</td>
<td>575</td>
<td>4.7</td>
<td>5.74</td>
<td>5.85</td>
<td>36.47</td>
</tr>
<tr>
<td>30</td>
<td>200</td>
<td>300</td>
<td>20</td>
<td>575</td>
<td>4.4</td>
<td>5.84</td>
<td>5.75</td>
<td>30.36</td>
</tr>
</tbody>
</table>
APPENDIX 4

Table A.3 Composition and pH of model processed cheese containing oil droplets stabilised by calcium caseinate.

<table>
<thead>
<tr>
<th>Oil concentration (%)</th>
<th>Stock emulsion (g)</th>
<th>Protein (g)</th>
<th>ES (g)</th>
<th>Water (g)</th>
<th>Citric acid (g)</th>
<th>pH TSC</th>
<th>pH TSPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>258.06</td>
<td>25.8</td>
<td>741.94</td>
<td>6.45</td>
<td>5.60</td>
<td>5.72</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>243.91</td>
<td>24.4</td>
<td>631.09</td>
<td>6.12</td>
<td>5.52</td>
<td>5.55</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>229.76</td>
<td>23.0</td>
<td>520.24</td>
<td>5.8</td>
<td>5.64</td>
<td>5.55</td>
</tr>
<tr>
<td>15</td>
<td>375</td>
<td>215.60</td>
<td>21.5</td>
<td>409.40</td>
<td>5.48</td>
<td>5.48</td>
<td>5.62</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
<td>201.45</td>
<td>20.1</td>
<td>298.55</td>
<td>5.16</td>
<td>5.82</td>
<td>5.71</td>
</tr>
<tr>
<td>25</td>
<td>625</td>
<td>187.30</td>
<td>18.7</td>
<td>187.70</td>
<td>4.84</td>
<td>5.70</td>
<td>5.65</td>
</tr>
<tr>
<td>30</td>
<td>750</td>
<td>173.15</td>
<td>17.3</td>
<td>76.85</td>
<td>4.52</td>
<td>5.62</td>
<td>5.83</td>
</tr>
</tbody>
</table>
### APPENDIX 5

**Table A.4** Composition and pH of model processed cheese containing oil droplets stabilised by whey protein isolate (WPI).

<table>
<thead>
<tr>
<th>Oil concentration (%)</th>
<th>Stock emulsion (g)</th>
<th>Protein (g)</th>
<th>ES (g)</th>
<th>Water (g)</th>
<th>Citric acid (g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>258.06</td>
<td>25.8</td>
<td>741.94</td>
<td>6.45</td>
<td>5.43</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>245.11</td>
<td>24.5</td>
<td>631.09</td>
<td>6.12</td>
<td>5.56</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>233.59</td>
<td>23.4</td>
<td>520.24</td>
<td>5.8</td>
<td>5.52</td>
</tr>
<tr>
<td>15</td>
<td>375</td>
<td>219.35</td>
<td>21.9</td>
<td>409.40</td>
<td>5.48</td>
<td>5.66</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
<td>206.46</td>
<td>20.6</td>
<td>298.55</td>
<td>5.16</td>
<td>5.75</td>
</tr>
<tr>
<td>25</td>
<td>625</td>
<td>193.56</td>
<td>19.4</td>
<td>187.70</td>
<td>4.84</td>
<td>5.88</td>
</tr>
<tr>
<td>30</td>
<td>750</td>
<td>180.64</td>
<td>18.1</td>
<td>76.85</td>
<td>4.52</td>
<td>5.70</td>
</tr>
</tbody>
</table>
APPENDIX 6

Table A.5 Composition and pH of model processed cheese containing oil droplets stabilised by lecithin.

<table>
<thead>
<tr>
<th>Oil concentration (%)</th>
<th>Stock emulsion (g)</th>
<th>Protein (g)</th>
<th>ES (g)</th>
<th>Water (g)</th>
<th>Citric acid (g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>258.06</td>
<td>25.8</td>
<td>741.94</td>
<td>6.45</td>
<td>5.33</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>245.11</td>
<td>24.5</td>
<td>631.09</td>
<td>6.12</td>
<td>5.62</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>233.59</td>
<td>23.4</td>
<td>520.24</td>
<td>5.8</td>
<td>5.63</td>
</tr>
<tr>
<td>15</td>
<td>375</td>
<td>219.35</td>
<td>21.9</td>
<td>409.40</td>
<td>5.48</td>
<td>5.89</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
<td>206.46</td>
<td>20.6</td>
<td>298.55</td>
<td>5.16</td>
<td>5.64</td>
</tr>
<tr>
<td>25</td>
<td>625</td>
<td>193.56</td>
<td>19.4</td>
<td>187.70</td>
<td>4.84</td>
<td>5.85</td>
</tr>
<tr>
<td>30</td>
<td>750</td>
<td>180.64</td>
<td>18.1</td>
<td>76.85</td>
<td>4.52</td>
<td>5.79</td>
</tr>
</tbody>
</table>