UNIVERSITY OF BIRMINGHAM

Emulsion Design for Protection of Chemically Sensitive Bioactive Ingredients

A Thesis Submitted to The University of Birmingham for the Degree of Doctor of Philosophy

By

Maryam Kargar

Department of Chemical Engineering
School of Engineering
The University of Birmingham

Abstract

Essential fatty acids (ω-3 and ω-6) are necessary for human health, but the body cannot synthesise them. Therefore, they must be provided through diet. The addition of these health promoting ingredients to food products is often hampered by oxidative damage that occurs during storage. Lipid oxidation is a major issue for food manufacturers resulting in alteration of taste and appearance of product, formation of off-flavors and rancidity and reduction in nutritional quality and shelf-life of product. This is normally reduced by adding chelating agents or synthesised antioxidants, such as EDTA (Ethylenediaminetetraacetic acid), to the product. In recent years, the food industry has been facing a tremendous pressure from consumers for using chemical agents to prevent lipid oxidation. Therefore, there is a global demand to reduce or exclude synthesised additives from food products.

The approach taken in this work is to physically separate the reactive species (hydroperoxides and pro-oxidants) by designing and using microstructure approaches. Specifically, microstructure engineering has been used to manipulate the physical properties of the emulsion, and form a thick interfacial layer around oil droplets to inhibit lipid oxidation.

Initially, oil-in-water emulsions were prepared with silica particles as a model system. These samples were stable for 40 days, and the lipid oxidative stability was improved by up to 50%, compared with the emulsions stabilised by surfactant (Tween 20). However, silica particles are not “label friendly” hence; Modified Starch (MS) and colloidal Microcrystalline Cellulose (MCC) were used as the “food-grade” Pickering particles. It was shown that these samples had great stability against coalescence and the oxidation rate was

reduced by up to 75%, compared with the emulsions stabilised by Tween20 surfactant. In addition, when the structure of the interfacial layer around droplets was viewed by Cryo-electron microscopy the particles were seen at the interface covering the surface of droplets.

Since food-grade particles are able to significantly increase the oxidative stability, the next step was to control the packing of particles at the interface. To study this hypothesis, oil-in-water emulsions were stabilised in presence of both "food-grade particles" (MS or MCC) and small molecule surfactants (Tween 20). These emulsions were not just able to increase the physical stability of emulsions but also extensively enhanced the oxidative stability of food-grade emulsions.

The potential ability of fat crystal at the droplet interface (Pickering emulsion) to enhance the oxidative stability was also investigated. This was carried out by stabilising oil-in-water emulsions with saturated monoglyceride in the presence of various concentrations of xanthan gum. A bench scale scraped-surface heat exchanger was used to produce these emulsions. Solid fat crystals "shells" were created around the oil droplets by oscillating the temperature during emulsification. Cryo-electron microscopy micrographs confirmed the existence of smooth fat crystal "shells" around the oil droplets. It was shown that these "shells" were capable of inhibiting the lipid oxidation by preventing pro-oxidants to come into close contact with the oil droplets.

Acknowledgements

I would like to express my gratitude to my supervisors, Prof Ian Norton and Dr Fotios Spyropoulos for their continuous support during my study. I would like to thank them for their patience, enthusiasm and guidance throughout my PhD.

I would like also to express my deep and special appreciation to staff members of the school of Chemical Engineering and technicians in the Biochemical Engineering and Material and Metallurgy School for their valuable assistance during this project. Special thanks to Mrs Theresa Morris for helping me with preparing samples for scanning electron microscope imaging.

Special thanks are extended to all my colleagues from the microstructure group for their support by sharing their time and knowledge which contributed significantly to my project.

I would like to acknowledge Parastoo Jamshidi, Dr. Fideline Tchuenbou and Dr. Julia Hofinger for their constant support and friendship who made the PhD experience a pleasurable one. A very special thank you to Taiwo Saola for being such a good friend as well as for proofreading all parts of this dissertation.

Most of all, I would like to thank my sister, Leila, for her love, support over the years and my mum and dad for their unconditional love and interest they have shown for everything I do. You have always been there for me with encouraging words during difficult times.

Finally, special thanks my beloved husband, Alireza, who has always stood by my side to help and inspire me. Thank you so much for your support and patience. Without your love and understanding I would not be able to make it.

Table of Content

Table of Content v

List of Figures xi

List of Tables xxi

Chapter 1: Introduction 1

1.1 Background 1

1.2 Objectives 3

1.3 Thesis Layout 4

1.4 Publications and Conferences 5

Chapter 2: Literature Survey 8

2.1 Emulsions 8

2.1.1 Definitions 8

2.2 Mechanism of Emulsification 9

2.3 Emulsifiers 11

2.3.1 Surfactant 11

2.3.2 Protein 14

2.3.3 Solid Particles 16

2.3.4 Fat Crystals 22

2.4 Emulsion Stability 27

2.5 Physical Instability.....27
2.5.1 Creaming/ Sedimentation 27
2.5.2 Flocculation 28
2.5.3 Coalescence 28
2.5.4 Partial Coalescence.....29
2.5.5 Ostwald Ripening 32
2.6 Chemical Instability.....33
2.7 Lipid.....33
2.8 Mechanisms of Lipid Oxidation 34
2.8.1 Lipid Oxidation in Oil-in-Water Emulsion.....36
2.9 Lipid Oxidation Measurements Techniques.....37
2.9.1 Primary Lipid Oxidation Product 39
2.9.2 Secondary Lipid Oxidation Products.....41
2.10 Factors influencing oxidative stability.....42
2.10.1 Type of Lipid 42
2.10.2 Temperature.....43
2.10.3 Pro-oxidants.....43
2.10.4 Antioxidants.....44
2.10.5 Interfacial Characteristics 47
2.10.6 Surfactant Micelles 49
2.11 Controlling Lipid Oxidation by Microstructure Engineering.....49

Chapter 3: Experimental 51
3.1 Materials 51
3.1.1 Dispersed Phase 51
3.1.2 Continuous Phase 52
3.1.3 Emulsifier 52
3.1.4 Thickening Agents 53
3.1.5 Pickering Particles 53
3.1.5.1 Silica Particles 53
3.1.5.2 Colloidal Microcrystalline Cellulose (MCC) 54
3.1.5.3 Chemically Modified Food Starch (MS) 55
3.1.6 Saturated Monoglycerides (MG) 55
3.1.7 Reagents 56
3.1.8 Solvent 56
3.2 Emulsion Preparation 56
3.2.1 Surfactant Stabilised Emulsion 56
3.2.2 Protein Stabilised Emulsion 57
3.2.3 Pickering Particles Stabilised Emulsion (Pickering Emulsion) 57
3.2.3.1 Silica Particles Stabilised Emulsion 57
3.2.3.2 Food-grade Particle Stabilised Emulsions 57
3.2.4 Mixed-Emulsifier Emulsion 58
3.2.5 Fat Crystal Stabilised Emulsions 58

3.3 Analytical Methods....61
3.3.1 Droplet size Measurement....61
3.3.2 Creaming Stability Measurement....62
3.3.3 Light Microscopy....64
3.3.4 Particle Size Measurement....64
3.3.5 Particle Charge Measurement....66
3.3.6 Cryo Scanning Electron Microscopy (Cryo-SEM)....68
3.3.7 Rheology....69
3.3.8 Differential Scanning Calorimetry (DSC)....70
3.3.9 Spectrophotometry....71
3.4 Lipid Oxidation Measurement....72
3.4.1 Peroxide Value Method (PV)....73
3.4.2 p-Anisidine Value (p-AnV)....75
Chapter 4: The Effect of Interfacial Microstructure on the Lipid Oxidation Stability of Oil-in-Water Emulsions....77
4.1 Effect of Physical Properties of Emulsions on Coalescence Stability....79
4.1.1 Effect of Emulsifier Type and Concentration....79
4.1.2 Effect of Oil Phase Volume....81
4.2 Effect of Physical Properties of Emulsions on Lipid Oxidation....83
4.3 Effect of Continuous Phase Protein (CAS) on Oxidative Stability....91
4.4 Pickering Emulsions....97

4.4.1 Silica Particles Stabilised Emulsions 98

4.4.2 Oxidative Stability of Silica Stabilised Emulsions 100

4.5 Conclusion 104

Chapter 5: Effect of Food-Grade Particles on the Physical and Oxidative Stability of Oil-in-Water Emulsions 105

5.1 Characteristics of Food-Grade Particles 107

5.1.1 Particle Size 107

5.1.2 Particles Charge 108

5.2 Food-Grade Particles Stabilised Emulsions 109

5.2.1 Effect of Particle Concentration on Physical Stability 109

5.2.2 Effect of Charge of Particles on Coalescence and Creaming Stability 115

5.3 Oxidative Stability of Food-Grade Particles Stabilised Emulsions 121

5.4 Conclusions 128

Chapter 6: Physical and Oxidative Stability of Oil-in-Water Emulsions Stabilised by Both Food-Grade Particles and Low Molecular Weight Surfactant 130

6.1 Surfactant Stabilised Emulsions 132

6.2 Particles-Surfactant Stabilised Emulsions 135

6.2.1 Physical Stability 135

6.2.2 Oxidative Stability 146

6.3 Conclusions 151

Chapter 7: Physical and Oxidative Stability of Oil-in-Water Emulsion Stabilised with Fat Crystals 152

7.1 Characterisation of MG Fat Crystal 154

7.1.1 Determination of the Thermal Properties of MG 154

7.2 Emulsion Stability 156

7.2.1 Effect of Shaft Speed 157

7.2.2 Effect of Throughput Rate 158

7.2.3 Effect of Fat Crystal Concentration 159

7.2.4 Effect of Thickening Agent 167

7.3 Oxidative Stability 173

7.3.1 Effect of Fat crystals on Oxidative Stability 173

7.4 Fat Crystals Versus Food-Grade Particles 177

7.5 Conclusion 178

Chapter 8: Conclusions 180

8.1 Microstructural Design to Reduce Lipid Oxidation 180

8.2 Food-Grade Particles Stabilised Emulsions 181

8.3 Emulsions Stabilised by Both Food-Grade Particles and Low Molecular Weight Surfactant 182

8.4 Emulsions Stabilised by Fat Crystals 183

8.5 Future Work 184

References 186

List of Figures

Figure 2- 1: The schematic diagram of the forces acting on the water molecules in the bulk liquid and at the interface of liquid. 9

Figure 2- 2: Schematic of a surfactant molecule structure (*25*). 12

Figure 2- 3: Schematic of surfactant aggregates (*25*). 14

Figure 2- 4: Position of a small spherical particle at oil-water interface depending on their hydrophobicity (*70*). 18

Figure 2- 5: Stabilisation of solid particles at droplet interface (A) Formation of particles layer around droplets (B) Formation of bridging particles (*72*). 19

Figure 2- 6: Chemical structure of starch octenyl succinate (OSA). 21

Figure 2- 7: Schematic illustration of partial coalescence phenomena (*25*). 30

Figure 2- 8: Chemical structure of (A): omega-3 (ω-3) (alpha-linolenic acid (ALA)) and (B) omega-6 (ω-6) (linoleic acid). 34

Figure 2- 9: Schematic representation of lipid oxidation in oil-in-water emulsions. Mn+ is pro-oxidants in the continuous phase, ROOH is hydroperoxides at droplets interface and RO^{o} is an alkoxyl radical (*161*). 37

Figure 2- 10: Reaction of p-anisidine with aldehydes (*156*). 41

Figure 2- 11: “Layered-by-Layered Electrostatic Deposition” mechanism for producing multilayer emulsions (*13*) 50

Figure 3- 1: Scheme of "A" and "C" unit set-up…….. 60

Figure 3- 2: Principle of particle sizing Static Light Scattering (Mastersize 2000). The focal plane detector is for detecting small scattered angles (< 10^{o}), the large angle detectors are

measuring higher angles (10º - 90º) and the back scatter detectors are detecting very high angles (100º - 135º) (*220*). 61
Figure 3- 3: Scheme of creaming process occurring in oil-in-water emulsions. 63
Figure 3- 4: Schematic illustration of the DLS instrument. 65
Figure 3- 5: Schematic of illustrating Huckel and Smoluchowski's approximations used for the conversion of electrophoretic mobility into zeta potential Adapted from http://www.silver-colloids.com/Tutorials/Intro/pcs21.html. 67
Figure 3- 6: Cryo scanning electron microscope: (1) insertion rod, (2) cylindrical chamber for transferring of the sample from freezing unit to cryo chamber, (3) binocular, (4) cryo-chamber and (5) lever to handle the fracture knife. 69
Figure 3- 7: Schematic of a parallel plate geometry. 70
Figure 3- 8: Schematic of (A) heat flux DSC (B) power compensation DSC (*225*). 71
Figure 3- 9: Calibration curve for Peroxide Value measurement. 74
Figure 3- 10: Reaction of p-Anisidine with aldehydes (*156*). 75
Figure 3- 11: Calibration curve for P-AnV. 76
Figure 4- 1: Effect of physical properties of emulsions (oil phase volume, emulsifier type and concentration) on lipid oxidation rate. (A&B) Hydroperoxide formation (primary lipid oxidation product) for emulsions stabilised with Tween 20 surfactant and CAS protein respectively. (C&D) p-Anv value (secondary lipid oxidation products) for emulsions stabilised with Tween 20 surfactant and CAS protein respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The pH of solutions is not adjusted. The error bars represent at least 3 different measurements on the same sample……..85

Figure 4- 2: Formation of hydroperoxide normalized to total oil droplets surface area over 7 days. (A) 0.1% and 2% CAS (B) 0.1% and 2% Tween 20. All samples held at 40˚C. The pH of emulsions is not adjusted. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 90

Figure 4- 3: Zeta-potential of 0.5% CAS protein as a function of pH. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.....91

Figure 4- 4: Effect of different concentration of CAS protein in the continuous phase of 20% sunflower oil-in-water emulsions stabilised with 0.6% Tween 20 on lipid oxidation at pH 3 and 7. (A&B) Primary lipid oxidation product (hydroperoxide formation) for pH 3 and pH 7 respectively. (C&D) Secondary products formation (AV) for pH 3 and 7 respectively. All samples held at 40˚C. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbol. The error bars represent at least 3 different measurements on the same sample. 93

Figure 4- 5: Change in pH of different concentrations of CAS in continuous phase of 20% oil-in-water emulsions stabilised with 0.6% Tween 20 over 7 days (A) Initially pH 3. (B) Initially pH 7.3. Data points represent mean values (n=3) ± ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 95

Figure 4- 6: Lipid oxidation of 20% sunflower oil-in-water emulsions stabilised with 0.6% Tween 20 and containing no CAS protein at pH 3 and 7 (A) Primary lipid oxidation product (PV) and (B) Secondary lipid oxidation products (AV). All samples held at 40˚C. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller

than symbols. The error bars represent at least 3 different measurements on the same sample. 97

Figure 4- 7: Droplet size of 20% oil-in-water emulsions stabilised with 0.5% to 2% silica particles at (A) pH 2 and (B) pH 7. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 99

Figure 4- 8: (A&B) Formation of hydroperoxide normalized to total oil droplets surface area over 7 days at pH 2 and pH 7 respectively.). (D & C) Formation of secondary products at pH 2 and pH 7 respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 101

Figure 4- 9: Formation of hydroperoxide normalized to total oil droplets surface area over 7 days. 20% oil-in-water emulsions stabilised with either silica particles or Tween 20. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 103

Figure 5- 1: The zeta potential of 0.5% MCC solution and 0.5% MS solution at different pH conditions. Data points represent mean values (n =3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample……108

Figure 5- 2: Droplets size distribution of 20% oil-in-water emulsions stabilised with 0.1% to 2.5% (immediately after formation). (A) MCC particles and (B) MS particles. The pH of emulsions is not adjusted. Data points represent mean values (n= 3) ± standard deviation.

Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 110

Figure 5- 3: Creaming stability of 20% oil-in-water emulsions stabilised with different concentrations of MCC particles (0.1% to 2.5%). The pH of emulsions was not adjusted. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols.....113

Figure 5- 4: Creaming stability of 20% oil-in-water emulsions stabilised with different concentrations of MS particles (0.1% to 2.5%). The pH of emulsions was not adjusted. Data points represent mean values (n=3)± standard deviation. Where not visible error bars are smaller than symbols.....114

Figure 5- 5: Droplets size distribution of 20% oil-in-water emulsions stabilised with 1.5% food-grade particles as a function of pH (immediately after preparation). (A) MCC particles and (B) MS particles. Data points represent mean values (n= 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 117

Figure 5- 6: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by 1.5% MCC particle.....120

Figure 5- 7: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by 1.5% MS particles. 121

Figure 5- 8: Effects of MCC concentration on lipid oxidation rate of 20% oil-in-water emulsions. Formation of lipid hydroperoxide /surface area in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MCC at pH 4 (A) and pH 6 (B). Formation of secondary products in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MCC at pH 4 (C) and pH 6 (D) respectively. Data points represent mean values (n = 3) ±

standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.....123
Figure 5- 9: Effects of MS particles concentration on lipid oxidation rate of 20% oil-in-water emulsions. (A&B) Formation of lipid hydroperoxide/ surface area in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MS particles at pH 4 and pH 6 respectively pH 6 (C & D) Formation of secondary products in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MS particles at pH 4 and pH 6 respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 126
Figure 5-10: Formation of hydroperoxide normalised to total oil droplets surface area for 20% oil-in-water emulsions. 127
Figure 6- 1: Droplet size stability of 20% oil-in-water emulsion stabilised with 0.1% to 2% Tweeen 20 at (A) at pH 4 and (B) pH 6. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample…… 133
Figure 6- 2: Effects of Tween 20 concentration on the creaming stability of 20% oil-in-water emulsions. Emulsions stabilised with (0.1% to 2%) Tween 20 at (A) pH 4 and (B) pH 6. The error bars represent at least 3 different measurements on the same sample. 134
Figure 6- 3: Droplet distribution of 20% oil-in-water emulsions stabilised with both 1.5% MCC particles and different Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6. The error bars represent at least 3 different measurements on the same sample. 136
Figure 6- 4: Droplet distribution of 20% oil-in-water emulsions stabilised with both 1.5% MS particles and different Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6.The error bars represent at least 3 different measurements on the same sample. 137

Figure 6- 5: Average emulsion droplet size as a function of emulsifier concentrations and pHs. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 142
Figure 6- 6: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by (A) 1.5% MCC particles and 2% Tween 20 surfactant at pH 4 (B) 1.5% MS particles and 2% Tween 20 surfactant at pH 4. 143
Figure 6- 7: Effect of increasing Tween 20 concentrations on the cream layer of 20% oil-in-water emulsions stabilised with 1.5% MCC particles and various Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6 after 3 days. 145
Figure 6- 8: Effect of increasing Tween 20 concentrations on the cream layer of 20% oil-in-water emulsions stabilised with 1.5% MS particles and various Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6 after 3 days. 146
Figure 6- 9: Effects of mixed-emulsifier system on lipid oxidation rate of 20% oil-in-water emulsions stabilised with both 1.5% MCC particles and (0.5% to 2% Tween 20): (A) Formation of hydroperoxide/surface area (B) Secondary lipid oxidation products. The pH of emulsions was not adjusted. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 148
Figure 6- 10: Effects of mixed-emulsifier system on lipid oxidation rate of 20% oil-in-water emulsions stabilised with both 1.5% MS particles and (0% to 2% Tween 20): (A) Formation of hydroperoxides/surface area and (B) Secondary lipid oxidation products. The pH of emulsions was not adjusted. Data points represent mean values (n = 3) ± standard deviation.

Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 149

Figure 6- 11: Formation of hydroperoxide normalised to total oil droplets surface area for emulsions stabilised with both 1.5% particles and (0.5 to 2%) Tween 20, emulsions stabilised by either 0.6% Tween 20 alone and emulsions stabilised with 0.6%. The error bars represent at least 3 different measurements on the same sample. 150

Figure 7- 1: DSC curves of different concentrations of MG. The Samples were heated at 10 ºC/min from 0 to 100 ºC..... 155

Figure 7- 2: Effect of shafts speeds on droplets size of 20% oil-in-water emulsions stabilised with 2.5% MG. The error bars represent at least 3 different measurements on the same sample.....158

Figure 7- 3: SEM micrograph of an emulsion stabilised by 2.5 % monoglyceride which was collected from "A" unit.....161

Figure 7- 4: Droplet size distributions of 20% sunflower oil-in-water emulsions stabilised with 0.5 to 2.5% MG- collected from "C" unit. The error bars represent at least 3 different measurements on the same sample. 164

Figure 7- 5: (A) Cryo-SEM image of 20% oil-in-water emulsion stabilised with 2.5% MG. (B) light microscope image of 20% oil-in-water emulsion stabilised with 2.5% MG. Sample was collected from "C" unit.....166

Figure 7- 6: Effect of different concentrations of XG in the continuous phase of 20% sunflower oil-in-water emulsions stabilised with different concentrations of MG (0.5-2.5%) on droplet size. Samples were collected from "A" unit (Left column), and "C" Unit (Right Column). Data points represent mean values (n = 3) ± standard deviation. Where not visible

error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 169
Figure 7- 7: 20% oil-in water emulsions stabilised with 2.5% MG containing 0.25% XG: (A& B) Cryo-SEM image and Light microscope image for samples from “A” unit respectively. a(C&D) Cryo-SEM image and Light microscope image for samples from “C” unit respectively. 171
Figure 7- 8: Cryo-SEM image of 20% oil-in water emulsions stabilised with 2.5% MG containing 1% XG (A) Samples were collected from “A” unit. (B) Samples were collected from “C” unit. (C) Light microscope image for samples from “C” unit. (D) Image D is magnification of image B.....172
Figure 7-9: Effect of XG concentration on the flow curves of 20% oil-in water emulsions stabilised with 2.5% Mg (A) Samples were collected from “A” unit. (B) Samples were collected from “C” unit. The error bars represent at least 3 different measurements on the same sample. 173
Figure 7-10: Lipid oxidation rate of 20% oil-in-water emulsions stabilised with (0.5%, 1.5% and 2.5%) MG and containing 1% XG. Samples were collected from “A” unit (A) Hydroperoxide formation/surface area (B) Secondary lipid oxidation products. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 176
Figure 7-11: Lipid oxidation rate of 20% oil-in-water emulsions stabilised with (0.5%, 1.5% and 2.5%) MG and containing 1% XG. Samples were collected from “C” unit (A) Hydroperoxide formation /surface area (B) Secondary lipid oxidation products. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller

than symbols. The error bars represent at least 3 different measurements on the same sample. 176

Figure 7-12: Hydroperoxide formation/surface area of 20% oil-in-water emulsions, containing 0.07 mM ferrous. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample. 177

List of Tables

Table 2- 1: Method used to monitor oxidative stability of a sample 39

Table 3- 1: Fatty acid composition of regular sunflower oil……52

Table 3- 2: The specification of Silica particles provided by the Degussa (UK). 54

Table 4- 1: Mean droplet size (D3,2), and standard deviations of 5% oil-in-water emulsions stabilised with different concentrations of Tween 20 surfactant or CAS protein. The error bars represent at least 3 different measurements on the same sample…… 80

Table 4- 2: Mean droplet size (D3,2) and standard deviations of 30% oil-in-water emulsions stabilised with different concentrations of Tween 20 surfactant or CAS protein. The error bars represent at least 3 different measurements on the same sample……82

Table 4- 3: Charge of 0.5% Silica particles dispersion at different pH conditions. The error bars represent at least 3 different measurements on the same sample. 98

Table 5- 1: The particle sizes of 0.5% food-grade particles dispersed in water with either magnetic bar or high shear mixer. The pH of solutions is not adjusted. The error bars represent at least 3 different measurements on the same sample……...107

Table 5- 2: Droplet size of emulsions stabilised with different concentrations of MCC or MS particles. The pH of emulsions was not adjusted. The error bars represent at least 3 different measurements on the same sample. 112

Table 5- 3: Droplet size of oil-in-water Pickering emulsions containing 1.5% either MCC or MS particles at different pH conditions. The error bars represent at least 3 different measurements on the same sample. 118

Table 6- 1: Mean droplet size ($D_{3,2}$) of 20% oil-in-water emulsions stabilised with both 1.5% food-grade particles (MCC or MS) and various Tween 20 (0.1% to 2%) concentrations at pH 4 and 6. The error bars represent at least 3 different measurements on the same sample 140

Table 7- 1: The melting properties of different concentrations of MG. The Samples were heated at 10 ºC/min from 0 to 100 ºC…… 156

Table 7- 2: Effect of pump rate on droplet size of 20% oil-in-water emulsions stabilised with 2% Mg. The error bars represent at least 3 different measurements on the same sample. ...159

Table 7- 3: Details of experimental set up for perpetration of 20% oil-in-water emulsions stabilised with different concentrations of MG (0.5 to 2.5%). The error bars represent at least 3 different measurements on the same sample. 160

Abbreviations

HLB	Hydrophilic-Lipophilic Balance
CAS	Sodium Caseinate
o/w	Oil-in-water
w/o	Water-in-oil
MG	Saturated Monoglycerides
MCC	Colloidal Microcrystaline cellulose
MS	Modified starch
SEM	Scanning electron microscopy
XG	Xanthan gum
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
EDTA	Ethylenediaminetetraacetic acid
A unit	Scraped surface heat exchanger
C unit	Pin stirrer heat exchanger
DSC	Differential scanning calorimetry
CMC	Critical micelle concentration
OSA	Octenyl succinic anhydride
SFA	Saturated fatty acids
MUFAS	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids
EFA	Essential fatty acids

ω-3	Omega-3
ω-6	Omega-6
ALA	Alpha-linolenic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
RH	Unsaturated lipids
ROO°	Peroxyl radicals
ROOH	Hydroperoxide
RO°	Alkoxyl radical
PV	Peroxide Value Method
p-AnV	p- Anisidine Value
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric ions
PG	Propyl gallate
SDS	Sodium dodecyl sulphate
DTAB	Dodecyl trimethyl ammonium bromide
WPI	Whey protein isolate

Nomenclature

A	Surface area (m^2)
R	Radius of the droplet (m)
ρ	Density (kg/m^3)
g	Acceleration due to gravity (m/s^2)
η	viscosity (Pa.s)
V	Velocity (m/s)
T	Temperature (ºC)
M	Molecular weight (g/mol)
$D_{3,2}$	Surface weighted mean diameter (µm)
$D_{4,3}$	Volume weighted mean diameter (µm)
θ	Contact angle(degree)
K	Boltzmann constant (1.38×10^{-23} J/K)
N	Power law
$\dot{\gamma}$	Shear rate (1/s)
τ	Shear stress (N/m^2)
N_d	Number of droplets

Chapter 1: Introduction

1.1 Background

Statistics have shown that heart attack and heart disease are the leading cause of death worldwide. Annually, more people die from heart disease than any other diseases. The UK national statistics showed one in five men and around one in six women died from heart disease including heart attacks, in 2007 (*1*). The dietary choices that people make may either increase or decrease the risk of coronary heart disease. Hence, diet quality and food consumption are directly related to the human health.

The human body needs fat to provide energy, produce hormones, carry fat soluble vitamins and help the brain and nervous system to develop correctly. However, high consumptions of certain types of fats (saturated) are linked to heart disease and cancers. Furthermore, high fat diets contribute to weight gain and obesity. On the other hand, alternative fats (omega 3-polyunsaturated fatty acid and omega-6 polyunsaturated fatty acid) appear to decrease a risk of having high cholesterol and heart disease over time *(2).*

In last three decades, omega-3 fatty acids have received an increasing level of attention due to their several health benefits including, prevention of coronary heart disease, diabetes and arthritis, reduction of blood pressure and cholesterol and improvement of brain function in infants. These types of polyunsaturated fats (alpha-linolenic acid (omega-3 fatty

acid) and linoleic acid (an omega-6 fatty acid)) are known as *essential fatty acids* and they must be obtained through diet as body cannot make them (*3, 4*).

In past few years, consumers have become more interested and aware of the "healthy" fats. Therefore, food manufacturers are rising to meet consumer demand by adding these ingredients to their products. An effective approach to include omega fatty acids into consumers' dietary regime is to use emulsions as a medium for delivering these health promoting ingredients into food and beverage products. However, the addition of these ingredients to foods is not a trivial task as some of them can easily undergo lipid oxidation. Lipid oxidation is a major problem and economic concern in the food industry because it changes taste, texture and appearance of products, develops undesirable flavours and decreases nutritional value and shelf-life of products. Hence, food manufacturers have been seeking for a reliable and feasible method to incorporate fish oils into food products and specifically emulsion-based foods (*5, 6*).

There are various factors that can influence the rate of lipid oxidation in emulsion, including the molecular structure of lipids, heat, light, the physical characteristics of emulsion droplets, processing conditions, and the presence of antioxidants or pro-oxidants (transition metals such as, iron) in the system (*7*). A great deal of research has been carried out to explain lipid oxidation mechanisms in emulsified oils. One of the major mechanisms that causes lipid oxidation in emulsions is the interaction between lipid hydroperoxides located at the droplet surface and transition metals (sited in the aqueous phase), which accelerates the breakdown of lipid hydroperoxide into free highly reactive radicals, such as alkoxyl and peroxyl radicals (the main causes for the off-flavour and off-odour generation in foods) (*8*). The common approach taken so far, by food scientists to prevent lipid oxidation, is using antioxidants (*9*).

Antioxidants have the ability to inhibit lipid oxidation by various mechanisms, such as free radical scavenging which quenches reactive radicals and metal chelating agents which convert transition metals (pro-oxidants) into stable products (*10*). Currently, most of antioxidants used in the food industry are synthetic including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ehylenediaminetetraacetic acid (EDTA) *(11)*. In recent years, there has been an increase in the food safety issues surrounding the use of synthetic antioxidants. This has resulted in the decrease in acceptability of these ingredients by customers. There is, therefore, a need to find alternatives as the inclusion of bioactive ingredients can have several health benefits *(10)*.

Since the lipid oxidation reactions in food-based emulsions take place at droplet interfaces, the importance of interfacial properties has been studied. Recently, many researchers have improved the lipid oxidation stability of oil-in-water emulsions by using different types of emulsifiers (oppositely charged) to form multilayered membranes around the oil droplets, so called "Layer-by-Layer Deposition Technique" (*12*). However, this technique has some limitations, mainly the high tendency for the droplets to aggregate and flocculate when preparing samples. Furthermore, the use of different types of emulsifiers in order to create multilayer membranes around oil droplets increases the total cost of the product (*13*). Although it has been demonstrated that lipid oxidation can be inhibited by droplet engineering, alternative microstructure approaches must be developed in order to avoid problems that were mentioned above.

1.2 Objectives

It has been well established that the oxidation of unsaturated fatty acids is undesirable process for both manufacturers and consumers. Although lipid oxidation can be

reduced with the addition of synthetic antioxidants, on the other hand it failed to produce "additive free", natural products. The main objective of this study is to find alternative ways to overcome lipid oxidation in "food-based" emulsions (Oil-in-Water) instead of adding any synthetic additives.

The approach taken in this project is to physically separate the reactive species by designing and using microstructures. Specifically, microstructure engineering has been used to manipulate the physical properties of the emulsion, and form a thick interfacial layer around oil droplets to inhibit lipid oxidation.

Specifically, the main objectives of this study are:

- Design a structure around droplets to protect the oil droplets from pro-oxidation in the continuous phase by using different types of emulsifiers and Pickering particles
- Find suitable food-grade Pickering particles to be effective in controlling lipid oxidation
- Control the packing of food-grade particles at droplet surface to create a "thicker membrane" around oil droplets
- Build thick fat crystal "shells" around the oil droplets using a bench scale scraped-surface heat exchanger

1.3 Thesis Layout

- Chapter 1 introduces the subject and the objectives of this work.
- Chapter 2 covers a detailed review on emulsion principle, mechanism of emulsification, physical instability of emulsion, lipid oxidation mechanism and the effect of different parameters (light, pro-oxidants, antioxidants, interfacial properties) on oxidative stability.

- Chapter 3 contains all the material, experimental design, operating procedures and techniques used to characterise the physical and oxidative stability of samples.
- Chapter 4 reports the effect of different types of emulsifiers (protein, small molecule surfactant and Pickering particles) on oxidative stability.
- Chapter 5 shows the properties of aqueous dispersions containing various kinds of "food-grade" Pickering particles and their ability to enhance the physical and oxidative stability.
- Chapter 6 investigates the ability of both "food-grade" particles and small molecule surfactant (particle-surfactant stabilised emulsion) to inhibit lipid oxidation as a function of pH and emulsifier concentration.
- Chapter 7 demonstrates whether fat crystal "shells" (Monoglyceride crystals) at the droplets' interface are capable of increasing the physical and oxidative stability of the emulsion at different processing conditions.
- Chapter 8 contains conclusions and future work recommendations.

1.4 Publications and Conferences

Publication

- M. Kargar, F. Spyropoulos, I.T. Norton, (2011), **The Effect of Interfacial Microstructure on the Lipid Oxidation Stability of Oil-in-Water Emulsions,** *Journal of Colloid and Interface Science,* 357, 527-533.

- M. Kargar, F. Spyropoulos, I.T. Norton, (2011), **Microstructural Design to Reduce Lipid Oxidation in Oil-in-Water Emulsions**, *11th International Congress on Engineering and Food (ICEF11)*, 1, 104-108.
- M. Kargar, Kh. Fayazmanesh, M. Alavi, F. Spyropoulos, I.T. Norton, (2012), **Investigation into the Potential Ability of Pickering Emulsions to Increase the Oxidative Stability of Oil-in-Water Emulsions**, *Journal of Colloid and Interface Science*, 366 209–215.
- M. Kargar, F. Spyropoulos, I.T. Norton, (2010), **Effect of Physical Characteristic of Droplets on the Oxidation Stability of Sunflower Oil in Water Emulsions Stabilised with Protein,** *Proceedings of 5th World Congress on Emulsions*, Lyon, France**.**
- M. Kargar, Kh. Fayazmanesh, M. Alavi, F. Spyropoulos, I.T. Norton **The Ability of Oil-in-Water Emulsions Stabilised by Both Low Molecular Weight Surfactants and Food-Grade Particles to Reduce Lipid Oxidation Rate.** *Journal of Food Engineering***,** (Under preparation).
- M. Kargar, F. Spyropoulos, I.T. Norton, **Physical and Oxidative Stability of Oil-in-Water Emulsion Stabilised with Fat Crystals**, *Food Hydrocolloids,* (Under preparation).

Conferences

- M. Kargar, F. Spyropoulos, I.T. Norton, **Emulsion Design for Protection of Chemically Sensitive Bioactive ingredients,** *15th Gums & Stabilisers for the Food Industry Conference*, 2009 Wrexham, UK.
- M. Kargar, F. Spyropoulos, I.T. Norton, **Effect of Physical Characteristic of Droplets on the Oxidation Stability of Sunflower Oil-in-Water Emulsions**

Stabilised with Protein and Surfactant, *Controlled Release in Food, Pharmacy and Agrochemical Systems*, 2010, London, UK.

- M. Kargar, F. Spyropoulos, I.T. Norton, **Effect of Physical Characteristic of Droplets on the Oxidation Stability of Sunflower Oil-in-Water Emulsions Stabilised with Protein**, *The 5th World Congress on Emulsion*, 2010, Lyon, France.
- M. Kargar, F. Spyropoulos, I.T. Norton, **Microstructural Design to Reduce Lipid Oxidation in Oil-in-Water Emulsions**, *11th International Congress on Engineering and Food*, 2011, Athens, Greece

Chapter 2: Literature Survey

This chapter discusses the most current and relevant published literature regarding the "food-based" emulsions and their physicochemical stabilities. Initially, the general principles and applications of emulsion are introduced then the physical (in)stability of emulsion is discussed in detail. Finally, the lipid oxidation phenomena (chemical (in)stability of emulsions) and its impact on emulsion quality are discussed.

2.1 Emulsions

2.1.1 Definitions

An emulsion is a colloidal system containing droplets of one liquid (dispersed phase) dispersed in another immiscible liquid (continuous phase). Emulsions are extensively used in a wide variety of industries including, food, pharmaceutical, cosmetic, petroleum and paint (*14, 15*). Many processed foods such as, salad dressings, ice cream, infant formula, margarine and some natural foods such as milk, are also emulsions.

Oil and water are the two main immiscible liquids in food emulsions (*16*). Emulsions can be categorised based on different criteria. For instance, emulsions can be characterised according to their dispersed phase droplet size; such as macro-emulsion (0.2-50 μm), mini-emulsion (0.1-0.4 μm) and micro-emulsion (10-100 nm) (*17*). Emulsions are also classified as oil-in-water (O/W) or water-in-oil (W/O) emulsions depending on which liquid is dispersed in the other. Some examples of oil-in-water emulsions include milk, salad dressings, soups, coffee whiteners and meat products (*18*). Furthermore, in the food industry more complex emulsions known as "double emulsions" also exist. Double emulsions can be

used for protecting certain ingredients (*19, 20*), controlling the release of ingredients (*21*) or for producing low-fat products (*22*). These emulsions have an interior emulsion as the dispersed phase in a continuous phase. The two main types of double emulsions are water-in-oil-in-water ($W_1/O/W_2$) and oil-in-water-in-oil ($O_1/W/O_2$) (*23*). This study is solely concerned with oil-in-water emulsions; hence the main focus of this chapter is oil-in-water emulsions.

2.2 Mechanism of Emulsification

Since the contact between oil and water molecules is thermodynamically unfavourable, o/w emulsions tend to separate into a system that consists of a layer of oil (lower density) on top of a layer of water (higher density). As shown in Figure 2-1, attractive forces from all directions are exerted on a water molecule in the centre. Hence, the net force on this molecule is zero. However, the forces are not in equilibrium for the water molecules at the interface. Since there are no water molecules above, the molecules at the interface experience an imbalance of forces. The net forces at the water molecule interface are called *interfacial tension (24)*.

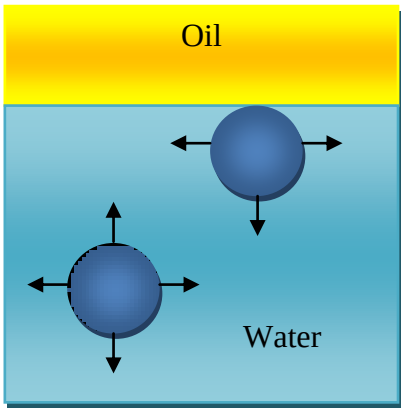


Figure 2- 1: The schematic diagram of the forces acting on the water molecules in the bulk liquid and at the interface of liquid.

In emulsions, the process of dispersion of one liquid in the other results in an increase in surface area and surface free energy which can be represented as follows (*25*):

$$\Delta G = \gamma \Delta A \qquad \text{Equation 2- 1}$$

Where ΔG is the increase in free energy, γ is the interfacial tension and ΔA is the increase in surface area. In general, emulsions are thermodynamically unstable. They tend to separate into two layers (oil layers on top of the layer of water) to reduce the free energy and the total surface area between the two immiscible liquids. Consequently, in order to form stable emulsions ("kinetically stable") for a reasonable amount of time (e.g weeks, months), a third substance known as an *emulsifier* is required to reduce the interfacial tension by adsorbing to the surface of freshly formed droplets (*26*).

Emulsification process can be separated into two main stages: First is to break down large drops into small uniform droplets (known as "break up stage"). In order to break up a drop into smaller droplets (break up disperse phase), mechanical energy is required to overcome the Laplace pressure (is the pressure difference between the inside and the outside of the droplet, across the oil-water interface), which increases with decreasing emulsion droplet size. Normally, intense agitation supplies this energy. The most common devices for emulsification in the food industry are high-speed mixer, colloid mill, or high-pressure valve homogenizer (*24, 27*). As mentioned above, at this stage there is a large increase in surface area. Hence, dispersed droplets tend to coalesce (known as "coalescence phenomena") in order to reduce surface area, therefore, complete phase separation occurs ultimately. For that reason, it is necessary to use emulsifiers to produce stable emulsions (prevent "coalescence phenomena"). In the second stage of emulsification, emulsifier agents rapidly absorb at the surface of the freshly formed droplets, reduce interfacial tension and prevent droplets from

coalescing with their neighbours by forming a stable interfacial layer around the droplets. It is worth mentioning that, all these stages have their own time scale (in microseconds). The balance between droplet break up and droplet coalescence determines the size of the droplets produced in an emulsion. The size of the emulsified droplets is an important parameter for food manufacturers because it determines the stability, appearance and taste of the final product (*25, 28*).

2.3 Emulsifiers

As aforementioned, emulsifiers not only delay or prevent coalescence of the emulsion droplets but also facilitate droplet disruption ("break up" stage) by reducing the interfacial tension. Therefore, emulsifiers play a significant role in producing stable emulsions. Emulsifiers can be broadly classified as surfactants, proteins and finely divided solids. Since the particles do not significantly lower the interfacial tension, they are known as "mechanical emulsifiers" or stabilisers (*29-31*). Each of these emulsifiers is discussed in greater details below.

2.3.1 Surfactant

A surfactant (a contraction of the term surface-active agent) is a substance that consists of a hydrophilic head (high affinity for water) and a hydrophobic tail (high affinity for oil) (Figure 2-2). This type of structure is known as an *amphipathic* or *amphiphilic* structure. Surfactants have been used in a wide range of applications in petroleum, pharmaceutical and food industries and can also be found in the household products due to their exclusive properties. Surfactants are able to absorb at the interface and significantly alter interfacial free energies. Naturally occurring surfactants such as lecithin (from egg yolk) is used for the preparation of many food products, including mayonnaise. Despite the

availability of naturally occurring surfactants, numerous synthetic surfactants such as sorbitan esters (Tween series) have recently been used in food emulsions (*30, 32*).

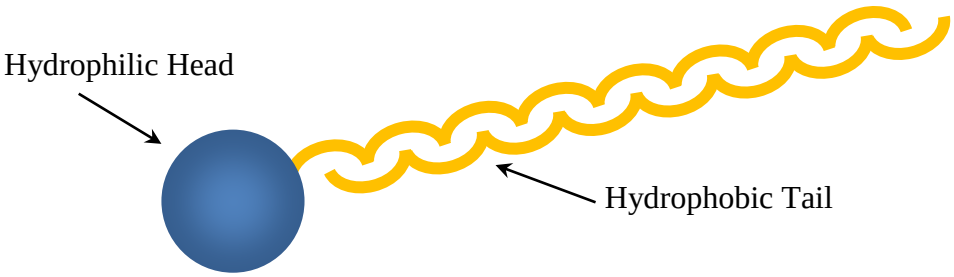


Figure 2- 2: Schematic of a surfactant molecule structure (*25*).

Surfactants are generally categorised based on the charge of their head group. It is common to divide surfactants into five main types (*18, 33*) :

- Anionic: the head group of surfactant is negatively charged.
- Cationic: the surface-active portion bears a positive charge.
- Non-ionic: no charge on their hydrophilic head group.
- Zwitterionic: have both positive and negative charges on their surface-active part of system.
- Amphoteric: charge on their surface-active part changes with pH.

Surfactants can also be characterised based on their hydrophile-lipophile balance (HLB) number which was first introduced by Griffin (*34*). The HLB number demonstrates the overall tendency of the surfactant for either an oil phase or an aqueous phase. The HLB value of a particular surfactant can be calculated according to the hydrophilic and hydrophobic regions of the surfactant molecule (*15, 25, 33, 35*).

$$HLB=7+\sum(\text{hydrophilic group numbers})-\sum(\text{lipophilic group numbers}) \qquad \text{Equation 2-2}$$

A surfactant with high HLB (10-18) value is mostly hydrophilic and dissolves in water and acts as a good oil-in-water stabiliser. However, a surfactant with low HLB value (3-6) is mainly hydrophobic, dissolves in oil and stabilises water-in-oil emulsions. A surfactant with an intermediate HLB value (7-9) has no particular affinity to either oil or water and acts as a good wetting agent. One of the first empirical techniques to define the type of emulsion is proposed by Brancroft. Based on Brancroft's rule the phase in which that the surfactant is most soluble will form continuous phase of an emulsion (*18*).

Micelles can form thermodynamically stable colloidal clusters as a result of spontaneous aggregation of surfactant molecules when sufficient amount of surfactant is available in the system. Micelles are typically spherical and contain 50-100 monomers. However, they can be found in other forms (cylindrical, lamellar) once the surfactant concentration increased (Figure 2-3). Micelles are formed due to their affinity to minimise the unfavourable contact between water molecules and the non-polar tail parts of surfactant molecules. The concentration of surfactant at which the formation of micelle begins is called the critical micelle concentration (CMC). At low surfactant concentration (below CMC), surfactant (dispersed mostly in the form of monomers) will tend to absorb at the interface which leads to reduced interfacial tension. As the surfactant concentration increases, these interfaces become saturated and surfactant molecules tend to form micelles. At high surfactant concentration (above CMC), increasing the total surfactant concentration does not change the concentration of monomer, therefore, no further reduction in the interfacial tension occurs (*36, 37*).

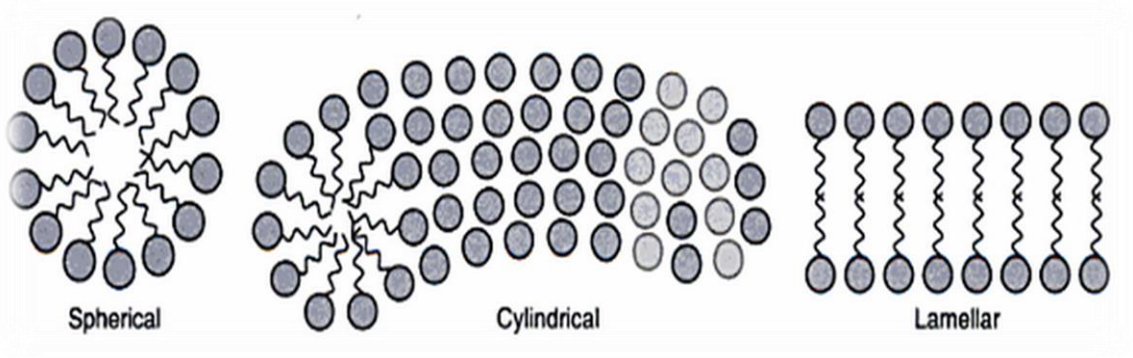


Figure 2- 3: Schematic of surfactant aggregates (*25*).

2.3.2 Protein

Proteins are surface-active compounds which consist of chains of amino acid residues, linked to each other by peptide bonds. Proteins are used in a broad range of food products as emulsifier agents in order to improve the emulsion stability and nutritional value of the product. Proteins are able to absorb to the surfaces of newly formed oil droplets and reduce the interfacial tension. They can also produce protective viscoelastic layers around the droplets which slow down droplet coalescence. It should be noted that, a protein must be soluble to be able to diffuse and absorb at droplet interface, otherwise it tends to form large aggregates (*25, 38-40*).

The formation of an interfacial layer is achieved in three steps. Initially, soluble protein diffuses at the droplet interface during the emulsification process. Tornberg (1978) reported that soy protein diffused slowly to the interface compared to the sodium caseinate and argued that it was due to the large particle size of soy protein (*40*). In contrast to soy proteins, whey proteins consist of smaller molecules thereby diffused quickly to the interface

(*41*). Since proteins are larger molecules than small molecule surfactants, their diffusion to the interface is much slower than small molecule surfactants (*42*).

Once the protein is absorbed, its structure alters in a way that hydrophobic amino acids are in contact with the oil-water interface. This leads to a further reduction in the interfacial tension (*43*).

Finally those protein molecules that are absorbed will unfold and reshape their original arrangement to form an interfacial membrane around oil droplets. However, this arrangement can be influenced by the nature and flexibility of the protein (*25*). For instance, β-casein is able to rapidly unfold at the interface and reduces the interfacial tension faster than β-lactoglobulin due to its flexible structure (*44*). Finally, the interfacial layer formed at the droplet interface can prevent coalescence through repulsive interactions (steric repulsive and electrostatic forces) between oil droplets (*38*).

Milk proteins are widely utilised in the food industry for preparation of dairy products and other processed foods due to their functional properties, such as emulsification, thickening, gelling and flavour binding. Milk proteins can be classified into two main groups: caseins (which consist of α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein) and whey proteins (β-lactoglobulin, bovine serum albumin, α-lactalbumin and immunoglobulins) (*45, 46*). Caseins are known for their unique emulsifying properties due to their hydrophilic and hydrophobic characteristics. They are flexible and tend to rapidly unfold at the oil-water interface. Amongst the four major fractions of casein, β-casein is the most effective emulsifier as it can lower the interfacial tension more significantly than the other caseins. Moreover, caseins are not sensitive to heat and are stable against thermal denaturation due to their structure (*47*).

Sodium caseinate (CAS) is the most commonly used casein preparation in the food industry. Numerous studies have shown that CAS is able to stabilise oil-in-water emulsions (*48*). However, the concentration of CAS plays a key factor in stabilising or destabilising emulsions (*49*). It has been reported that at low CAS concentration (<1%), the emulsion was destabilised by bridging flocculation because of low surface protein coverage (droplet surfaces were only partially covered). Emulsions became stable when they contained 2% CAS as a result of the formation of a thick uniform layer around oil droplets (protect the droplets against coalescence via steric repulsive forces). However, when the CAS concentration was increased to above 3.0%, non-adsorbed CAS gave rise to a depletion flocculation (*38, 50*).

2.3.3 Solid Particles

The ability of solid particles to stabilise emulsions was first discovered by Ramsden (1903) (*51*) and shortly afterwards by Pickering (1907) (*52*). This type of emulsion is known as a *Pickering emulsion*. A broad range of solid particles have been used as stabilisers for Pickering emulsions, including silica (*53*) clay (*54*), latex (*55*), etc.

Recently, Pickering emulsions have been receiving increasing level of attention due to a variety of reasons such as, their remarkable stability against coalescence (*56-58*), their high stability against changes to processing conditions (pH, salt concentration, temperature, etc.) (*59, 60*) and their “emulsifier-free” character which assist to reduce the use of hazardous surfactants (particularly important in health care and pharmaceutical products) (*61, 62*).

The great stability of Pickering emulsions against coalescence is due to the fact that solid particles absorb strongly at the droplets interface and create a rigid barrier around each droplet (*58*). Therefore, such emulsions have been used in a variety of industries such as, cosmetic, pharmaceutical, oil recovery, ceramic and food industries (*63*).

Many researchers have reported that the effectiveness of particles in stabilising emulsions depends on several parameters, such as the particle wettability (*64*), particle concentration (*59*), shape (*65*) and size (*66*).

The most important factor which defines the emulsion type (oil-in-water or water-in-oil) is the wettability characteristic of the particles which is expressed by contact angle (θ) between the solid particles and the liquid interface (*64, 67, 68*). Similar to surfactants, the adsorption of a particle at the oil-water interface depends on its degree of hydrophobicity. Particles with contact angles (<90º) are hydrophilic (preferably wetted by water) and tend to stabilise oil-in-water emulsions, while particles with large contact angles (>90º) are deemed hydrophobic (preferably wetted by oil) and tend to stabilise water-in-oil emulsions (Figure 2-4). However, if the particles used are completely wetted by oil (θ=180º) or water phase (θ=0º), they become fully dispersed in the phase thereby not likely to absorb at the oil-water interface (*64, 69*). Many research groups have suggested that the optimum contact angles for the stabilisation of oil-in-water emulsions and water-in-oil emulsions are 70<θ<86º and 94<θ<110º, respectively (*62*).

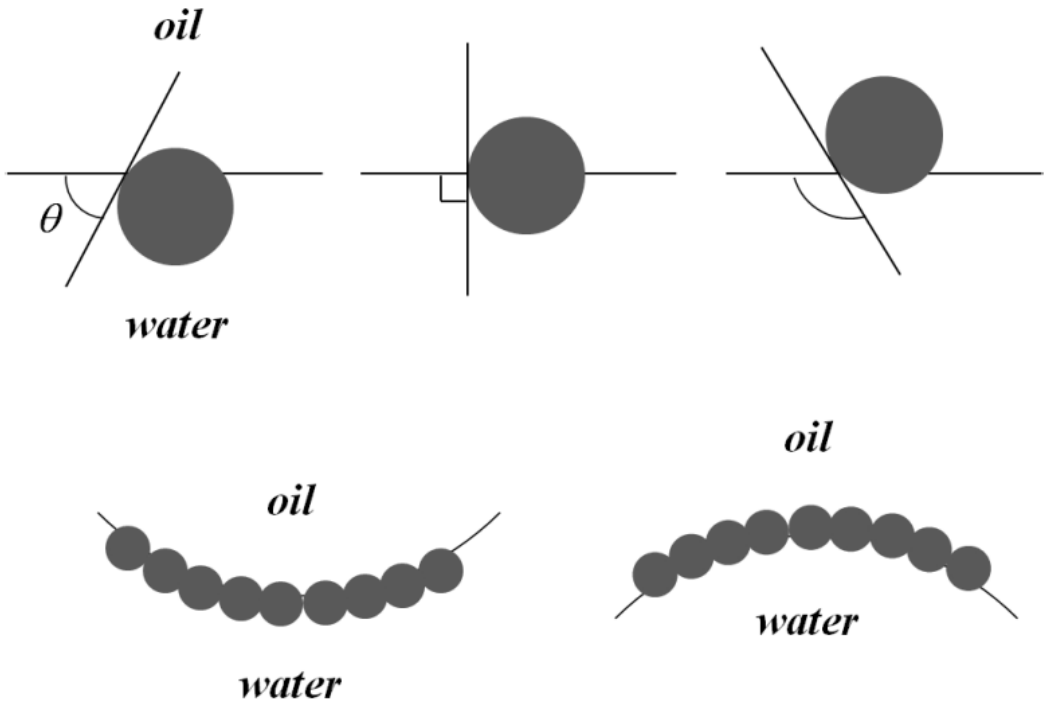


Figure 2- 4: Position of a small spherical particle at oil-water interface depending on their hydrophobicity (*70*).

As mentioned above, Pickering particles strongly absorb at the oil-water interface and are irreversibly trapped at the fluid interface. The amount of energy required to remove particles from interface is much greater than the energy needed for removing a surfactant molecule in "simple" emulsion. Such energy (for particle removal) depends on contact angle interfacial tension and particle size (Equation 2-3) (*71*).

$$E = \pi.r^2.\gamma.(1 \pm \cos\theta) \qquad \textbf{Equation 2-3}$$

Where r is the radius of the particle and γ is the interfacial tension between oil and water.

Based on Equation 2-3, energy required to remove particles is at maximum when the contact angle is close to 90º, thereby particles are equally wetted by both liquids (held at the oil-water interface) (*56, 57*). Moreover, the energy of attachment will drop significantly, if the particle size decreases. Binks (2002) reported that very small particles (<0.5 nm) can be easily detached from the oil-water interface and may not be as effective as larger particles to stabilise emulsions (*70*).

The stabilisation of Pickering emulsions involves two mechanisms: (i) Solid particles adsorb at the oil-water interface and form a packed layer at the interface which protects droplets from coalescence via a steric (mechanical) barrier (Figure 2-5 A) (*58, 70*). It should be noted that the magnitude of this barrier depends on difficulty level of removing particles from oil-water interface (*69*). (ii) Particle bridges are formed in a three dimensional networks in the continuous phase (Figure 2-5 B) (*72, 73*). Horozov and Binks (2006) have also shown that the silica particles could form a dense monolayer bringing which leads to stabilise oil-in-water emulsions (*74*).

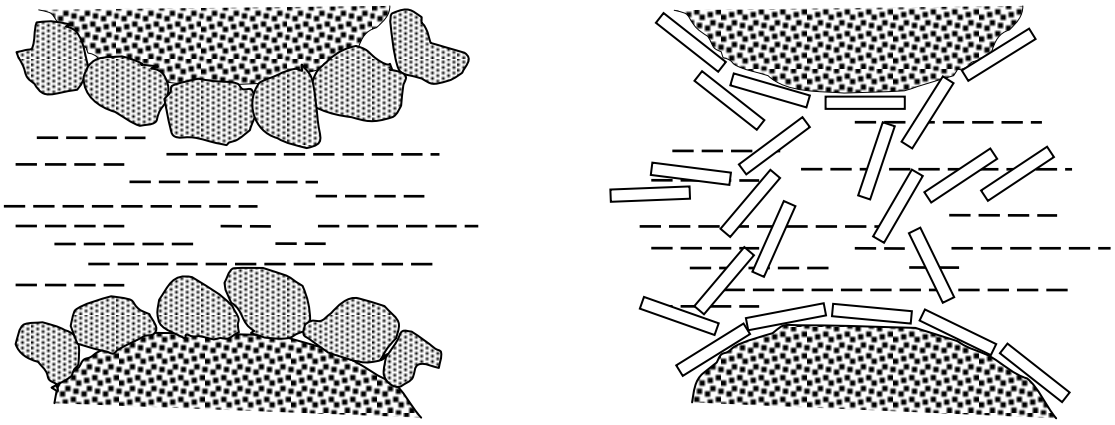


Figure 2- 5: Stabilisation of solid particles at droplet interface (A) Formation of particles layer around droplets (B) Formation of bridging particles (*72*).

Many research groups have shown that using co-stabiliser such as surfactant and/or polymers can improve the stability of Pickering emulsions by: (1) altering the wettability properties (2) modifying the charge on particles surface (3) encouraging flocculation of solid particles in the continuous phase (4) reducing interfacial tension *(75-80).*

Bausch *et al.,* (2002) reported the addition of hydrophilic surfactant with opposite charge to the particles in the aqueous phase enhanced particle adsorption at the surface of oil droplets (*78*). Midmore (1998) also showed that the addition of hydroxypropyl cellulose polymer promoted the silica particles flocculation which led to the formation of very stable oil-in-water emulsions (*79*). It has been demonstrated that the silica particles surfaces became more hydrophobic as non-ionic C12E7 surfactants were added to the aqueous phase (*80*). Furthermore, Pichot *et al*., (2009) reported that emulsions stabilised with both surfactant (monoolein) and hydrophilic silica particles provided long-term stability against coalescence visa "synergistic two part mechanism". The authors argued that, small surfactant molecule absorbed in few seconds at droplet interface (due to its smaller size) and reduced the interfacial tension (induce break up). This provided sufficient time for silica particles to assemble at the interface and form a densely packed layer around the oil droplets which prevented droplets from coalescing (*81*).

So far, there have been numerous studies carried out on the ability of silica particles to stabilise emulsions (*53, 61, 76*). Although, silica particles are able to provide long-term physical stability, they are not "label friendly". Therefore, in order for Pickering emulsions be applicable to the food products, "food-grade" Pickering particles must be used. Starch (including hydrophobically modified starch), Microcrystaline cellulose and wax crystals are some of the examples of food-grade particles available.

Starch has a great nutritional value for human body and it is often used as thickening agent in the food products. It should be noted that "native" or unprocessed starch is not hydrophobic, hence, not suitable to absorb to the oil-water interface. However, by chemical modification of starches, the hydrophobicity can be increased. Thus, modified starch can absorb to the oil-water interface and stabilise an emulsion. Octenyl succinic anhydride (OSA) modification introduces both hydrophilic and hydrophobic groups to starch to make it amphiphilic and surface-active ingredient. OSA starch (Figure 2-6) is an accepted food ingredient and widely used in food and pharmaceutical products due to its emulsion-stabilising properties. Several studies have shown that OSA starch is a surface-active substance and able to produce stable emulsions when sufficient amount of OSA starch is available in the system (*82-86*).

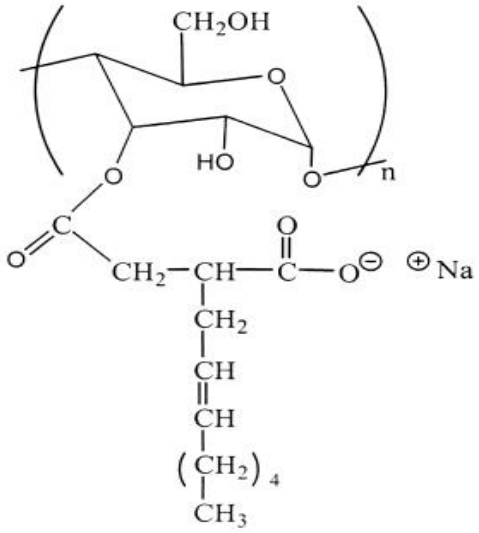


Figure 2- 6: Chemical structure of starch octenyl succinate (OSA).

Microcrystalline cellulose (MCC) is also able to stabilise oil-in-water emulsions. It was proposed MCC particles could stabilise emulsions by locating at oil-water interface and forming three dimensional networks around oil droplets, which increased the viscosity of the

water phase, thereby, preventing close approach of oil droplets and subsequent coalescence (*68, 87, 88*).

2.3.4 Fat Crystals

In many emulsified foods (e.g. chocolate, margarine, etc) fat crystals are used as Pickering particles to stabilise emulsions (*89*). Fat crystals must be located at the droplets interface in order to provide a rigid barrier against coalescence (*90*). Many research groups have shown that fat crystals can also destabilise emulsions when located in the disperse phase. These fat crystals can penetrate to the interfacial layer which results to droplet coalescence and ultimately phase inversion. This process is also known as partial coalescence. Partial coalescence is essential in the production of ice-cream and butter *(91).* In butter, extensive partial coalescence eventually results in phase inversion, whereas in ice cream the aggregated fat droplets form a network around the air cells and within the aqueous phase that provides the mechanical strength needed to produce stable product *(91-9*3). Partial coalescence will be discussed in more details in section 2.4.4.

Fat crystallisation (liquid-solid phase transition) can be divided into two main stages: nucleation and crystal growth. In order to start the crystallisation process, the liquid phase must be cooled at temperatures below its melting point (sub-cooling) (*94, 95*). The driving force for nucleation is the degree of undercooling. Generally, nucleation process can be classified as primary (homogeneous or heterogeneous) and secondary nucleation. Based on nucleation theory, in order to form crystalline lattice from a liquid phase a free energy barrier for formation of solid-liquid surface must be overcome (*96*). It has been reported that the energy needed to form a stable nucleus is influenced by the interfacial tension and the surface area (*97*). During homogeneous nucleation, there is no impurity (i.e. dust particles,

vessel walls) in the system to reduce the activation energy required for nucleation. Thus, the surface energy required for the creation of new surfaces is high (*98*). Conversely, during heterogeneous nucleation, impurities are present to act as nucleation catalysts. Hence, the free energy required for nucleation is decreased as these impurities can provide some of the energy required to overcome the formation of the crystal surface. These can explain why heterogeneous nucleation could be spontaneous and requires low crystallisation driving force (minimal sub cooling) (*99*).

Secondary nucleation is the process where new crystal nuclei form on contact with existing crystals. This is promoted by agitation which leads to collision between a crystal and its neighbours or with the agitator or vessel walls. Consequently, secondary nucleation involves the production of new crystals in a solution containing pre-existing crystals. (*100,101*). Timms (2005) reported that if the crystalline elements (separated from an existing crystal surface) are smaller than the critical size (the minimum size of a crystal that is stable at the existing temperature), they re-dissolve, however, if larger then can act as secondary nuclei. Fine crystals have an enormously increased solubility and require a lot of supercooling to make them crystallise *(102).*

One of the main parameters that can influence the nucleation rate is temperature. It has been demonstrated that at the temperature closer to the melting point (low driving force) the rate of formation of nuclei is almost zero, while if fat is cooled below a certain temperature (high driving force) numerous nuclei will form (*97, 103*).

Following stable nuclei formation, crystals nuclei will continue to grow. Crystal growth occurs via two steps: firstly, molecules diffuse from liquid phase into the crystal surface (growth units). Secondly, they will rearrange to integrate into crystal lattice at

suitable locations (*96, 103*). The crystal growth rate depends on the rate of molecule diffusion and the time needed to integrate with crystal lattice. As mentioned above, the nucleation process needs high driving force (several degree sub-cooling), while crystal growth is achievable with lower driving force (little sub-cooling) (*16, 104*).

During storage or cooling, several crystallisation processes may occur, such as nucleation of new crystals, crystal growth, polymorphic transformation, Ostwald ripening (section 2.4.5) and solid bridging formation (sintering). These processes are known as post-crystallisation. For instance, Cebula and Zieglede (1995) reported that fat bloom chocolate was achieved by the transition of polymorphic form (V) to form (IV) during storage (*105*). During post-crystallisation stage, nucleation and crystal growth may lead to the formation of solid bridging (sintering) which creates tight fat crystal network. The fat crystals network is stabilised by Van der Waals and solid bridging. However, Johansson and Bergenstahl (1995) reported that Van der Waals forces were too weak to produce tight network and argued that adhesion force in the fat dispersion was responsible for the formation of strong crystal networks (*106*).

Fat crystallisation kinetics depends on the processing conditions such as, crystallisation temperature (sub-cooling), cooling rate and shear rate. Thus, the process parameters during crystallisation must be controlled to achieve the desired crystalline properties (*97*).

As sub-cooling increases (crystallisation at temperatures within several degrees of the melting temperature), nucleation rate increases and the induction time for crystallisation is decreased. However, it should be noted that if the crystallisation temperature is too low, the crystal growth will decrease as the molecular mobility is decreased. It has been reported that

crystallisation temperatures can also influence the crystal size and morphology of the system (*101, 107, 108*). Herrera and Hartel (2000) found that when the crystallisation temperature was high, large crystals and a broader crystal size distribution were observed (*109*). Furthermore, Tamime (2007) reported that when anhydrous milk fat was crystallised at 27.5 ºC, only large spherulites shape crystals were found leading to the formation of larger crystals. While the crystallisation temperature was 5 ºC, only granular microstructures with small crystals were observed (*110*).

Cooling rate influences the nucleation rate, crystal structure and crystal size. Campos *et al.*, (2002) observed that the crystallisation rate of samples cooled rapidly was significantly higher than the crystallisation rate of the samples cooled slowly. They argued that the driving force of crystallisation was high for samples cooled rapidly (*111*). Thus, rapid cooling led to the formation of many crystal nuclei in a shorter period of time as lower free energy was needed to be overcome. However, during slow cooling the nucleation rate was low; hence, larger induction time for crystallisation was observed as a result of higher energy barriers of nucleation. This means crystallisation begins at higher temperature for the slow cooling process (*109, 111, 112*).

It has been shown that a slow cooling rate results in the formation of large crystals, whereas at rapid cooling rate, formation of smaller crystals was observed. This was attributed to the short induction time for crystallisation in rapidly cooled cases. When samples were cooled rapidly, there was not enough time for crystals to grow (diffuse and rearrange themselves into clusters), therefore, more nuclei were formed leading to the formation of several small crystals (*21, 90, 109*). Campos *et al.*, (2002) reported that as the system is crystallised rapidly, higher melting fat will be rapidly undercooled and initially crystallised,

developing a solid within the liquid phase. This fast crystallisation is accompanied by a rapid increase in viscosity, thus limiting molecular diffusion and crystal growth (*111*).

It is worth mentioning that cooling occurs more rapidly in the scraped surface heat exchangers than in the traditional batch process (*113*). The structure of the crystal depends on the cooling rates (*109*). Generally, fat can be found in one of 3 different crystalline forms based on processing condition and composition. This phenomenon is called *polymorphism* (*103*) (*114*).

Monoglycerides structure can exist in three polymorphic forms α, β′ and β (*114*). The α-crystal is the least stable and has the smallest size and lowest melting point which rapidly transforms to the β′ form. The β′ has intermediate properties which is more stable than α polymorph but less stable than β polymorph. The β crystal is the most stable and has the highest melting point. It should be noted that distilled monoglycerides do not form β′ polymorph (*94, 97, 104*).

Rapid cooling encourages the formation of unstable crystal polymorphs whereas slow crystallisation can produce more stable crystal structure (*109, 111*). Ojijo *et al*., (2004) reported that rapid cooling leads to the formation of unstable α polymorphs in monoglyceride while slow cooling leads to the formation of the more stable β polymorph. Therefore, when samples cooled slowly they have denser crystal structure. Since α polymorphs has low activation energy for nucleation, several α-form crystals will form in a short period of time (*115*).

Shear rate is another key crystallisation parameter that can influence the crystal polymorph, size and nucleation rate. High shear rate promotes the nucleation process and leads to the formation of small crystals. This is due to the mechanical disturbance which

supplies the energy required for nucleation. As mentioned earlier, high shear mixing also promotes secondary nucleation by detachment of crystallites from crystal structures (*116-118*).

2.4 Emulsion Stability

Emulsion stability is a critical issue for food manufactures. Emulsions can destabilise due to physical mechanisms or chemical processes (*17*). The main processes by which an emulsion may become physically unstable are creaming (*119*), flocculation (*120*), coalescence (*121*), partial coalescence (*122*) and Ostwald ripening (*123*), while the two main processes leading to chemical instability are lipid oxidation (*124*) and biopolymer hydrolysis (*125*).

2.5 Physical Instability

2.5.1 Creaming/ Sedimentation

As a result of density differences between the oil and water phases, creaming and sedimentation can occur due to the movement of emulsion droplets. Creaming occurs in oil-in-water emulsions in which the less dense oil droplets move to the surface, creating a "cream" layer. On the other hand, sedimentation takes place in water-in-oil emulsions where the more dense water droplets sink to the bottom (*126*). Both creaming and sedimentation can be considered as reversible processes since the emulsion can be easily re-dispersed by gentle shaking. The creaming rate of a single, spherical droplet (v_s) is given by Stokes' law (*25*):

$$v_s = \frac{2r^2(\rho_0 - \rho)g}{9\eta_\circ}$$ **Equation 2-4**

Where r is the radius of drop, ρ_0 and ρ are the density of continuous phase and dispersed phase respectively, g is the acceleration due to gravity and $\eta_\circ$ is the Newtonian viscosity of the continuous phase.

Based on Stokes' law the creaming can be inhibited by reducing the drop size (introducing the high energy input in the system e.g high shear mixer), increasing the viscosity of the continuous phase (using a thickening or a gelling agent, e.g. biopolymers xanthan gum) and by reducing the density difference of the two phases (adding a weighting agent to the oil phase e.g. ester gum and sugar) (*127-129*).

2.5.2 Flocculation

Emulsion droplets are in constant motion due to Brownian motion, gravity or mechanical agitation and as a result they may collide with each other (*128*). Flocculation is the process in which the droplets stick to each other without breaking of their surfaces. Although the droplets are attached to each other, they are still separated by a continuous phase film. Flocculation is weak and reversible. Creaming rate can be affected by flocculation since the flocss are able to move faster due to their larger effective radius. Manipulating the collisions frequency between droplets can control the flocculation by increasing the viscosity of the continuous phase (using thickening agents such as, Xanthan gum) (*25, 50, 130-133*).

2.5.3 Coalescence

Once a combination of two or several droplets form a single large droplet coalescence has occurred. Coalescence happens when the thin film between the droplets is ruptured. In oil-in-water emulsions, coalescence eventually leads to formation of a layer of oil on top, which is referred to as "oiling off" (*121, 134-136*). Therefore, the stability of an emulsion depends on the rate of coalescence and the properties of this film. The effective

approach to slow down or prevent coalescence is to add sufficient amount of emulsifier into the system. Sufficient amount of emulsifier can fully cover the oil-water interface, while, for emulsions stabilised with insufficient amount of emulsifier there are gaps in the interfacial membrane. Consequently, if the two gaps on different droplets move close to each other coalescence will occur.

The viscoelasticity of the interfacial layer also has great influence on film rupture. The thicker the membranes around the droplets, the more stable the droplets are. This is because they are less likely to be ruptured and they induce a greater static repulsion between droplets. Furthermore, the presence of charged emulsifier (either positive or negative) at droplets interface provides electrostatic repulsion between two identically charged droplets which decreases the contact between droplets (*18, 35, 38, 136*).

2.5.4 Partial Coalescence

"True" or complete coalescence involves the complete merging of two or more droplets to form a single larger droplet. However, partial coalescence occurs when two partially crystalline droplets come in close contact and join together to form a single irregular aggregate (Figure 2-6). Partial coalescence (clumping) only occurs when solid particles are present in the disperse phase.

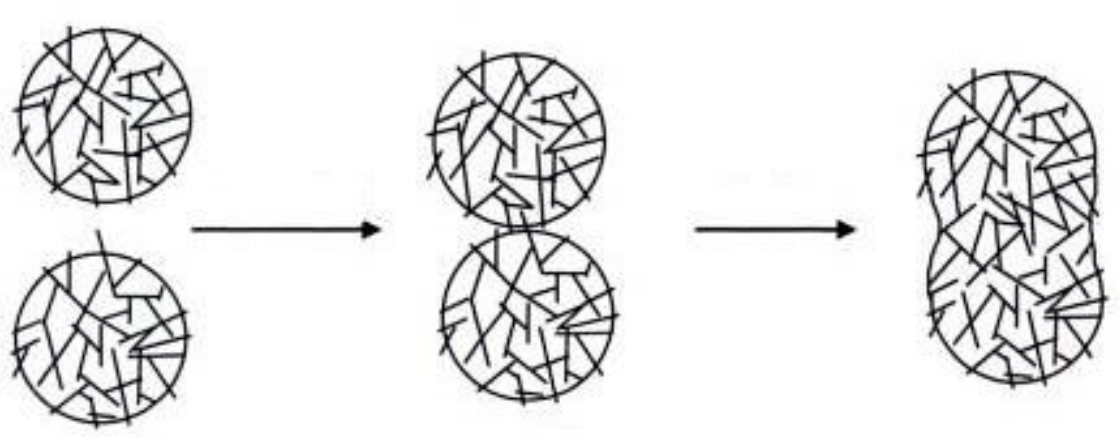


Figure 2- 7: Schematic illustration of partial coalescence phenomena (*25*).

When two partially crystalline droplets collide, a crystal from one of the crystalline may penetrate to the adjacent droplet's interface and protrudes into liquid part of the other droplet. This is how partial coalescence occurs in the oil-in-water emulsions. When the crystal pierces another droplet, it will preferably be wetted by the oil phase rather than by the continuous phase and since this arrangement is thermodynamically favourable the droplets remain aggregated. It is worth mentioning that, partial coalescence is not always an undesirable process. For instance, in ice cream production partial coalescence leads to formation of a network that gives the product a desirable texture (*18, 122, 137, 138*)

There are many factors that can influence partial coalescences such as, shear, fat volume fractions, colloidal repulsion and rheology of continuous phase (*139*).

- Shear: The extent of partial coalescence increases as the collision frequency increases. Therefore, oil-in-water emulsions containing fat crystals are destabilised once shear is applied. This can be achieved when hydrodynamic mechanical forces are present in the system. Shear increases the collision rate and decreases the minimum separation

distance between droplets (increase collision efficiency). The shear force also brings droplets closer together, thus it increases the probability of protruding crystals to pierce the film (*91, 140, 141*).

- Fat volume fractions: Emulsions with a high fat volume fraction are more susceptible to partial coalescence than those with a low fat content. When more crystals are available in the system the possibility of protruding fat crystals piercing adjacent droplet's interface will increase (*131, 137*).
- Colloidal repulsion: Colloidal repulsion strongly stabilises emulsions against partial coalescence. Two droplets would easily come close to each other, when the repulsion is weak, which permits fat crystals to penetrate. The magnitude of this repulsion force between droplets depends on the types of emulsifier used to stabilise the oil-in-water emulsions. It has been reported that droplets stabilised with protein are more stable against partial coalescence than those stabilised with small molecular surfactants. This is mainly due to the fact that proteins induce stronger steric repulsion compared to most small molecular surfactants and often produce more viscoelastic membrane at the oil-in-water interface (*115, 142, 143*).
- Rheology of continuous phase: As the viscosity of the continuous phase increases the collision frequency of the droplets decreases, thus decreasing the rate of partial coalescence. The presence of polysaccharides (Xanthan gum, guar gum and locust bean gum) in the system can be an effective approach to increase the partial coalescence stability by providing a sufficiently thick continuous phase and/or by supplying a protective coating around the globule (*139, 144*).

2.5.5 Ostwald Ripening

Ostwald ripening is the growth of large droplets at the cost of smaller ones. The driving force for this process is the difference in solubility between the small and larger droplets. This is an effect of increased chemical potential in small particles resulting from the high surface curvature and surface/interfacial tension *(123).* With time, the smaller droplets dissolve, and their molecules diffuse in the bulk and become deposited on the larger ones *(123).* These results in a shift of the droplet size distribution to larger value. Oswald ripening should lead to integration of all droplets into a single drop (phase inversion), unless this process is arrested *(131).* Solid particles are able to prevent Ostwald ripening process. Unlike surfactant molecules which are reversibly adsorbed at interface particles are irreversibly attached to the droplets due to the extremely high energy of particle detachment *(70).* There is therefore a huge energy barrier for droplet shrinkage, as this would result in particles being forced off the interface and for this reason Ostwald ripening virtually ceases in Pickering emulsions *(145).*

Ostwald ripening can occur for both oil-in-water and water-in-oil emulsions and it proceeds more rapidly when the size distribution of the droplets becomes wider. Moreover in most food emulsions the mutual solubility of oil and water are so low that Oswald ripening is negligible. However increasing the solubility of dispersed phase in the continuous phase enhances the Ostwald ripening rate. For instance, alcohols are able to increase water solubility of lipids in water which results in higher the Ostwald ripening rate (*123, 145, 146*).

2.6 Chemical Instability

Lipid oxidation and biopolymer hydrolysis are common types of chemical instability. Since the main focus of this work is on lipid oxidation, the biopolymer hydrolysis process is not investigated in this study.

2.7 Lipid

In many food products lipids exist as the primary component due to their source of energy and essential nutrients. Lipid in the form of liquid at room temperature is known as *oil* and it is referred as *fat* at solid state. The main components of food lipids are fatty acids which contain carbon, hydrogen and oxygen atoms. Fatty acids can be classified based on following: (*2, 147*)

- Chain length: Chain length of fatty acids is identified by the number of carbon atoms. Normally, short chain fatty acids consist of fewer than 8 carbons, whereas, medium chain fatty acids are formed of 8 to 12 carbons and long-chain fatty acids have more than 12 carbons) (*147, 148*).
- Carbon bond: Fatty acids containing only single carbon bonds are known as *saturated fatty acids* (SFA). However, fatty acids with one double bond are monounsaturated fatty acids (MUFAS) and those with more than one double bonds are polyunsaturated fatty acids (PUFAs) (*16, 89*).
- Essential and nonessential fatty acids: The human body is able to produce most of the various types of fatty acids. For instance, the body synthesises oleic acid by removing hydrogens from saturated stearic acid. These types of fatty acids synthesise by human body are known as *nonessential fatty acids*. On the other hand, *essential fatty acids* (EFA) are polyunsaturated fatty acids that cannot be produced by human

body, thus must be provided by diet. Omega-3 (ω-3) (alpha-linolenic acid (ALA)) and omega-6 (ω-6) (linoleic acid) are two types of EFA (Figure 2-8). Within the body, both can be converted to other PUFAs such as arachidonic acid, or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (*149*, *150*).

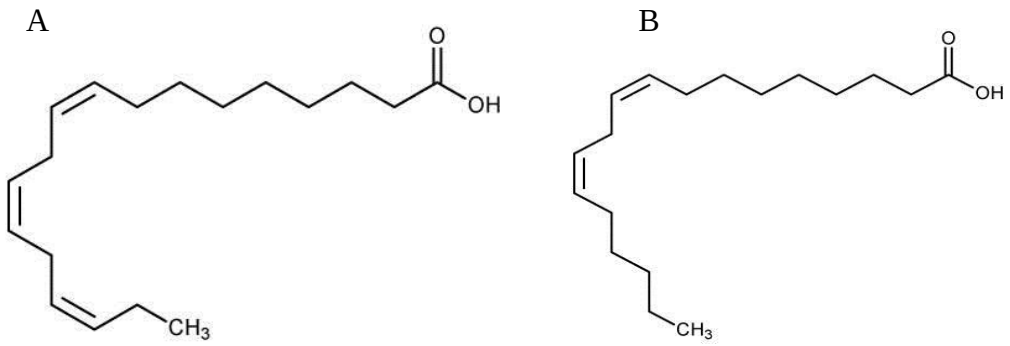


Figure 2- 8: Chemical structure of (A): omega-3 (ω-3) (alpha-linolenic acid (ALA)) and (B) omega-6 (ω-6) (linoleic acid).

EFAs have high nutritional value and various health benefits linked to the development of eye and brain function in infants (*151*), treatment of rheumatoid arthritis (*152*), coronary artery disease (*153*), hypertension (*154*), diabetes and cancer (*3*).

2.8 Mechanisms of Lipid Oxidation

As mentioned above, omega fatty acids have several health benefits. Hence, food manufacturers are interested in adding these ingredients to their products. However, addition of these ingredients to foods is not a trivial task as some of them can easily undergo lipid oxidation. This results in food products that become unstable and lose their appearance, taste,

texture, shelf-life, and nutritional profile (*5, 155*). Lipid oxidation, therefore, is a serious matter for food manufacturers.

Lipid oxidation can normally occur through different mechanism such as, auto-oxidation, photo-oxidation, thermal oxidation and enzymatic oxidation. Auto-oxidation is the most common process when it comes to food products which happens through a free radical chain reaction involving three following steps (*156*):

$$RH \xrightarrow{\text{Initiator}} R^{\circ} + H^{\circ}$$ **Equation 2-5**

$$R^{\circ} + O_2 \longrightarrow ROO^{\circ}$$
$$ROO^{\circ} + RH \longrightarrow ROOH + R^{\circ}$$ **Equation 2-6**

$$R^{\circ} + R^{\circ} \longrightarrow RR$$
$$ROO^{\circ} + R^{\circ} \longrightarrow ROOR$$ **Equation 2-7**

- Initiation (formation of free radicals): In first stage, free radicals (R°) known as the alkyl radical are formed when hydrogen atom is removed from unsaturated lipids (RH) in the presence of initiators such as, transition metals (Equation 2-5).
- Propagation (free-radical chain reaction): at this stage, alkyl radical reacts rapidly with the oxygen to form peroxyl radicals (ROO°), which can abstract a hydrogen atom from another adjacent unsaturated fatty acid, leading to a lipid hydroperoxide (ROOH) and other radicals (Equation 2-6).
- Termination (formation of non-radical species): Termination is the final step of autoxidation where free radicals begin to react with each other to form a more stable, non-radical product (Equation 2-7).

Although hydroperoxide is the primary product of lipid oxidation, it is not the main cause of off-flavour and rancid smell of food products. The lipid hydroperoxides decompose via β-scission reaction to alkoxyl radicals. Alkoxyl radicals then tend to decompose into a wide variety of volatile, low molecules weight components (known as secondary lipid oxidation products), such as aldehydes, ketones, alcohols, and hydrocarbons. These secondary products are responsible for off-odour and off-flavour (*5, 9, 16, 124, 157*).

2.8.1 Lipid Oxidation in Oil-in-Water Emulsion

In oil-in-water emulsions lipid droplets are dispersed in an aqueous phase. Many studies have shown that the transition metals (pro-oxidants) such as, iron or copper are mostly located in the aqueous phase, whereas, hydroperoxide can be found at oil-water interface due to the fact that hydroperoxide are surface-active compounds. Iron is one of the major pro-oxidants which can be found in water, certain food ingredients and packaging materials (*158-160*). Consequently, the lipid oxidation takes place at the droplet interface, where the pro-oxidant in the continuous phase comes in close contact with the hydroperoxide located at the droplet surface to breakdown the lipid hydroperoxide into free highly reactive radicals such as alkoxyl and peroxyl radicals (Figure 2-5) (*8*).

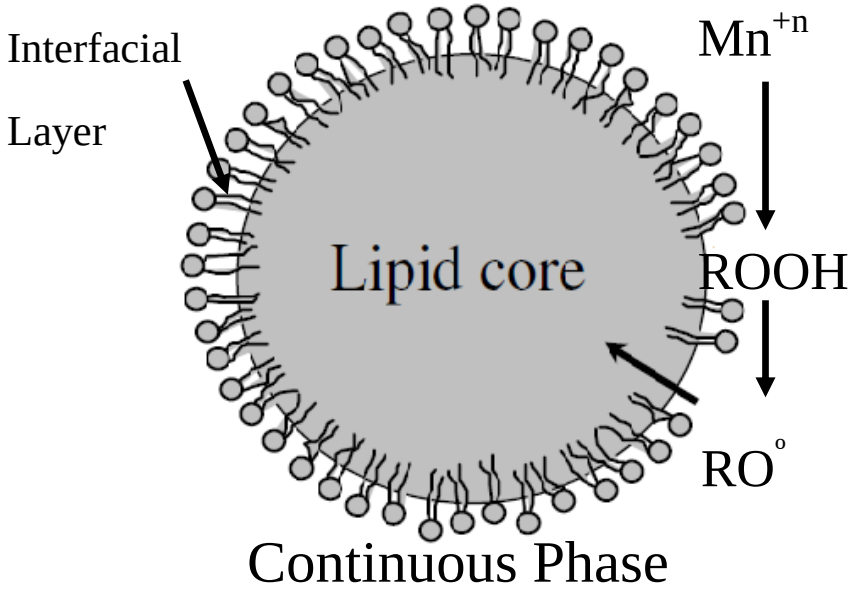


Figure 2- 9: Schematic representation of lipid oxidation in oil-in-water emulsions. Mn+ is pro-oxidants in the continuous phase, ROOH is hydroperoxides at droplets interface and RO° is an alkoxyl radical (*161*).

The interaction of lipid hydroperoxides (ROOH) with iron (Equation 2-8 and Equation 2-9) can produce highly reactive peroxyl (ROO°) and alkoxyl (RO°) radicals which are able to react with another unsaturated lipid or promote β-scission reactions. Subsequently, the presence of pro-oxidants in the continuous phase is a major factor in accelerating lipid oxidation (*6, 162*). Griotti (1998) reported that, the Fe^{2+} ions were more reactive than Fe^{3+} ions and decomposed hydrogen peroxide over 100 times faster (163).

$$Fe^{3+} + ROOH \longrightarrow Fe^{2+} + ROO^{\circ} + H^{+} \qquad \textbf{Equation 2-8}$$

$$Fe^{2+} + ROOH \longrightarrow Fe^{3+} + RO^{\circ} + OH^{-} \qquad \textbf{Equation 2-9}$$

2.9 Lipid Oxidation Measurements Techniques

There are numerous techniques available for assessing the oxidative stability of a sample of lipid. According to Shahidi and Zhong (2005) these techniques can be roughly divided into five main categories depending on what they measure: the absorption of oxygen,

the loss of initial substrates, the formation of free radicals and formation of primary and secondary lipid oxidation products (*156*). Table 2-1 shows a list of chemical tests or instrumental analyses that have been developed to monitor/detect the lipid oxidation parameters (*156, 164, 165*).

As aforementioned, lipid oxidation is a complex process due to the continuous formation of hydroperoxides (primary products) which can easily decompose to a range of volatile and non-volatile components (secondary products). Therefore, in this study two methods were used to determine both primary and secondary lipid oxidation products.

Table 2- 1: Method used to monitor oxidative stability of a sample

What is measured?	Techniques
Absorption of oxygen	Oxygen electrode (Oxidograph)
loss of initial substrates	Gas Chromatography
Primary Lipid oxidation Product	Spectrometric Assay Fourier transform infrared (FTIR)
Secondary Lipid oxidation products	Spectrometric Assay Gas Chromatography High Pressure Performance Liquid Chromatography (HPLC)
Free-radical type and concentration	Electron Spin Resonance (ESR) Spectrometric

2.9.1 Primary Lipid Oxidation Product

The most common method used to measure the hydroperoxide formation in the food emulsion is *peroxide value* (PV). A number of measurement techniques have been established to determine PV such as, idometric titration and ferric thiocyanate methods.

For measuring PV value based on idometric titration method, potassium iodide (KI) is used to react with hydroperoxides (Equation 2-10). The concentration of the peroxide in the system is related to the amount of released iodine (I_2), which is determined titrimetrically

with sodium thiosulphate ($Na_2S_2O_3$), using a starch indicator (Equation 2-11). In this method PV is stated as milliequivalents of oxygen per kilogram of sample (meq/kg). This method has several disadvantages such as, time consuming, requires a large amount of sample and lack of sensitivity (*156, 164, 166*).

$$ROOH + KI \rightarrow ROH + KOH + I_2$$ **Equation 2-10**

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$ **Equation 2-11**

The ferric thiocyanate method is an alternative approach to determine peroxide values. This method is based on the oxidation of ferrous iron (Fe^{2+}) to ferric ions (Fe^{3+}) when is in contact with lipid hydroperoxides, which is determined colorimetrically as ferric thiocyanate. Initially, hydroperoxide react with Fe^{2+} to produce Fe^{3+} (Equation 2-12 and Equation 2-13). Ferric ions then react with thiocyanate (as a reactant) to form ferric thiocyanate (red-violate complex) which can be determined spectrometrically (Equation 2-14). This method requires small amounts of sample, is more sensitive and less time consuming than idometric titration method. Consequently, throughout this work ferric thiocyanate method was used to evaluate peroxide values (*9, 27, 167*).

$$Fe^{2+} + ROOH \longrightarrow Fe^{3+} + ROO^{\circ} + H^{-}$$ **Equation 2-12**

$$RO^{\circ} + Fe^{2+} + H^{+} \rightarrow ROH + Fe^{3+}$$ **Equation 2-13**

$$Fe^{3+} + [SCN]^{-} \longrightarrow Fe(SCN)$$ **Equation 2-14**

Red-violet complex

2.9.2 Secondary Lipid Oxidation Products

As mentioned in section 2.5.2, carbonyl compounds such as aldehydes, are one of the main secondary lipid oxidation products. *p*-Anisidine value (*p*-AnV) test can be used to determine the content of α and β- unsaturated aldehydes (mainly 2-alkenals and 2,4-alkadientals) produced during the decomposition of hydroperoxides during oxidation. This method is based on the reaction of aldehydes with *p*-anisidine reagent (in the presence of acetic acid) to form yellowish products that can be measured spectrometrically (Figure 2-6) (*10*, *167*).

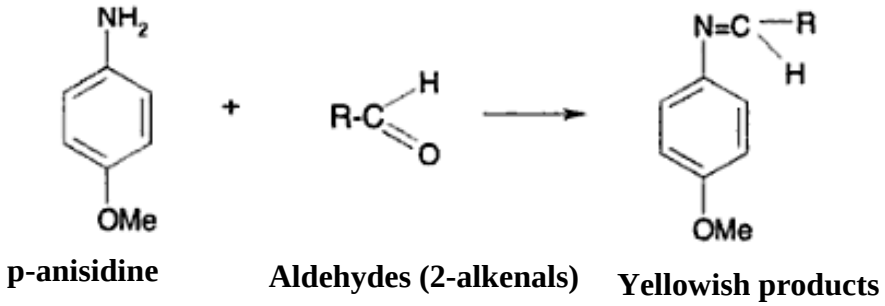


Figure 2- 10: Reaction of p-anisidine with aldehydes (*156*).

The 2-thiobarbituric acid (TBA) assay is alternative method to determine degree of secondary oxidation products. During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids is formed as a result of the degradation of polyunsaturated fatty acids *(151).* In this assay, one mole of MA is reacted with two moles of thiobarbituric acid (TBA) to form a pink MA-TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm *(156).* Many other substances may react with the TBA reagent and contribute to absorption, causing an overestimation of the intensity of colour complex. Furthermore, the presence of barbituric acid impurities in the TBA reagent may produce TBA-MA-barbituric acid and MA-barbituric acid adducts that absorb at 513 nm and

490 nm, respectively, indicating that thiobarbituric acid should be purified before use. Therefore, due to the limitations of TBA method, *p*-anisidine method was used to determine the secondary lipid oxidation products in this study (*156*).

2.10 Factors influencing oxidative stability

There are various factors that can influence the rate of lipid oxidation in emulsion, including the molecular structure of lipids, heat, light, the physical characteristics of emulsion droplets and the presence of antioxidants or pro-oxidants (transition metals) in the system.

2.10.1 Type of Lipid

The rate of lipid oxidation is influenced by the type of lipid (saturated or unsaturated), number and location of the double bonds. Only unsaturated lipids are susceptible to lipid oxidation (*5*). Therefore simplest way to inhibit lipid oxidation in food products is to use saturated lipids instead of unsaturated lipids. However, this is not a practical approach as saturated lipids have been associated with increased risk of heart disease, stroke, and even cancer (*168*). Therefore, food manufacturers must use unsaturated lipids in their products and find alternative techniques to prevent lipid oxidation. Additionally, lipid oxidation is promoted as the number of double bonds increases, thus, long chain polyunsaturated fatty acids are more prone to the oxidation (*169*). Nawar (1996) also reported that double bonds in the *cis* configuration oxidised faster than their *trans* isomers (*124*).

2.10.2 Temperature

Temperature is another parameter that can accelerate lipid oxidation rate. In general, the auto-oxidation rate increases as the temperature is increased. It has been established that every 10 ºC of temperature increase would approximately double the rate of oil oxidation (*170*) (*171*).

2.10.3 Pro-oxidants

The presence of pro-oxidants such as, transition metals[1] in the continuous phase of oil-in-water emulsions has been shown to be a key factor in the promotion of lipid oxidation. As mentioned in section 2.8, transition metals are able to breakdown lipid hydroperoxide into free highly reactive radicals such as alkoxyl and peroxyl radicals (*172*). Trace amounts of transition metals such as, iron and copper can be found in edible oils (edible oils may also be contaminated with the metals during the production process and contact with storage materials), meet foods, water and packaging materials (*5, 172*). It has been reported that even small amounts of copper (0.01 to 0.03 ppm) and iron (0.03 ppm) were sufficient to lower the oxidative stability of soy bean oil (*173*). Therefore, one effective approach to increase oxidative stability is to reduce the concentration of these metals in the system. This could be accomplished by purchasing high-purity ingredients (removing metal traces from oil during alkali refining) or using stainless steel vessels for the transportation and storage of raw materials to avoid the transition of metal ions from the vessel into the ingredients. However, these techniques are not economically practical for food manufactures. Thus, the most feasible method to reduce the amount of transition metals in the system is to use antioxidants (chelating agents) (*5*).

[1] The term transition metal refers to elements in the d-block of the periodic table which include groups 3 to 12 on the periodic table.

2.10.4 Antioxidants

The common approach to lower down the rate of lipid oxidation is to incorporate either synthesis or natural antioxidants into the food products. According to Reische *et al.,* (1998) antioxidants can be classified as either primary or secondary antioxidants based on their mechanism of action (*174*).

***Primary Antioxidants*:** primary antioxidants or "chain breaking" antioxidants can hinder lipid oxidation by delaying the initiation step or interfering with the propagation step. Primary antioxidants are able to react with lipid radicals (peroxyl and alkoxyl radicals) by rapidly donating a hydrogen atom to a lipid radical and subsequently converting them to more stable radicals. Thus, the mechanism of primary antioxidants is based on scavenging free radicals.

The effectiveness of these antioxidants is dependent on their physical location in the system and their chemical properties. Commonly, antioxidants can be physically classified into hydrophilic (polar) and lipophillic (non-polar) group. According to the "polar paradox" theory polar antioxidants are more effective in bulk oils as they accumulate at the air-oil interface (where lipid oxidation is most dominant). However, non-polar antioxidants are more effective in emulsion because they concentrate on the oil-water interface where interactions between hydroperoxides at the droplet surface and pro-oxidants originating in the aqueous phase take place. Thus, based on this phenomenon, antioxidants that are effective at hindering lipid oxidation in emulsified oils may not be as effective in the bulk oil (*175, 176*). It has been shown that non-polar α-tocopherol and propyl gallate were more efficient antioxidants in an oil-in-water emulsion compared to bulk oil, whereas polar Trolox and gallic acid showed the opposite behaviour (*177, 178*).

The most efficient free radical scavengers which can enhance oxidative stability are compounds that contain hydroxyl (phenolics), sulfhydryl (cysteine) and amino groups (proteins) (*11*). A number of studies suggest that the natural sources (tochopherols (Vitamin E, C), caffeic acid and ferulic acid) or synthetic phenolic antioxidants (propyl gallate (PG), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)) are able to reduce lipid oxidation because of their ability to donate hydrogen and form stable phenolic free radicals (*11, 171, 179-181*).

So far, a great deal of research has been carried out on the ability of continuous phase proteins in oil-in-water emulsions to inhibit lipid oxidation by scavenging free radicals through their sulfhydryl and/or amino acid residues (*8, 182, 183*). Tong *et al.,* (*2000*) reported that whey protein sulfhydryls reduced lipid oxidation in salmon oil-in-water emulsions stabilised with Tween 20 (*184*). Faraji *et al.,* (2004) also found that the sulfhydryl groups of soy protein isolate could act as free radical scavengers, thereby inhibited lipid oxidation rate (*185*). It has been reported that the antioxidant activity of continuous protein can be increased by thermal processing which exposes sulfhydryls residues hidden in the protein (*186, 187*).

While, all 20 amino acids found in protein can be involved in free radical scavenging, histidine, tryptophan, cysteine and tyrosine seem to be the most effective free radical scavengers (*11, 188*). Elias *et al*., (2005) studied the oxidative stability of cysteine, tryptophan and methionine residues in continuous phase protein (β-lactoglobulin) and argued that tryptophan and cysteine could enhance the oxidative stability in greater extent than methionine residues. They argued that methionine residues were inaccessible to aqueous phase pro-oxidants as buried within protein core, hence less effective than cysteine, tryptophan residues (*189*).

Secondary Antioxidant: Chelators are secondary antioxidants components that can inhibit lipid oxidation by various mechanisms including prevention of metal redox cycling, formation of insoluble metal complexes and steric hindrance of metal-lipid interactions. It should be noted that none of these mechanisms convert free radicals to more stable products (*5, 171*).

One potential approach to enhance the lipid oxidation stability is to inhibit pro-oxidants ability by using metal chelators to alter physical location of pro-oxidant in the continuous phase (prevent pro-oxidant from the close contact with the droplet surface). The most common food chelators are phosphoric acid and ethylenediaminetetraacetic acid (EDTA) (*190*). EDTA is capable of reducing lipid oxidation rate by removing the transition metals from the surface of oil droplets (*191*). It has been demonstrated that EDTA reduces the rate of lipid oxidation in corn oil-in-water emulsions at both pH 3 and 7 conditions (*192*). It should be noted that the concentration of EDTA in the system plays an important role on its antioxidant performance. Mahoney and Graf (1986) found that EDTA can behave as a pro-oxidants when EDTA:iron ratio of ≤ 1, in contrast, at an EDTA:iron ratio of ≥ 1, can act as effective as metal chelator (*193*).

The demand for using 'natural' antioxidants has increased due to the potential hazardous effects of synthetic antioxidants (*8*). The chelating ability of different types of protein (whey protein isolate, soy protein isolate and sodium caseinate) was investigated by Faraji *et al.*, (2004) (*185*). The authors showed that sodium caseinate had the greatest iron-binding ability due to its phosphorylated serine residues and whey protein isolate was less effective at chelating iron (*185, 194, 195*). In addition to proteins, polysaccharides have also been found to enhance the oxidative stability through chelating pro-oxidants and increasing

the viscosity of continuous phase (*196*). For instance, xanthan gum was found to chelate metal ions through its negatively charged pyruvate sites (*197, 198*).

2.10.5 Interfacial Characteristics

As mentioned in section 2.9, lipid oxidation takes place at the droplet interface where surface-active hydroperoxides and water soluble pro-oxidants interact with each other. Consequently, the properties of droplets interface can play crucial role on oxidative stability. The interfacial characteristics such as charge and thickness can influence iron's ability and thereby alter lipid oxidation rate.

Droplet Interfacial Charge: A number of studies suggest that the rate of lipid oxidation in oil-in-water emulsions can be controlled by the charge of the droplets interface (*199-202*). The attractive or repulsive forces between emulsion's droplets and positively charged pro-oxidants depend on surface charge of droplets. The type of emulsifier used to stabilise an emulsion and/or the pH of the system can influence the surface charge of droplets. Mancuso *et al.,* (1999) observed that the lipid oxidation rate of salmon oil-in-water emulsions was the highest for negatively charged droplets (stabilised with sodium dodecyl sulphate (SDS)), intermediate for uncharged droplets (stabilised with Tween 20), and the lowest for positively charged droplets (stabilised with dodecyl trimethyl ammonium bromide (DTAB)) (*191*). These results indicated that negatively charged droplet interfaces were electrostatically attracted to positively charged pro-oxidant (iron), hence, pro-oxidants were in closer proximity to hydroperoxides and subsequently oxidation was promoted.

In case of protein stabilised emulsions, pH of the system plays significant role to hinder lipid oxidation. The oxidation stability increased when proteins carried positively charged around droplets (pH<pI), whereas, oxidative stability reduced when a net anionic

charge was established around droplets (pH>pI) (*162*). Hu *et al*., (2003) showed that the rate of lipid oxidation in salmon oil-in-water emulsions stabilised with either whey protein isolate (WPI), sweet whey, β-lactoglobulin or α-lactoglobulin was slower at pH 3 (proteins have positive charge) than pH 7 (negative charge) and argued that this was due to the repulsive forces at pH 3 between the positively charged droplets and the positively charged pro-oxidants in the continuous phase (*203*).

Droplet Interfacial Thickness: Another effective approach that can decrease the lipid oxidation rate in emulsion is to form thick layer around oil droplets. The thick layer at the droplet interface can act as a physical barrier that separates lipid substrates from pro-oxidants in the aqueous phase, thereby, inhibiting pro-oxidants-hydroperoxides interactions (*5, 16*). The interfacial thickness on oil droplets is dependent on the size of the emulsifier structure (emulsifier head and tail group). For instance, Silvestre *et al*., (2000) compared the oxidation rate in salmon oil-in-water emulsions stabilised with Brij 76 or Brij 700. They reported that the lipid oxidation rate of emulsions stabilised with Brij 700 was slower than those stabilised by Brij 76, and argued that this was due to the fact that Brij 700 produced a thicker interfacial layer as a consequence of its larger head group (10 times larger Brij 76) (*204*). Moreover, increasing size of hydrophobic tail group of surfactant can also enhance oxidative stability. Chaiyasit *et al.,* (2000) found that lipid oxidation rate was slower for emulsions stabilised with Brij-stearyl (C18 tail group) than those stabilised with Brij-lauryl (C12 tail group) (*205*). In addition to small molecule surfactants, proteins can also build thick layer around oil droplets (*206, 207*).

2.10.6 Surfactant Micelles

As mentioned in section 2.3.1, surfactants can form micelles when the concentration of surfactant exceeds its CMC. So far, a great deal of research has been carried out on the ability of surfactant micelles in the continuous phase to inhibit the rate of lipid oxidation by solubilising pro-oxidants and/or hydroperoxides. It should be noted that the surfactant micelles have insignificant free radical scavenging activity; therefore, their ability to inhibit lipid oxidation is not due to their antioxidant activity, nevertheless it is through altering the physical location of pro-oxidant transition metals (*6, 16, 208*). Cho *et al*., (2002) found that Brij micelles could hinder lipid oxidation through their ability to solubilise pro-oxidant (iron) and promote movement of iron out of oil droplets (*209*). In addition, surfactant micelles are able to solubilise lipid hydroperoxides and remove it from oil droplets (*210*).

2.11 Controlling Lipid Oxidation by Microstructure Engineering

Recently, researchers have shown that a droplet interfacial engineering technology, based on electrostatic deposition of charged biopolymers on the surfaces of charged droplets, can be used to control lipid oxidation. This technique is known as "layer-by-layer electrostatic deposition". In order to create multilayer emulsions, initially, an ionic emulsifier should quickly migrate to the surface of lipid droplets during emulsification process (known as "primary emulsion"). Then an oppositely charged polymer is added to the system that absorbs to the droplets surfaces to produce "secondary emulsions" (containing droplets coated with a two-layer interfacial membrane) (Figure 2-7). These steps can be repeated to form oil droplets coated by interfacial membranes containing three or more layers (*211-213*).

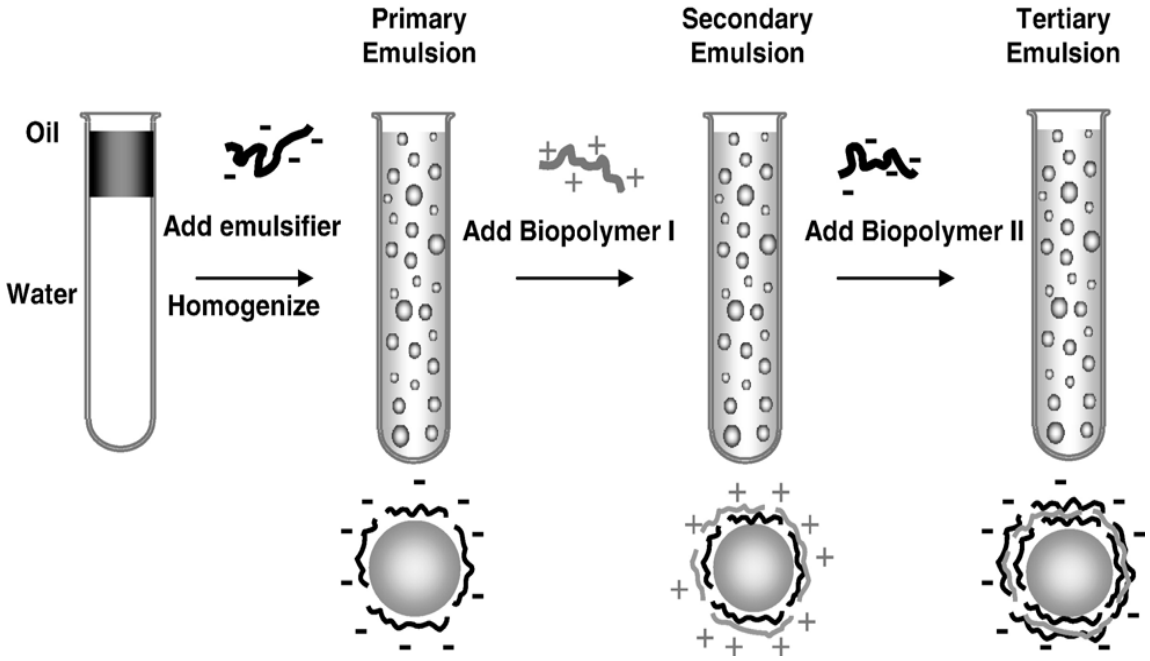


Figure 2- 11: "Layered-by-Layered Electrostatic Deposition" mechanism for producing multilayer emulsions (*13*)

A number of studies suggest that the oxidative stability of oil-in-water emulsion can be increased by using an electrostatic layer-by-layer deposition technique due to the formation of a cationic surface charge and/or thick interfacial layer around oil droplets (*214-216*). Klinkesorn *et al.*, (2005) reported that the lipid oxidation rate was slower for tuna oil-in-water emulsions coated by lecithin and chitosan than emulsions stabilised by lecithin alone. They argued that this was due to the formation of cationic droplets (repelling pro-oxidants) and thicker interfacial layer (separating pro-oxidants from hydroperoxides) (*12*). It is worth mentioning that this technique has some limitations, mainly because of the high tendency for the droplets to aggregate and flocculate when preparing samples. Furthermore, the use of different types of emulsifiers in order to create multilayer membranes around oil droplets increases the total cost of the product (*13*).

Chapter 3:Experimental

This chapter has been divided into three main sections: Materials, Emulsion Preparation and Equipment. The first section, “Materials”, includes a description of all the chemical compounds used throughout this work. The second section, “Emulsion Preparation”, consists of the procedures followed to prepare each sample. Finally, in the last section, “Equipment”, all equipment employed to characterise samples is described in details. The content of this chapter is organised according to the order in which the results are presented in following chapters.

3.1 Materials

The concentrations of all materials used in this study were calculated as the percentage of weight of a component per weight of the final product (wt/wt %). However, for simplification the “%” symbol without any other designation means weight per weight percent, unless stated otherwise. All materials were used with no further purification or modification of their properties.

3.1.1 Dispersed Phase

The oil used in this work was commercially available sunflower oil and purchased from local supermarket. The same brand and type of oil was used throughout the study. Density and refractive index of sunflower oil are 918.8 kg/m^3 and 1.46 at 25 ºC respectively. Sunflower oil contains mostly triacylglycerols (98–99%), and a small fraction of

phospholipids, tocopherols, sterols, and waxes. Table 3-1 shows the fatty acid composition of regular sunflower oils (*169, 217*).

Table 3- 1: Fatty acid composition of regular sunflower oil

Fatty Acids	**Fatty acid composition of regular sunflower oil (%)**
Palmitic acid (C-16:0)	5-8
Palmitoleic Acid (C-16:1)	<1
Stearic Acid (C-18:0)	1.5-7
Oleic Acid (C-18:1)	14-40
Linoleic Acid (C-18:2)	48-74
Arachidonic Acid (C-20:0)	<0.2
Other	1

3.1.2 Continuous Phase

Double distilled water was used for the preparation of all samples in this study. Density and refractive index of double distilled water are 998.23 kg/m^3 and 1.33.

3.1.3 Emulsifier

Different types of emulsifier such as, small molecular surfactant (Tween 20) and protein (sodium caseinate (CAS)) were used as emulsifier agents in this study to stabilise oil-in-water emulsions. Tween 20 (polyoxyethylene (20) sorbitan monolaurate, CAS number 9005-64-5, HLB 16.7) was supplied by Sigma Aldrich (UK). Spray-dried sodium caseinate (EM 7, milk protein type, CAS number 9005-46-3, HLB 14) was kindly provided by DMV International.

3.1.4 Thickening Agents

Xanthan gum (XG, from *Xanthomonas campestris*, CAS number 11138-66-2) was obtained from Sigma Aldrich (UK). This product occurs in dry, white powder which is soluble in cold and hot water, although intensive agitation was needed to prevent the formation of lumps. In this study XG was used as a thickening agent to increase the viscosity of the continuous phase.

Xanthan gum is a high molecular weight anionic polysaccharide derived from the microorganism *Xanthomonas campestris* (an organism originally isolated from the rutabaga plant). Xantham Gum is being extensively used in wide range of industries such as, food, cosmetics, pharmaceuticals and home care due to its unique rheological properties.

3.1.5 Pickering Particles

A range of Pickering particles were used in this study as the solo-emulsifier and or mixed-emulsifier system. The properties of each particle will be discussed in more detail in the following sections.

3.1.5.1 Silica Particles

Hydrophilic fumed silica particles (Aerosil ® 200, CAS number 112 945-52-57631-86-9) were kindly provided by Degussa (UK). The particles were in the white dry powder form. The specifications of silica particles were provided by the manufacturer and can be seen in Table 3.2. These hydrophilic particles are used in a wide range of industries such as, paint, food, cosmetic and agricultural.

Table 3- 2: The specification of Silica particles provided by the Degussa (UK).

Properties	Unit	Typical Value
Specific surface area	m^2/g	200±25
Average primary particle size	nm	12
pH (in 4% dispersion)	------	3.5-4.7
SiO_2-Content	Wt.%	≥ 99.8

3.1.5.2 Colloidal Microcrystalline Cellulose (MCC)

Colloidal microcrystalline cellulose (MCC, Sodium Carboxymethyl Cellulose content 10 - 20%, CAS number 9004-34-6, pH 6-8, ρ=600 kg/m^3) was purchased from Sigma Aldrich (UK). The MCC particles were provided in the white, dry powder.

Cellulose is a linear polymer of β-D-glucose which is linked by β (1-4) glycosidic bonds. Cellulose is the major structural material of plants. The hydroxyl groups of cellulose can partially or completely react with various reagents to produce derivatives with different properties *(218).* Microcrystalline cellulose is driven from cellulose which has been partially de-polymerised via hydrolysis in hydrochloric acid. Microcrystalline cellulose was supplied as a white and odourless powder which is insoluble in water and used mainly in the pharmaceutical and food industries. Sodium carboxymethyl cellulose is also produced by treating cellulose with sodium hydroxide and sodium monochloroacetate. Sodium-carboxymethyl cellulose (CMC) is an anionic cellulose derivative *(219).*

The Colloidal Microcrystalline Cellulose (MCC) which was used for this study is a mixture of powdered microcrystalline cellulose and sodium carboxymethylcellulose. MCC is

an anionic polymer which mainly used as thickening agent, ice and sugar crystal growth controller, emulsifier agent, non-adhesive binder, etc. (*218, 219*).

3.1.5.3 Chemically Modified Food Starch (MS)

Chemically modified food Starch refined from waxy maize (Octenyl succinic anhydride (OSA), N-creamer 46, E1450, CAS 66829-29-6, pH 4, ρ=580 kg/m^3) was supplied by National Starch Food Innovation (UK). This product is available in fine, white powder which is dispersed in cold water.

Starch molecules are composed of a combination of D-glucose polymers consisting of both amylose (linear) and amylopectin (branched) structure *(85).* Starch could be modified in order to perform further functions. Since the original starch is not suitable to absorb to the oil-water interfaces as they are not naturally hydrophobic, their hydrophobicity can be increased by chemical modifications. When modified starch remain intact and is predominantly wetted by the aqueous phase they could stabilise o/w emulsion via Pickering mechanism. There are several methods available to modify starch. In this study, the waxy maize starch has been modified with alkenyl succinic anhydrides in order to increase its hydrophobic nature. As such, this modified starch (octenyl succinate anhydrides) possesses certain surface-active properties which allow it to be used as an emulsifier/stabiliser for oil-in-water emulsions. N-CREAMER 46 finds application in fat-containing, spray-dried powders, such as coffee creamers, beverage emulsions and salad dressings.

3.1.6 Saturated Monoglycerides (MG)

Dimodan HP saturated monoglycerides (90% monoglyceride, CAS number 97593-29-8, HLB value 3-6) was obtained from Danisco, UK. Dimodan HP is a distilled monogylceride made from edible, fully hydrogenated palm based oil which has a wide range

of applications in food industry mainly in margarine, coffee whiteners, bread and whipping gels.

3.1.7 Reagents

Ferrous sulphate (Iron (II) sulfate heptahydrate for analysis 99.5%, CAS number 7782-63-0), hydrochloric acid (CAS number 7647-01-0) and ammonium thiocyanate (99%, CAS number 1762-95-4) were all provided from Fisher Scientific (UK) in order to measure lipid oxidation rate. Cumene hydroperoxide (α,α-Dimethylbenzyl hydroperoxide 80%, CAS Number 80-15-9), para anisidine (p-Anisidine 99%, CAS number 104-94-9), 2,4-decadienal (85% CAS number 25152-84-5) and barium chloride dehydrate (≥99%, CAS Number 10326-27-9) were supplied by Sigma-Aldrich (UK) and used as reagents for measuring lipid oxidation.

3.1.8 Solvent

Isooctane (2,2,4-Trimethylpentane 99.5+% HPLC, CAS number 540-84-1), Propan-2-ol (99.5+% HPLC, CAS number 67-63-0), butanol (Butan-1-ol 99+%,CAS number 71-36-3) and methanol (99.5+%, CAS number 67-56-1) were all used as solvents during the measurements of lipid oxidation and provided by Fisher Scientific (UK).

3.2 Emulsion Preparation

3.2.1 Surfactant Stabilised Emulsion

Tween 20 (0.1% to 2%) was added to double distilled water and the aqueous solution mixed with a magnetic bar for 15 minutes at room temperature until Tween 20 completely dissolved. The oil phase (5% and 20%) was then added to the aqueous solution

and the oil-in-water emulsions were prepared using a Silverson L4RT with a fine emulsion screen of 19 mm diameter and a speed of 10,700 rpm for 7 minutes.

3.2.2 Protein Stabilised Emulsion

For preparing oil-in-water emulsions stabilised with CAS, the CAS aqueous solution was stirred gently for 2 hours at 45-50 ºC to ensure complete dispersion. Then the desired amount of sunflower oil was added to the CAS solution. Finally, the mixture was emulsified with Silverson at 10,700 rpm for 7 minutes.

3.2.3 Pickering Particles Stabilised Emulsion (Pickering Emulsion)

3.2.3.1 Silica Particles Stabilised Emulsion

Initially, the required amount of silica particles was added to the double distilled water. Then the pH of the aqueous silica dispersion was adjusted (using Mettler Toledo pH meter, Beaumont Leys Leicester, UK) to 2, 3, 5 and 7 with 0.1 M HCl or NaOH. Lastly, high intensity ultrasonic vibracell processor (Jencons-PLS) operating at 20 kHz and 700 W was used for 3 minutes to disperse silica particles in the aqueous phases. Then 20% sunflower oil was added to the aqueous silica dispersions and emulsified with a high shear mixer for 7 minutes. It should be noted that the silica-in-water dispersion was kept in the ice bucket during dispersion to keep the sample temperature around 25 °C.

3.2.3.2 Food-grade Particle Stabilised Emulsions

(a) Dispersion of Particles

The dispersions of MCC and/or MS particles were prepared by adding different concentrations of particles into double distilled water. The pH of the solution was adjusted by

using 0.1 M HCl or NaOH. The pH of solution was varied from 2 to 8. Two methods were used to disperse these particles in order to obtain the smallest average particle size:

i. For the first method high shear mixer (Silverson L4RT, equipped with a fine emulsor screen, 19 mm of diameter) at 4000 rpm was used within different mixing time to disperse particles.
ii. For the second method, MCC and MS particles were added in distilled water and the resulting aqueous dispersion were dispersed with a magnetic stirrer for 45 minutes at 45–50 ºC.

(b) Particles Stabilised Emulsions

Initially, food-grade particles dispersions (0.1% to 2%) were prepared and their pH was adjusted at 2, 4, 6, and 8, as described above (section 3.2.3.2). The oil phase was then added to the aqueous dispersion and the mixture was emulsified using the high shear rate mixer.

3.2.4 Mixed-Emulsifier Emulsion

Firstly, 0.5% to 2% Tween 20 was added to 20% sunflower oil and mixed for 20 minutes at room temperature with a magnetic bar. Then the oil phase was added to the 1.5% food-grade dispersion solution at specific pH condition (prepared as described in section 3.2.3.2) and finally emulsified with the Silverson 10,700 rpm for 7 minutes.

3.2.5 Fat Crystal Stabilised Emulsions

Scraped surface heat exchanger (“A” unit) followed by pin stirrer (“C” unit) were used to prepare oil-in-water emulsions stabilised with fat crystals. The “A” unit was a tubular heat exchanger cooled by a liquid (in this study water) with volume of 40 ml and two 10 mm

blades mounted on a central shaft. The “C” unit was a larger pin stirrer tube with volume of 210 ml with sixteen 5 mm pins mounted on a rotating central shaft.

The oil phase was prepared by adding the required amount of monoglycerides and mixed slowly with a magnetic bar at 70-75 ºC. The continuous phase was then prepared separately by dissolving measured quantities of xanthan gum powders into distilled water at ~ 70 ºC, followed by intensive stirring for 4 hours to ensure complete dispersion. The pre-emulsion was prepared by mixing two phases with high shear mixer (Silverson) for 1 minute. Then this pre-emulsion was placed on the hot plate (60-65 ºC) and passed through a bench-scale scraped-surface heat exchanger (“A” unit) and a pin stirrer (“C” unit) with a peristaltic pump (Masterflex L/S, Cole Parmer Instrument Ltd, UK) through a silicon tubing (outer diameter 6.3 mm, inner diameter 3.2 mm; ESCO, SLC, UK) (Figure 3-1). The pump speed was set at 25, 75 and 110 ml/min. Both A and C units were cooled with water bath (F25-ME Julabo, Julabo Ltd, UK) at 35, 40, 45 and 50 ºC. Following emulsification a sample of the emulsion was taken and immediately refrigerated.

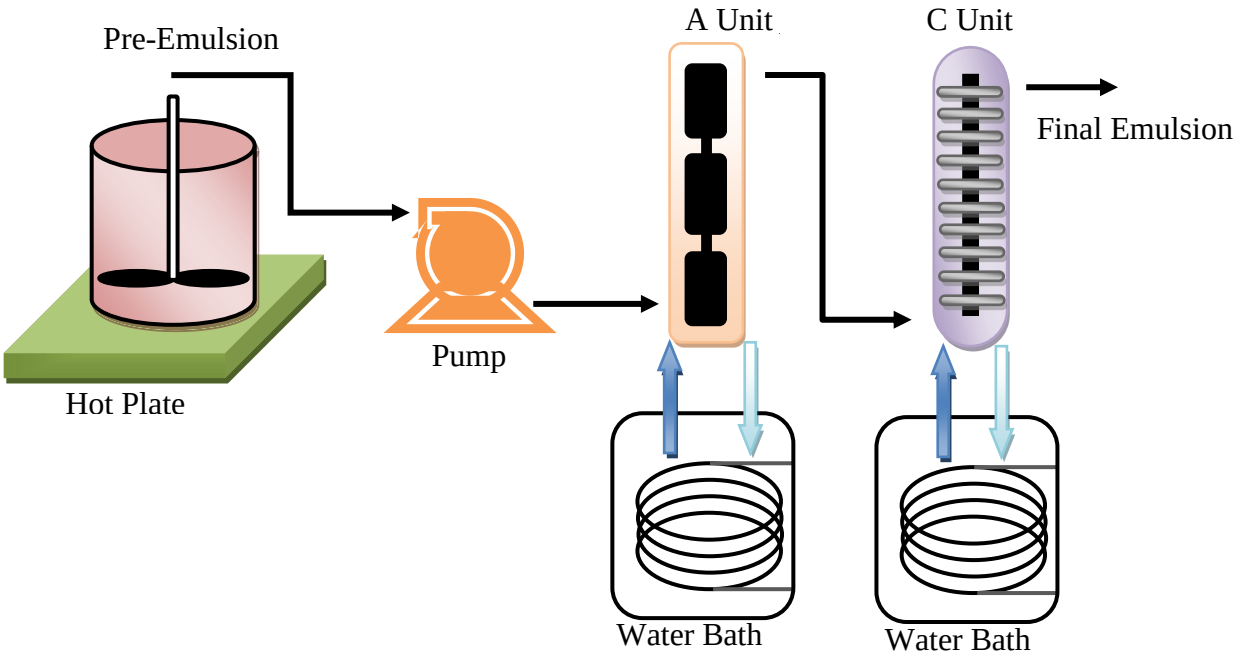


Figure 3- 1: Scheme of "A" and "C" unit set-up.

The main functions of “A” unit are (i) cool the hot pre-emulsion (promote rapid cooling) which leads to formation of crystal nuclei (start the crystallisation process) and (ii) remove the nucleated and crystallised fat from the wall with scraper blades mounted on a rotating central shaft which rotates continuously. The “C” unit provided residence time for the initial crystal growth and prevented formation of larger crystal networks by continuously shearing the sample. The “A” unit shaft speed was kept constant at either 1320 rpm or 800 rpm and the “C” unit speed was varied from 650 rpm, 800 rpm and 1200 rpm to identify the optimum shafts speeds that yields the stable droplet size. The temperatures at inlet and outlet of both “A” and “C” unit were all measured.

3.3 Analytical Methods

3.3.1 Droplet size Measurement

The Mastersizer HYDRO 2000 SM (Malvern, UK) was used to measure the droplet size and droplet size distribution. The Mastersizer is a static light scattering particle sizer (SLS) which covers the detection range from 0.02 to 2000 µm (*220*). When the particles pass through a laser beam, they scatter light at an angle which is in inverse relation with the size of particle; as the particle size decreases, the observed scattering angle increases. A number of photosensitive silicon detectors were used to measure the angular intensity of the scattered light. The particle size could also impact the observed scattering intensity. Small particles scatter light at wide angles but with low intensity, while, large particles scatter light at narrower angles with high intensity. Figure 3-2 demonstrates a typical measurement system consisting of a laser, a sample presentation system, and a series of detectors (*221*).

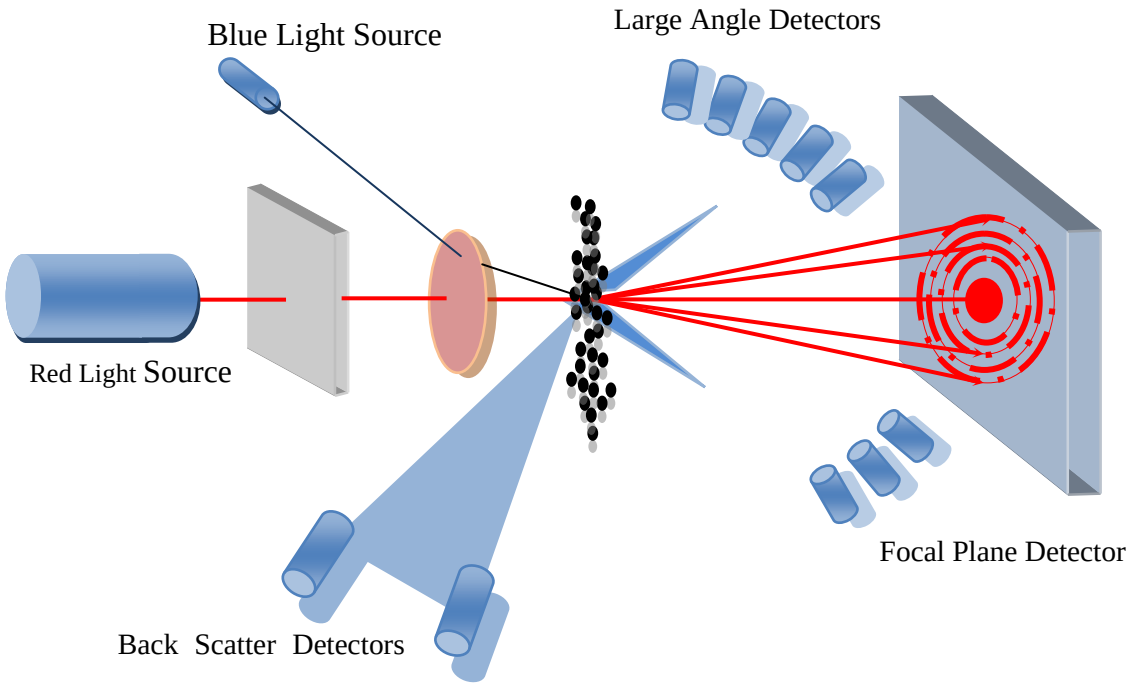


Figure 3- 2: Principle of particle sizing Static Light Scattering (Mastersize 2000). The focal plane detector is for detecting small scattered angles (< 10º), the large angle detectors are measuring higher angles (10º - 90º) and the back scatter detectors are detecting very high angles (100º - 135º) (*220*).

Prior to use the equipment, the "dispersion unit" was cleaned at least 4 times using distilled water at a stirring speed of 3000 rpm until the Mastersizer reading was in the green range (>77% laser light transmitted to the detector). Then the stirring speed was set at 1500 rpm and sample was added by pipette until the laser obscuration reached 12-16% of its total value. Finally, Mastersizer was switched on to start measuring the droplets size. Three measurements were carried out in order to verify reproducibility and accuracy. The machine was also cleaned between each test.

The reflective index values of 1.47 (for emulsions stabilised by surfactant), 1.46 (for emulsions stabilised with silica particles), 1.5 (for emulsions stabilised by either MCC or MS particles) and 1.44 (for emulsions stabilised by fat crystals) were selected.

Mastersizer analyses size of droplet and their distribution in a different ways regarding to the number, surface area or volume different values are obtained for the mean droplet diameter. $D_{(3,2)}$ (surface weighted mean diameter) was selected as the emulsion droplet size throughout this study.

3.3.2 Creaming Stability Measurement

There are several methods to measure creaming stability such as, visual observation, light scattering, conductimetry, ultrasounds and nuclear magnetic resonance (NMR). In this study, the emulsion stability was assessed by visual observation.

The prepared emulsion was poured into a glass vial (2.8 cm diameter 7.5 cm high) and sealed tightly to prevent evaporation. Then it was stored at room temperature for few days. After storage, the emulsion separated into three layers: a "cream" layer on the top, a "suspension" layer in the middle and a "serum" layer at the bottom (Figure 3-3) (*25*). These layers could be distinguished by their visual appearance. The cream layer was optically

"cloudy" and whiter than the original emulsions, the suspension layer had the same appearance as the original emulsions and the serum layer was either slightly "cloudy" or transparent. The creaming stability of the prepared emulsions was studied according to the Keowmaneechai and McClements method (*222*).The total emulsion height (H_T) and serum layer height (H_S) were measured. A creaming index (CI) was calculated as:

$$(CI) = \frac{H_S}{H_T} \times 100$$ Equation 3- 1

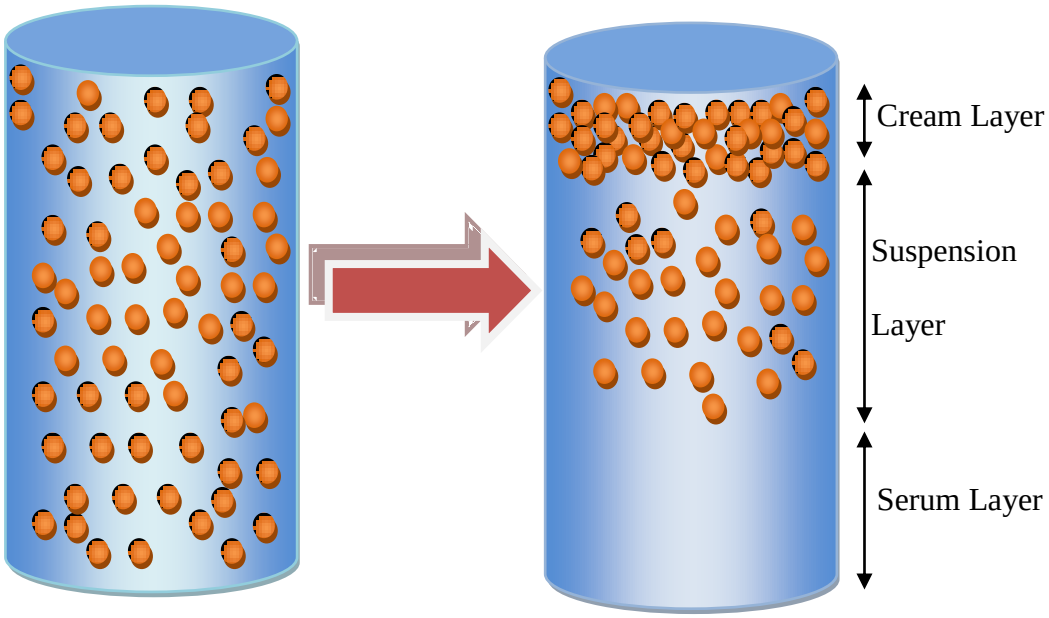


Figure 3- 3: Scheme of creaming process occurring in oil-in-water emulsions.

3.3.3 Light Microscopy

The light microscopy (Brunel microscopes Ltd, UK) was used to visualise the prepared emulsions droplets. The light microscopy was connected directly to a PC for real-time computer storage and image enhancement using Image analysis software (ImageJ).

3.3.4 Particle Size Measurement

The size of particles was measured using DelsaTM Nano C (Beckman Coulter, Brea, CA, USA). The DelsaNano employs Dynamic Light Scattering (DLS) also known as photon correlation spectroscopy technique to determine particle size which covers the detection range from 0.6 nm to 7 µm. Particles are illuminated by an incident laser beam as they diffuse through the sample cell via Brownian motion (Figure 3-4). The light is scattered in all directions giving a speckle pattern on a detector held close to the particles. The speckle pattern consists of bright areas where the light waves interfere constructively and dark areas where no light is detected (the phases are mutually destructive and cancel each other out).

The intensity of the bright and dark sides of speckle pattern varies since the particles are in motion. The frequency of such variation is dependent on the particles speed which can be connected to particle size by the diffusion coefficient known as D. The diffusion coefficient can be related to the particle size by the Stokes-Einstein equation (Equation 3-2):

$$D=\frac{K_B T}{3\pi\eta d}$$ Equation 3- 2

Where K_B is Boltzmann constant, T is the temperature, η is the viscosity of the dispersant phase and d is the diameter of the particle

Large particles tend to move slowly, which will result in the slow fluctuation of the intensity of the speckle pattern. The fluctuations of the scattered lights are recorded and then analysed using the autocorrelation function to provide the size (or distribution of size) of the particles in the sample (*25*, *223*).

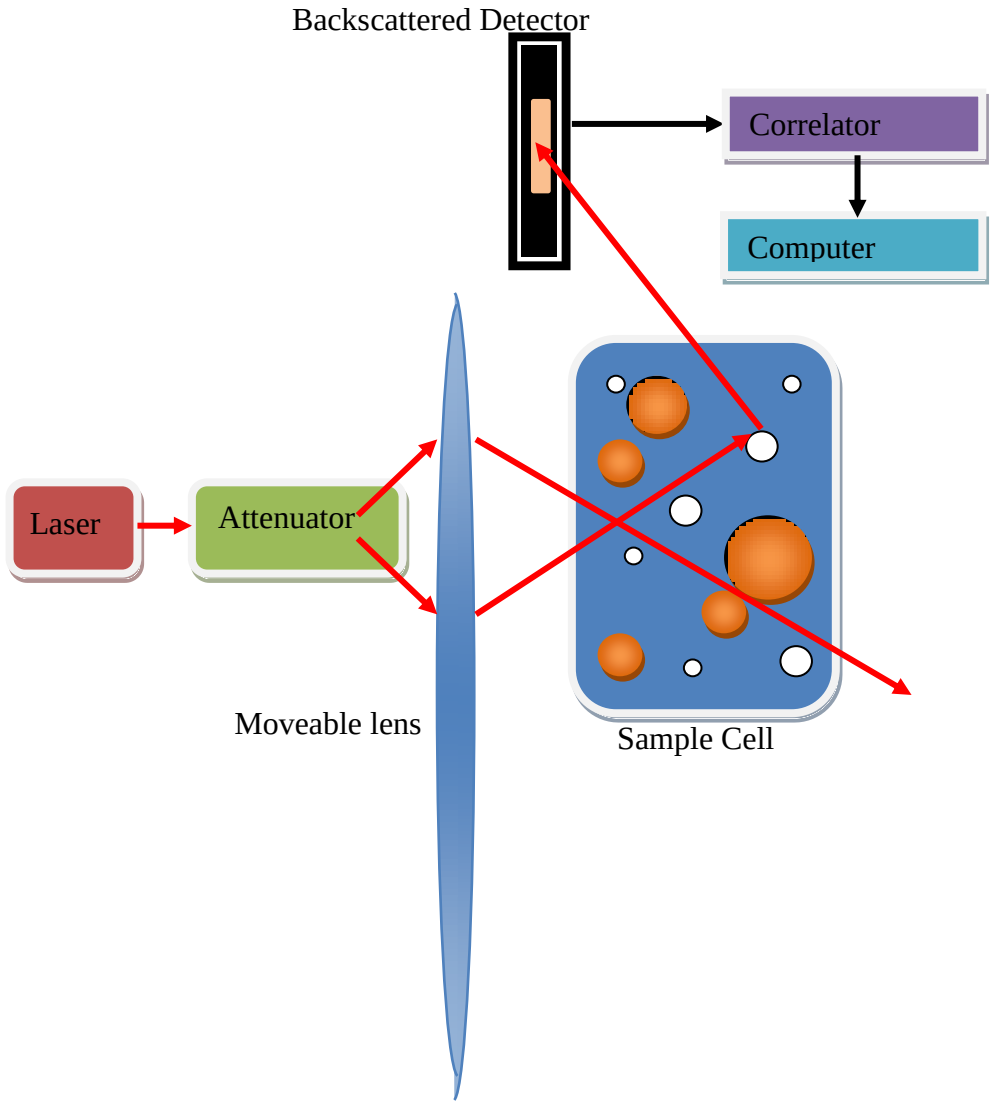


Figure 3- 4: Schematic illustration of the DLS instrument.

Initially, 0.5% of particles dispersion was prepared at the required pH as described in section 3.2.3.2. ~0.5 ml of sample was injected into the glass "size cell" (capacity of 0.9

ml) using a syringe. Filling must be done very slowly to avoid creating air bubbles in the cell. The cell was then placed into the device (sample chamber compartment). The measurement was carried out automatically. Scattered light was detected at 15° angle and analysed using a log correlator over 70 accumulations for each sample. The calculation of the particle size distribution (numerical basis) was performed using CONTIN particle size distribution analysis using the Delsa Nano C software. Each individual sample was tested in triplicate to ensure statistical significance.

3.3.5 Particle Charge Measurement

The zeta potential (charge) of particles dispersions was measured using DelsaTM Nano C (Beckman Coulter, Brea, CA, USA), which covers the detection range from -200 mV to +200 mV. Delsa Nano C uses electrophoretic light scattering (ELS) phenomenon to determine the zeta potential.

Colloidal particles with surface charge are attracted toward the opposite charge plate at the velocity proportional to the magnitude of zeta potential when an external electric field is applied. The direction and velocity (electrophoretic mobility) of the particles depends on the applied field *(224).* In order to measure the velocity of the particles, they are irradiated with a laser beam and the scattered light is then collected. The velocity of a particle in an electric field is dependent on: the strength of the electric field, the dielectric constant of the liquid, the viscosity of the liquid and the zeta potential *(25).* Electrophoretic mobility can be given as the velocity of particles move per unit of applied electric field. This can be measured from frequency shift (doppler shift) of the scattered light and this mobility can then be converted to the zeta potential (ζ) by using the Henry equation (Equation 3-3). Zeta potential can be estimated by measuring the velocity of the particles.

$$U_E = \frac{2\varepsilon\xi\ f(ka)}{3\eta}$$ Equation 3- 3

Where U_E is the electrophoretic mobility, ε is the dielectric constant of the medium, η is the viscosity of the medium and $f(ka)$ is the Henry's function. *ka* is the ratio of particle radius to the Debye length (1/k) and *a* is the particle radius.

The Debye length (1/k) is the "thickness" of the electrical double layer. For measuring zeta potential in aqueous solutions of moderate electrolyte concentration, a value of 1.5 is used and this is referred to as the Smoluchowski approximation. If measuring zeta potential in a non-polar solvent, the Huckel approximation is used, where f(Ka) is set to 1.0 (Figure 3-5). The increase in ionic strength (i.e. by a high solution ion concentration) decreases the double layer thickness and consequently the absolute value of zeta potential *(224).* Throughout this study the ionic strength was kept constant by using 0.03 mol/litter NaCl solution in all the experiments.

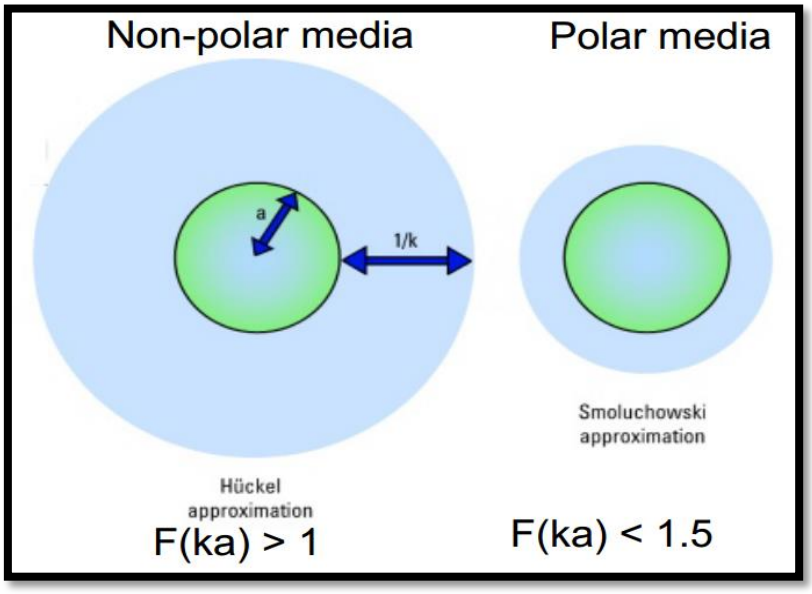


Figure 3- 5: Schematic of illustrating Huckel and Smoluchowski's approximations used for the conversion of electrophoretic mobility into zeta potential Adapted from http://www.silver-colloids.com/Tutorials/Intro/pcs21.html.

A small portion of particle dispersion (0.5 ml) was taken by a syringe, transferred to the glass “high concentration cell” (capacity of maximum 1 ml) and placed in the instrument for measurements. Similar to particle size measurement, care should be taken to avoid any air bubbles in the cell. The measurement was performed automatically and data was gathered simultaneously. Data were processed with the software included in the instrument. Each sample was measured in triplicate to assess reproducibility.

3.3.6 Cryo Scanning Electron Microscopy (Cryo-SEM)

Scanning electron microscopy (SEM) provides images of a surface of sample by scanning the sample surface using high energy beam of electrons, which interacts with the atoms that make up the sample producing signals containing information about the sample’s surface topography, composition and other properties.

SEM is unable to produce microstructure images of samples containing liquids since solvents present in samples may evaporate at vacuum condition in the SEM chamber. However, Cryo-SEM resolves this matter though rapid freezing in order to immobilise the liquid contained in samples and preserve microstructures of the samples.

In this study, Philips XL30 FEG Cryo Scanning Electron Microscopy (Figure 3-6) was used to visualise the microstructure of prepared emulsions. A small amount of prepared emulsion was placed in perforated holes of a brass stage mounted on a steel rod. The sample holder (rod) was plunged into “slushy” nitrogen to rapidly freeze the sample (the sample was frozen at -198 °C). The holder with frozen material was held under liquid nitrogen to be coupled to a rod and pulled back into a small cylindrical container. The sample holder was then withdrawn, under vacuum, into a device to be transferred to the cryo-preparation chamber. The sample was maintained at a low temperature of –180 °C for 2 minutes. Finally,

the sample was fractured using a metal knife and then dusted with gold particles in order to prevent damaging the electron beam. The sample was analysed using a 3 keV beam.

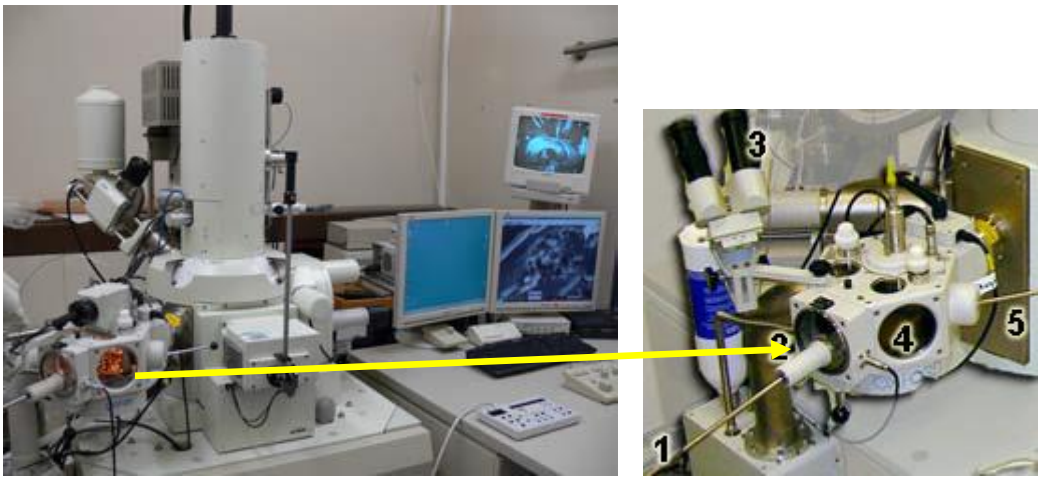


Figure 3- 6: Cryo scanning electron microscope: (1) insertion rod, (2) cylindrical chamber for transferring of the sample from freezing unit to cryo chamber, (3) binocular, (4) cryo-chamber and (5) lever to handle the fracture knife.

3.3.7 Rheology

A controlled stress Bohlin Gemini rheometer (Malvern Instruments, UK) was utilised to determine the viscosity of prepared emulsions. In this study, emulsion viscosity was measured at 25℃ over a shear rate range of 0.1 and 1000 s^{-1} with 60 mm acrylic parallel plate geometry (1 mm gap). Power-law model (Equation 3-4) was employed to analyse the emulsion flow curves.

$$\tau = K\dot{\gamma}^{n} \qquad \textbf{Equation 3- 4}$$

Where τ is shear stress, μ is viscosity, K is consistency coefficient, $\dot{\gamma}$ is shear rate, and n is flow behaviour (power law) index.

Parallel plate geometry consists of a stationary and a rotating flat, circular plate where a thin layer of sample is placed in between (Figure 3-7). In contrast to cone and plate

geometry, the shear rate varies and an average value is given by the rheometer software. However, unlike corn and plate system, parallel plate geometry has the advantage of a flexible gap.

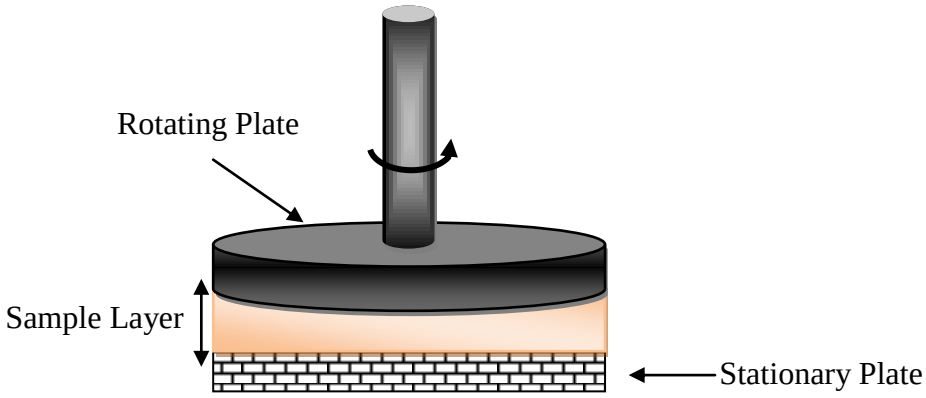


Figure 3- 7: Schematic of a parallel plate geometry.

3.3.8 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a measurement technique which determines the amount of energy (in the form of heat) absorbed or released by a sample as it is heated, cooled or kept at constant temperature. Commonly used DSCs are:

1) Power compensation DSC: the sample and reference pans are placed in separate furnaces and heated by separate heaters.
2) Heat flux DSC: the sample and the reference are interconnected by a metal disk that acts as a low-resistance heat flow path (Figure 3-8).

The heat flux is not as sensitive as the Power compensation method to small transactions, heating and cooling at slower rate and is less accurate because of its solo furnace design (*225, 226*).

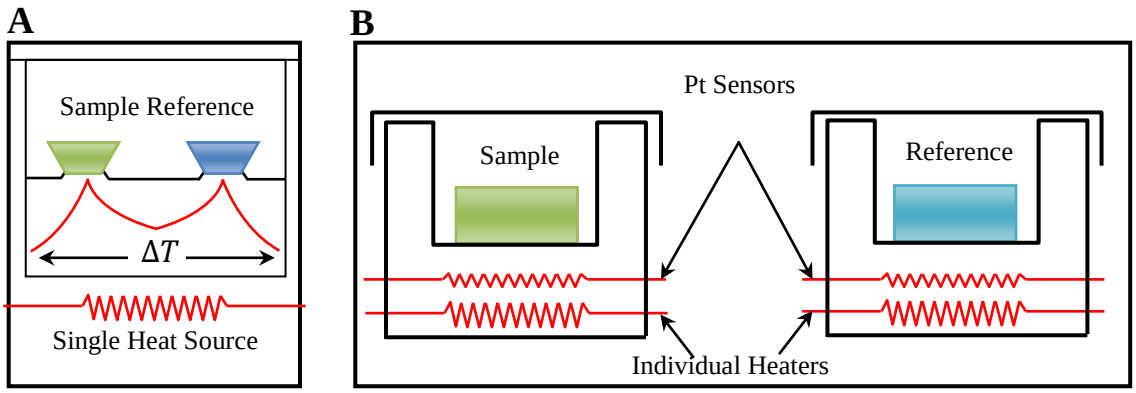


Figure 3- 8: Schematic of (A) heat flux DSC (B) power compensation DSC (*225*).

In this study, power compensation DSC Perkin-Elmer Diamond (Perkin- Elmer, UK) was used to monitor melting and crystallisation of different concentrations of fat crystals. An Intracooler III (capacity of -50 °C) with a cooling rate of 30 °C min^{-1} was used to cool the device. Nitrogen was used as the purge gas with a standard flow rate of 30 $ml.min^{-1}$. 10-12mg of samples (different concentrations of Monoglyceride in oil phase) was sealed in the Perkin Elmer 50 µl aluminium pans using the standard aluminium pan cover. 10-12 mg of pure sunflower oil was used as a reference. All samples were cooled to 0 °C for 10 minutes then heated up to 100 °C at 10 °C min^{-1} and held for 10 minutes and subsequently re-heated to 0 °C at the same rate. Data were processed using the Perkin Elmer Pyris software.

3.3.9 Spectrophotometry

In this work the level of primary and secondary lipid oxidation products were measured by spectrophotometric method. Spectrophotometry is an analytical technique widely used in laboratories to measure the absorbance or the transmittance of different samples. A spectrophotometer is employed to measure the amount of light that a sample absorbs. A small beam of light with a specific wavelength is emitted from the spectrophotometer which goes through the sample in a small glass container called a cuvette

(232). The spectrophotometer measures how much light is absorbed by the sample or how much of the light passes through the sample which is transmittance. In this study, UV/Vis Spectrophotometer from Biochrom (Libra S32) with a wavelength range from 190-1100 nm was used. Since other compounds in a solution (or the solvent itself) may absorb the same wavelengths as the compound being analysed, absorbance of the sample must be compared to a reference or blank. Ideally, the reference (blank) should contain everything found in the sample solution except the substance needed to measure. The amount of light transmitted through a solution is referred to as transmittance (T). The transmittance is defined as the ratio of the light energy transmitted through the sample (I) to the energy transmitted through the reference blank (I_0) The transmittance (T) and the absorbance (Abs) are estimated as follows *(232).*

$T = I/I_0$ **Equation 3- 6**

$Abs=-Log\ (T)$ **Equation 3-7**

3.4 Lipid Oxidation Measurement

As mentioned in chapter 2, lipid hydroperoxides are primary lipid oxidation products, which are relatively stable at low temperature and absence of metals. At high temperature or in the presence of metals, hydroperoxides could be decomposed to the secondary lipid oxidation products that impart rancidity (*16*). Therefore, it is necessary to measure both primary and secondary lipid oxidation products. In this study, the spectrophotometric Peroxide Value (PV) and p-Anisidine Value (p-AnV) methods were used to determine the level of primary and secondary lipid oxidation products respectively.

3.4.1 Peroxide Value Method (PV)

Emulsions (10 ml) immediately after formation were placed in a sealed screw cap glass tube, covered with aluminium foil and kept in an oven for 7 days at 40 ºC to accelerate oxidation. To measure of lipid hydroperoxides (primary lipid oxidation product), a method adapted from Shantha and Decker (1994) was applied (*167*). The PV was monitored as a function of time.

Prepared emulsion (0.3 ml) was added to a mixture of 1.5 ml of isooctane/2-propanol (3:1v/v), vortexed (10 s, 3 times) and followed by centrifugation for 5 minutes at 10,000 rpm. Then 0.2 ml of the clear upper solvent layer was mixed with a 2.8 ml of methanol/1-butanol (2:1, v/v) and 30 µl of thiocyanate/ferrous iron solution. Ferric thiocyanate is a red-violet complex which shows absorption at 500 to 510 nm. The thiocyanate/ferrous iron solution was prepared by mixing 15 µl of 3.94 M ammonium thiocyanate solution with 15 µl of 0.072 M ferrous iron solution. The ferrous iron solution was obtained from the supernatant of a mixture of 25 ml BaCl2 solution (0.132 M BaCl2 in 0.4 M HCl) and 25 ml of 0.144M FeSO4 solution. After each addition, the reaction tubes were vortexed for a few seconds. The tubes were kept in the dark at room temperature for 20 minutes. After 20 minutes, the absorbances of samples were measured at a wavelength of 510 nm using a spectrophotometer (Biochrom Ltd. PC32, UK).

A standard curve (calibration curve) made with cumene hydroperoxide was used to quantify the lipid hydroperoxides (Figure 3-9). The slope of calibration curve was used for calculating hydroperoxide concentration in each sample. For this purpose, initially 25 mg of cumene hydroperoxide was added to 25 ml of isooctane/2-propanol (3:1v/v) and vortexed (10 s, 3 times) to reach a concentration of 1 mg/ml. Then hydroperoxide solution was diluted

with isooctane/isopropanol (3:2 v/v) to various concentrations and absorbance of each dilution was measured 3 times at 510 nm with spectrophotometer.

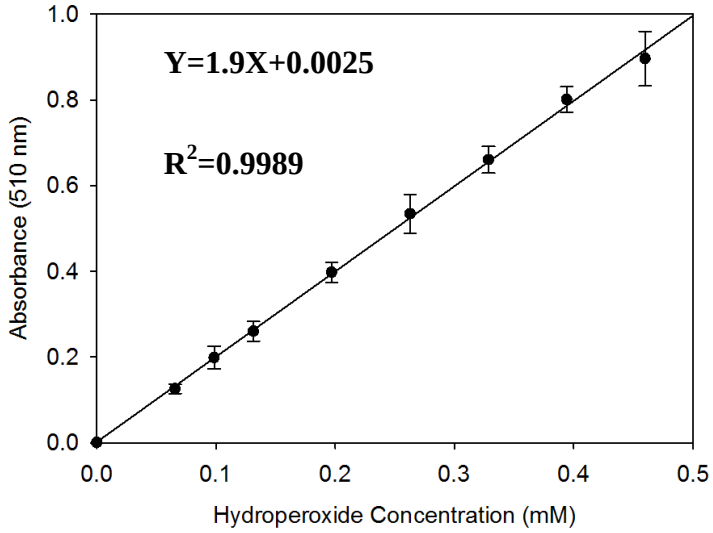
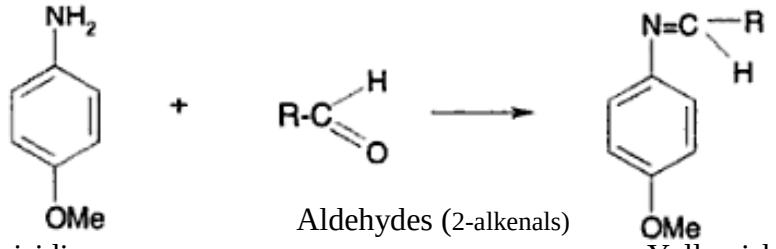


Figure 3- 9: Calibration curve for Peroxide Value measurement.

3.4.2 p-Anisidine Value (p-AnV)

The secondary products were measured using the p-anisidine value (p-AV) technique described in the American Oil Chemical Society (AOCS) CD 18–90 (1998) (*227*). It is based on the reaction of the p-anisidine with unsaturated aldehydes, resulting in the formation of a yellowish product that absorbs at 350 nm (Figure 3-10) (*228*).



p-anisidine Aldehydes (2-alkenals) Yellowish product (Absorb at 350 nm)

Figure 3- 10: Reaction of p-Anisidine with aldehydes (*156*).

One millilitre of the emulsion was mixed with 25 ml isooctane followed by centrifugation for 20 minutes at 4000 rpm. The absorbance of 5 ml of the clear upper solvent layer was measured with a spectrophotometer (Biochrom Ltd. PC32) at a wavelength of 350 nm (A_1 value). Again 5 ml of the clear upper solvent layer was added to 1 ml of the p-anisidine solution (prepared by mixing 0.25 g of p-anisidine in 100 ml of acetic acid). After 10 minutes the absorbance values of samples were measured at a wavelength of 350 nm (A_2 value). The AV values were calculated using the following relationship:

$$AV = \frac{25(1.2A_2 - A_1)}{\text{Mass of Sample}} \qquad \text{Equation 3- 5}$$

In order to check the accuracy of this method, 2,4-decadienal aldehayde was used to prepare the calibration curve (Figure 3-11). The reason this type of aldehayde was chosen is because 2,4-decadienal is a major decomposition product from the fatty acid composition of sunflower oil *(18)*. Initially, 25 mg of 2, 4-decadienal aldehayde was added to a 25 ml of isooctane and vortexed (10 s, 3 times) to reach a concentration of 1mg/ml. Then, a serial dilution was made from this solution and absorbance of each dilution was measured 3 times at 350 nm with spectrophotometer.

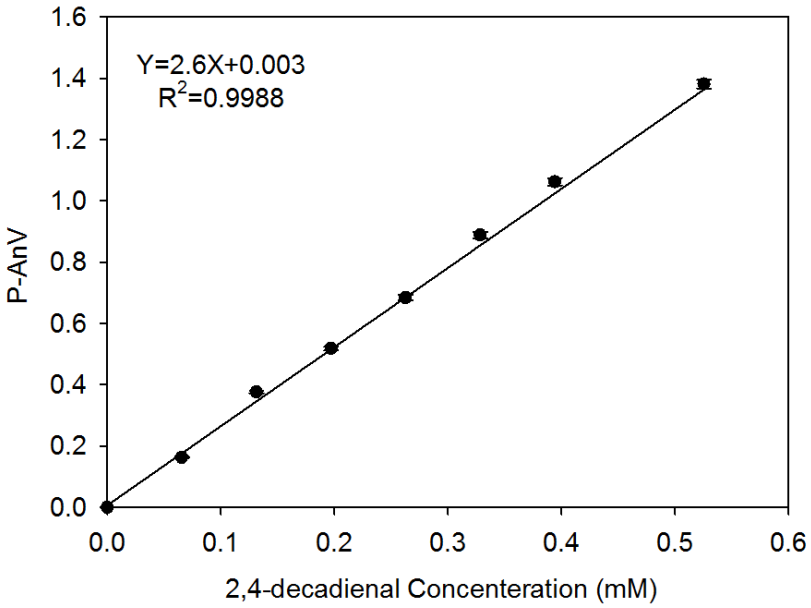


Figure 3- 11: Calibration curve for P-AnV.

Chapter 4: The Effect of Interfacial Microstructure on the Lipid Oxidation Stability of Oil-in-Water Emulsions

The common approach taken so far, by food scientists to prevent lipid oxidation, is to use chelating agents or synthesised antioxidants (e.g EDTA). However in recent years, there has been an increase in the food safety issues surrounding the use of synthetic antioxidants (*181*).

In oil-in-water emulsions, lipid oxidation reactions take place at the surface of the oil droplets, where pro-oxidants in the continuous phase come in close contact with the hydroperoxides located at the droplet surface (*5*). Hence, the properties of the oil-water interface can play a crucial role in the lipid oxidation rate.

The aim of this chapter is to reduce (control) the rate of lipid oxidation by the manipulation of emulsions' interfacial microstructure and physical properties of the emulsions in order to physically separate pro-oxidants from hydroperoxides.

In this chapter, protein (sodium caseinate (CAS)) and small molecular surfactant Tween 20 were used to stabilise oil-in-water emulsions. Then the effect of a variety of physical properties of these emulsions such as, oil fraction, emulsifier type and emulsifier concentration on the physicochemical stability of emulsions was investigated.

Furthermore, a microstructure engineering approach to reduce lipid oxidation rate, by using hydrophilic silica particles at the droplet interface to build a rigid layer around droplets, was employed in order to prevent the reactive species coming together.

4.1 Effect of Physical Properties of Emulsions on Coalescence Stability

The impacts of the environmental conditions such as thermal processing on the oxidative stability of emulsions have been studied (*186*). Nevertheless, lipid oxidation can be controlled by changing the physical properties of the emulsion such as emulsifier type and concentration. In order to develop the link between emulsion properties and emulsion physicochemical stability different concentrations of various emulsifiers such as, small molecular weight surfactant Tween 20 and protein CAS were used to stabilise emulsions.

4.1.1 Effect of Emulsifier Type and Concentration

The physical stability of emulsions containing 5% oil volume fraction and stabilised by either 0.1% or 2% Tween 20 and CAS was initially studied. The droplet size of these emulsions was measured immediately after emulsification and after 30 days in order to study the emulsions stability against coalescence.

It can be seen from Table 4-1, that emulsions stabilised with a high concentration (2%) of either Tween 20 surfactant or CAS protein were stable against coalescence and their mean droplets size remained stable for at least a month. However, at a low concentration (0.1%), emulsions were unstable and the size of droplets did change over time. For instance, the droplet size of emulsion stabilised with 0.1% Tween 20 was increased from 6.0±0.10 to 10.0±0.09 over a month. For emulsions stabilised with 0.1% Tween 20 surfactant or CAS protein no oil layer was observed at the top of the emulsions within the period of investigation (30 days).

Table 4- 1: Mean droplet size (D3,2), and standard deviations of 5% oil-in-water emulsions stabilised with different concentrations of Tween 20 surfactant or CAS protein. The error bars represent at least 3 different measurements on the same sample.

		Droplet size ($D_{3,2}$ µm)	
Emulsifier Type	**Emulsifier Concenteration (%)**	**Day 0**	**Day 30**
Tween 20	0.1	6.0±0.10	10.0±0.09
CAS		10.5±0.02	15.5±0.07
Tween 20	2	4.1±0.02	4.3±0.01
CAS		8.9±0.05	9.0±0.01

Emulsification is achieved by breaking up the oil phase into droplets (*229*). In order to stabilise the newly formed droplets, sufficient amount of emulsifier must be available in the system to rapidly absorb at the droplets interface, fully cover the surface of droplets and prevent newly formed droplets from coalescing. Consequently, at low concentrations there is not enough surfactant available in the system to completely cover the droplets interface; hence, the surface layers which begin to form are not able to prevent film rupture. Moreover, for emulsions stabilised by low concentration of higher molecular weight emulsifiers, such as protein, it is possible that protein will absorb to more than one droplet surface, leading to bridging flocculation (*38*). These results are in line with results of previous research (*230*). Su *et al.,* (2009) stabilised 40% v/v oil emulsion with 0.2 wt% whey protein isolate (WPI) and suggested that WPI content may not be enough to fully cover the oil droplets and form sufficiently dense adsorption layer. As a result, WPI acted as bridges among the oil droplets and led to droplets flocculation *(230).* The mean droplet size of emulsions stabilised with either 2% Tween 20 surfactant or CAS protein were 4.1±0.02 and 8.9±0.05 µm rescpectively. These results indicate that small molecular weight surfactants like Tween 20 are more

effective at producing smaller droplets than biopolymers (CAS) under similar emulsification conditions which is attributed to their ability to rapidly absorb to the droplet surfaces during emulsification.

4.1.2 Effect of Oil Phase Volume

The oil phase volume fraction is another key parameter that can alter the physiochemical properties of emulsions, such as droplet size, creaming and lipid oxidation. Therefore, in this section the effect of oil phase volume on emulsion stability was studied. As shown in section 4.1.1, all emulsions were prepared with 5% oil fraction, however, in this section all emulsions containing 30% oil fraction were produced and stabilised by either 0.1% or 2% Tween 20 surfactant and CAS protein. Table 4-2 shows the droplets size stability of 30% oil-in-water emulsions stabilised with different concentrations of Tween 20 and CAS.

By comparing Table 4-1 and Table 4-2, it can be seen that increasing oil-phase volume fraction from 5% to 30% increased the droplet size regardless of emulsifier types. This is probably because by increasing the oil phase volume, the number of oil droplets in the system increased. Since the amount of CAS is constant for both systems (5% oil phase volume and 30% oil phase volume), CAS present in the system with higher oil phase volume is insufficient to fully cover the oil-water interface. As a result, the protective interfacial layer that hinders droplets from coalescing with their neighbours was not formed. In addition, by increasing oil phase volume, the collision frequency among oil droplets is likely to increase and subsequently the rate of coalescence increases. For instance, the droplet size of 5% oil-in-water emulsion stabilised with 0.1% CAS protein increased from 10.5±0.02 μm to 15.1±0.03 μm as the oil volume fraction increased to 30%.

As expected, emulsions with low concentrations of surfactants and high oil volume fraction had larger droplets size with poor stability against coalescence. For instance, the

droplet size of 0.1% Tween 20 surfactant or CAS protein stabilised emulsions was 10.5±0.06 µm or 15.1±0.03 µm immediately after emulsification and around 18.0 µm or 21.2 µm after 1 month, respectively which suggests the occurrence of instability (coalescence or flocculation). On the other hand, increasing surfactant concentration to 2%, results in reduction in the droplets size and improvement of their stability against coalescence. There is no significant change in droplet size for these emulsions during storage (over 30 days). This is because at sufficient emulsifier concentrations the newly formed droplets were effectively covered by emulsifiers and therefore the protective layer that was needed around the droplets was formed. In addition, an increase in emulsifier concentration enhanced emulsifier migration to the droplet interface and surface coverage of oil droplets, which efficiently inhibited droplet aggregation or coalescence. Consequently, the emulsion droplets were stable against coalescence during collision.

Table 4- 2: Mean droplet size (D3,2) and standard deviations of 30% oil-in-water emulsions stabilised with different concentrations of Tween 20 surfactant or CAS protein. The error bars represent at least 3 different measurements on the same sample.

		Droplet size ($D_{3,2}$ µm)	
Emulsifier Type	**Emulsifier Concenteration**	**Day 0**	**Day 30**
Tween 20	0.1%	10.5±0.06	18.0±0.10
CAS	0.1%	15.1±0.03	21.2±0.01
Tween 20	2%	6.5±0.04	6.8±0.01
CAS	2%	12.5±0.10	11.22±0.2

Visual observation revealed that at low oil phase volume (5%) the CAS protein stabilised and Tween 20 surfactant stabilised emulsions creamed rapidly (after few hours). However, increasing oil phase volume to 30% delayed the development of a fully formed cream layer for 2 days. This could be due to the increase in volume fraction of oil droplets, which enhanced emulsion viscosity and lowered the creaming rate (*231, 232*).

4.2 Effect of Physical Properties of Emulsions on Lipid Oxidation

So far, the effect of properties of emulsion such as, emulsifier type, emulsifier concentration and oil phase volume on the physical stability of emulsion was studied. As discussed in chapter 2, the lipid oxidative stability depends on the physical properties of emulsions. Therefore, in order to investigate the effect of physical properties of emulsions (oil fraction, emulsifier type and emulsifier concentration) on oxidative stability, the lipid oxidation rate was measured for 7 days in emulsions containing either 5% or 30% oil fraction and stabilised by either 0.1% or 2% CAS protein or Tween 20 surfactant as described in section 3.4.

Lipid oxidation occurred in the oil-in-water emulsion (Figure 4-1), with a lag phase of lipid hydroperoxides and AV formation of 1 day. As shown in Figure 4-1, decreasing the oil fraction in the emulsions results in an increase in the rate of lipid oxidation regardless of the type of emulsifier (CAS or Tween 20) used. For instance, the hydroperoxide formation for 5% oil-in-water emulsions stabilised with 0.1% Tween 20 increased from 0.8 mM to 19 mM over 7 days whereas the hydroperoxide formation for 30% oil-in-water emulsion was increased from 0.1mM to 11.2 mM. The reason for this is because pro-oxidants, such as

metal ions, are present in the aqueous phase (*5*). Therefore, decreasing the oil volume results in an increase in the pro-oxidant concentration per interfacial area.

Another possible explanation could be found in the literature (5, 233) which is when the oil concentration increased; the higher number of unsaturated fatty acids may have migrated into the interior of the oil droplets. As a result these unsaturated fatty acids are less accessible to direct interaction with pro-oxidants in the continuous phase (*5, 233*). Osborn and Akoh (2004) reported that the 10% oil phase fraction emulsions had significantly higher rate of lipid oxidation compared to the 30% oil phase fraction ones and argued that this is due to the number of radicals generated per droplet which increases as the number of droplets decreases. Therefore, at 30% oil phase fraction fatty acids become less available for interacting with pro-oxidants compared to the 10% emulsions (*233*). The lower lipid oxidation rate for oil-in-water emulsions containing higher oil phase volume have also been previously reported for Menhaden oil and Safflower oil (*234, 235*).

Typically the reaction rate between lipid hydroperoxides and AV formation are different. Unlike hydroperoxides, aldehydes (secondary lipid oxidation product (AV)) do not decompose rapidly. This is because lipid hydroperoxides must first be formed before they decompose into secondary products *(7-8).* In the Tween 20 stabilised emulsions, 5% oil-in-water emulsions developed more aldehydes and had higher AV values than the 30% emulsions, which confirmed our first explanation for lower PV values in the 30% emulsions.

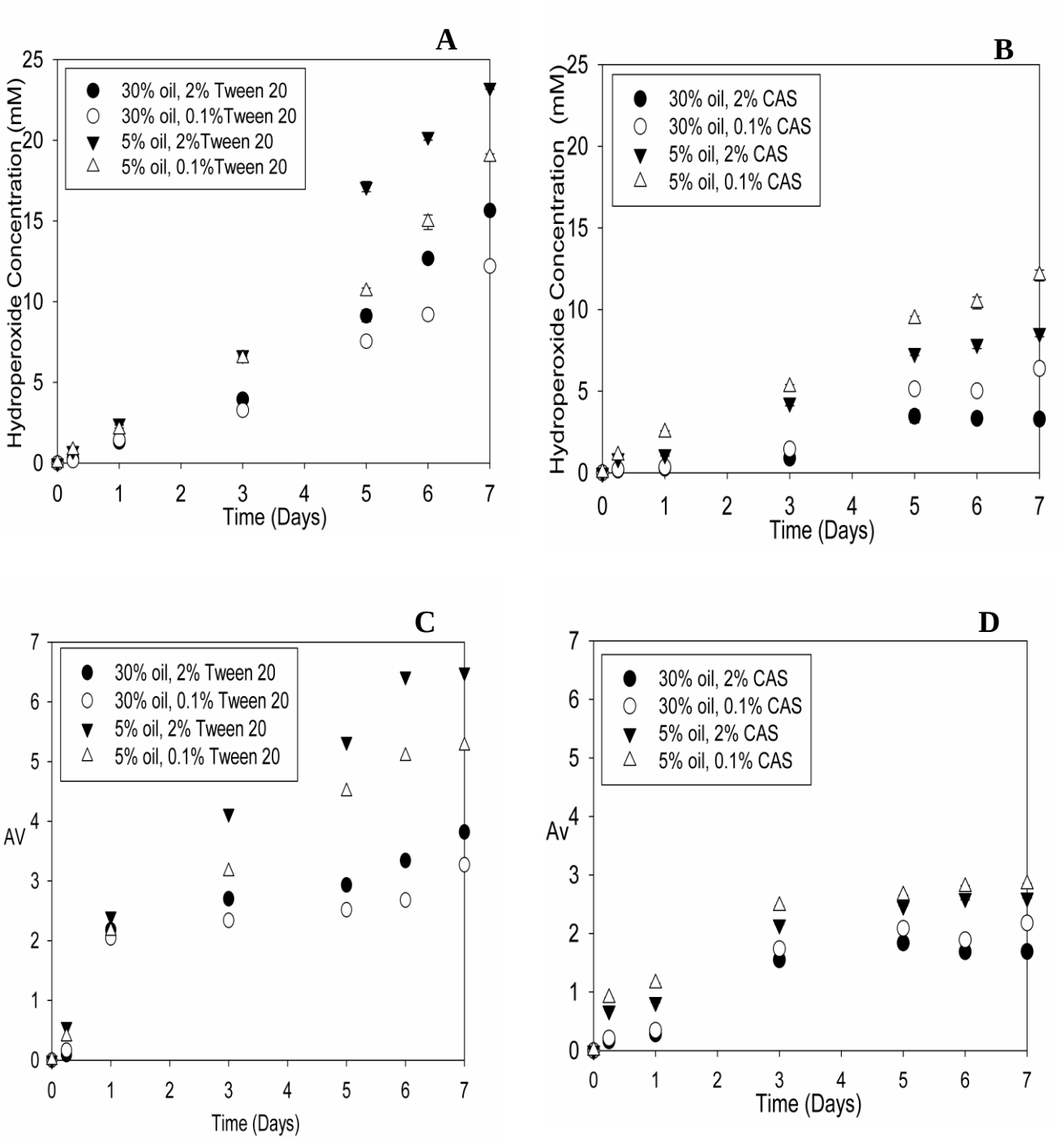


Figure 4- 1: Effect of physical properties of emulsions (oil phase volume, emulsifier type and concentration) on lipid oxidation rate. (A&B) Hydroperoxide formation (primary lipid oxidation product) for emulsions stabilised with Tween 20 surfactant and CAS protein respectively. (C&D) p-Anv value (secondary lipid oxidation products) for emulsions stabilised with Tween 20 surfactant and CAS protein respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The pH of solutions is not adjusted. The error bars represent at least 3 different measurements on the same sample.

As shown in Figure 4-1, the lipid oxidation rate is significantly affected by emulsifier type and concentration. The peroxide and p-AnV values for emulsions stabilised with CAS protein are much lower compared to those stabilised with Tween 20, irrespective of emulsifier concentration and oil fraction. For instance, the hydroperoxide formation for 5% oil-in-water emulsions stabilised with 0.1% CAS increased from 0.9±0.01 mM to 12.2 ±0.2 in 7 days. While the hydroperoxide formation of emulsion stabilised with 0.1% Twen 20 increased for 1.3±0.02 to 19.01±0.1 over a week. It is well known that proteins such as CAS, whey protein isolate (WPI) and β-lactoglobulin have antioxidant activity which inhibit lipid oxidation (*162*).

Previous studies have suggested that lipid oxidation progresses faster in emulsions with negatively charged oil droplets (*6,190, 200*). This is mainly attributed to the negatively charged oil droplets which attract positively charged pro-oxidants, hence increasing lipid oxidation. However, the results obtained here show that negatively charged CAS (pH>PI) stabilised emulsions inhibit lipid oxidation rate to a greater extent than non-ionic Tween 20 stabilised emulsions.

As mentioned in chapter 2, a thick interfacial layer may act as a barrier that decreases the ability of aqueous phase iron to decompose lipid hydroperoxides at droplets interface. Therefore, one possible explanation is suggested to be the ability of CAS protein to form a thick film around emulsions' droplets (oil-water interface) (*171*), which could form a barrier that decreases interactions between lipids and aqueous phase pro-oxidants. It has been reported that CAS can form a thick interfacial layer around dispersed oil droplets of up to 10 nm (*8*). However, small molecular surfactants can only produce a relatively thin interfacial layer around oil droplet (~1.4 nm) (*236*).

Moreover, CAS comprises of a mixture of four principal proteins: α_{s1}-, α_{s2}-, β-, and κ-caseins. α_{s1}-, α_{s2}- and β Caseins have polar domains that contain phosphorylated serine

residues (*237*). Many studies have reported that these caseins are able to inhibit lipid oxidation by binding to metal ions by their phosphoseryl residues, which results in partitioning of metal ions away from hydroperoxides (located in or at the interface of oil droplets) (*185*). Furthermore, CAS protein is able to scavenge free radicals through its anti-oxidative amino acid residues such as, histidine, methionine, phenylalanine, proline, tryptophan and tyrosine (*189*). Hence, CAS is more effective in inhibiting lipid oxidation than Tween 20 surfactant due to its antioxidant ability (chelate pro-oxidants and scavenge free radicals) and its ability to create thick emulsion droplet interfaces.

It can be seen from Figure 4-1, the emulsifier concentration potentially influenced the rate of lipid oxidation. The lipid oxidation rate of emulsions stabilised with 2% CAS was lower than those stabilised with 0.1% CAS. Possibly by increasing CAS concentration, the amount of nonabsorbed CAS molecules present in the aqueous phase of the emulsions may also increase. This could lead to less lipid oxidation rate due to the antioxidant ability (chelate pro-oxidants and scavenge free radicals) of nonabsorbed CAS in the continuous phase. Therefore, samples with higher concentration of CAS displayed lower rates of oxidation (Figure 4-1). On the other hand, as can be seen, the lipid oxidation rate increased as Tween 20 concentration increased (Figure 4-1). Some surfactants contain some levels of peroxide, such as Tween 20, Brij 10, Brij 35 and lecithin. Mancuso et al., (1999) reported that Tween 20 contained 13.7±0.9 μmol of peroxide/g of surfactant (*191*). They also suggested that these surfactants that contain some level of peroxide could serve as a source of free radicals and reduce oxidative stability (*191*). Therefore, adding more Tween 20 into the system could accelerate lipid oxidation, due to their close proximity to lipids at the emulsion droplet interface. This suggests that, increasing the surfactant concentration is not always the most effective way to inhibit lipid oxidation and it is depend on the nature of surfactant.

It is well established that the lipid oxidation reactions take place at the surface of the droplets (*5*). Hence, the surface area and droplet size would be potential factors affecting lipid oxidation. However, studies of the effect of droplet size on lipid oxidation in emulsions are contradicting. Some studies have been reported that for a constant droplet concentration, lipid oxidation rate increased as the droplet size decreased. They argued that when surface area increases more of the lipid phase is exposed to the surrounding aqueous phase, which could encourage lipid oxidation (*5, 238*). However, some studies found that the lipid oxidation rate was independent of droplet size and suggested that emulsion droplet size and surface area were not a major factor in the oxidative stability of emulsions (*182, 233*). Therefore, it seems that the effects of droplet size depends on the nature of the system, e.g., the type and concentration of emulsifiers, the amount and types of pro-oxidants and antioxidants exist in the system.

As can be seen in Table 4-1, Tween 20 stabilised emulsions had smaller droplets size than emulsions stabilised with CAS. Lipid oxidation rate was higher for Tween 20 stabilised emulsions compared to emulsions stabilised with CAS (Figure 4-1). This was attributed to smaller droplets having a larger surface area and thus "better access" to pro-oxidants. Hence, the increased oxidative stability of CAS stabilised emulsions might partly be due to the larger droplet sizes in these emulsions compared to Tween 20 stabilised emulsions. On the contrary, 2% CAS stabilised emulsions had smaller droplets sizes than those stabilised with 0.1% CAS (~8.9 and ~10.5 μm respectively) but showed lower lipid oxidation rate. Overall, droplet size (surface area) is not the only factor that can alter oxidation rate. The lipid oxidation in the food-based emulsions is the consequence of a combination of many factors such as, the type of emulsifier and the presence of antioxidants and pro-oxidants in the system.

Therefore, in order to eliminate the effect of the droplet size and surface area on lipid oxidation, the hydroperoxide formation was divided by the total surface area of the droplets. The total surface area (A_s) of a droplet can be calculated using Equations 4-1 to 4-3.

$$A_s = N_d \times S_{d_{3,2}} = \frac{6\phi}{d_{3,2}} \qquad \text{Equation 4- 1}$$

$$S_{d_{3,2}} = 4\pi(\frac{d_{3,2}}{2})^2 \qquad \text{Equation 4- 2}$$

$$N_d = \frac{V_{oil}}{V_{d_{3,2}}} = \frac{\phi}{\frac{4}{3}\pi(\frac{d_{3,2}}{2})^3} = \frac{6\phi}{\pi(d_{3.2})^3} \qquad \text{Equation 4- 3}$$

Where N_d is the number droplets in 100 grams of system, $S_{d3,2}$ and $V_{d3,2}$ is the surface area and volume of a single droplet, ϕ is the oil fraction and $d_{3,2}$ is the mean droplet size.

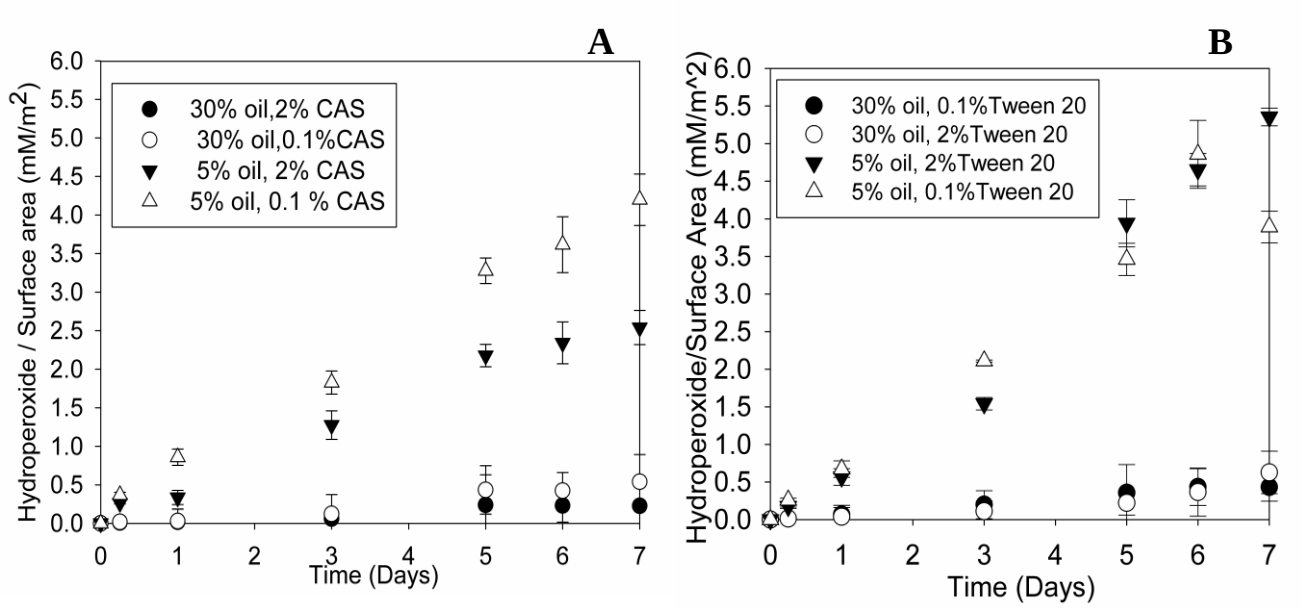


Figure 4- 2: Formation of hydroperoxide normalized to total oil droplets surface area over 7 days. (A) 0.1% and 2% CAS (B) 0.1% and 2% Tween 20. All samples held at 40˚C. The pH of emulsions is not adjusted. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

It can be seen that by taking into account the effect of surface area (Figure 4-2), emulsions stabilised with CAS are still more stable against lipid oxidation rate than those stabilised with Tween 20. The lipid oxidation is a complex phenomenon which can be influenced by many parameters. These results show that even after eliminating the effect of surface area similar trends were observed as previously described in Figure 4-1 (when the effect of droplet size wasn't eliminated). Hence, the effect of surface area can be modified, or even inverted due to the specific characteristics of the emulsion and the protective ability of the interface against oxidation, as found in a number of studies *(5, 11, 82, 233).*

4.3 Effect of Continuous Phase Protein (CAS) on Oxidative Stability

Many food manufacturers are keen to reduce the level of antioxidants used in the foods. This can be achieved by replacing some of these ingredients with natural alternatives such as protein. Results shown previously (section 4.2), clearly demonstrate that CAS protein is an effective emulsifier in producing stable emulsions (at least for 30 days) and reducing the rate of lipid oxidation. Therefore, based on the results obtained so far further investigation is needed to find out whether CAS in the continuous phase affect lipid oxidation. Hence, in this section the ability of continuous phase protein to alter oxidative stability was studied.

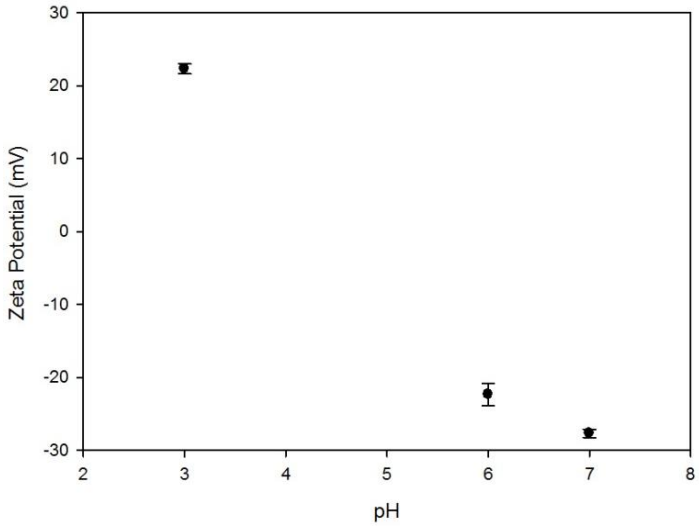


***Figure 4- 3*:** Zeta-potential of 0.5% CAS protein as a function of pH. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

The effect of variations in the concentration of CAS (0.2–1%), present in the continuous phase of oil-in-water (20% oil fraction) emulsions stabilised by 0.6% Tween 20 at both pH 3 and 7.3 on the lipid oxidation was studied. The reason that pH 3 and 7.3 were selected is because CAS protein is negatively charged at pH< pI and positively charged at pH> pI. Therefore, the impact of positively or negatively charged CAS protein located in the

continuous phase on lipid oxidation can be studied. As Figure 4-3 shows, at pH 4 the zeta potential was zero (CAS had no net charge), which corresponds to the isoelectric point (pI) of CAS, at higher pH (pH 7.3) CAS protein has negatively charged (~ -22 mV) and at low pH (pH 3) CAS has a positive charge (~20 mV). These data are in line with zeta potential measurements reported elsewhere (*239, 240*).

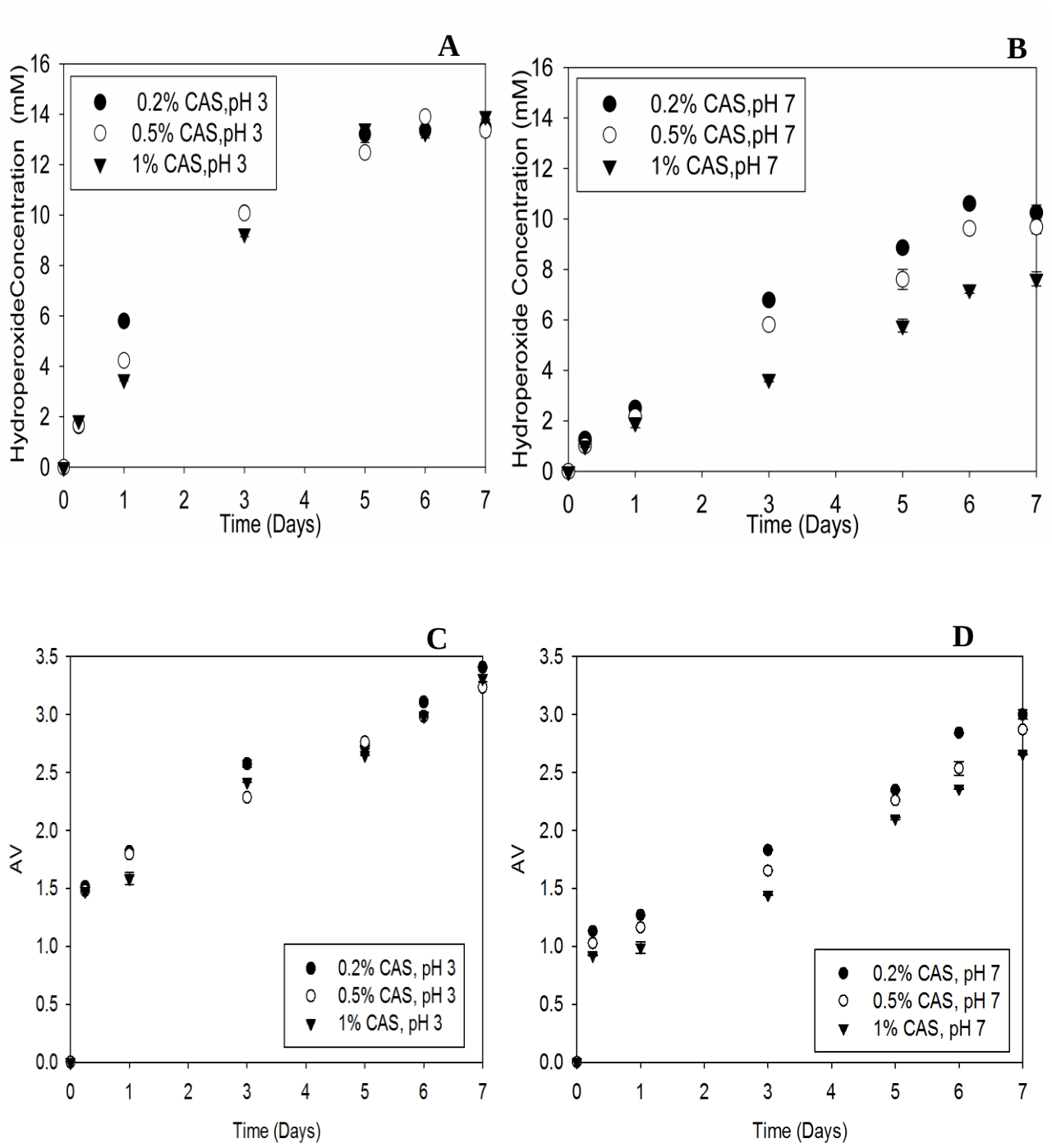


Figure 4- 4: Effect of different concentration of CAS protein in the continuous phase of 20% sunflower oil-in-water emulsions stabilised with 0.6% Tween 20 on lipid oxidation at pH 3 and 7. (A&B) Primary lipid oxidation product (hydroperoxide formation) for pH 3 and pH 7 respectively. (C&D) Secondary products formation (AV) for pH 3 and 7 respectively. All samples held at 40˚C. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbol. The error bars represent at least 3 different measurements on the same sample.

Figure 4-4 shows lipid oxidative stability of 0.6% Tween 20 stabilised emulsions containing various concentrations of CAS protein at both pH values of 3 and 7.3. The hydroperoxide formation of 0.6% Tween 20 stabilised emulsions containing 1% CAS at pH 3 increased from ~1.6 mM to ~ 13.7 mM in 7 days. However, the hydroperoxide formation of 0.6% Tween 20 stabilised emulsions containing 1% CAS at pH 7 increased from ~1.0 mM to ~6.5 mM. Therefore, the reaction rate (reaction rate=amount of product formed/time taken) of emulsions stabilised at pH 3 was 1.9 mM/day and the reaction rate of emulsions stabilised at pH 7 was 0.92 mM/day. Therefore, the rate of lipid oxidation is almost doubled at pH 7.3 than at pH 3. The ability of the continuous phase CAS to inhibit lipid oxidation at pH 7.3 but not at pH 3 is most likely due to the differences in the charge of the protein. As shown in Figure 4-4, at pH 7.3 CAS protein is negatively charged and thus would be able to chelate positively charged pro-oxidant (metals ions) in the continuous phase. However, at pH 3 the positively charged CAS proteins in the continuous repel metal ions located in the aqueous phase; hence CAS protein in the continuous phase could be more effective antioxidants at pH 7 than pH 3 due to greater binding of cationic transition metals to anionic proteins. Therefore, the presence of negatively charged proteins (CAS) in the continuous phase leads to increased oxidative stability by binding with cationic pro-oxidants This conclusion is also consistent with the findings of Faraji *et al.,* (2004) who reported that the continuous phase protein (Whey protein isolate) in 5% menhaden oil-in-water emulsions at pH 7 were better antioxidants than at pH 3 due to the differences in the charge of the proteins (*239*).

Figure 4-4 shows that increasing the CAS protein concentration in the continuous phase at pH 7.3, results in a decrease in the lipid oxidation rate. Conversely, increasing the concentration of CAS in the continuous phase at pH 3 had no significant effect on the lipid oxidation rate. This could be due to the fact that CAS protein becomes anionic at pH>pI (pH 7.3), thereby chelate positively charged pro-oxidants. However, at pH 3 CAS proteins

become positively charge hence, could not chelate pro-oxidants. By increasing CAS concentration (at pH 7.3) the amount of effective chelators in the system increased which leads to the removal of transition metals from the surface of emulsion droplets.

As Figure 4-4 (C&D) shows, the initial AnV for the emulsions at both pH 3 and pH 7 is low. This could be due to the fact that hydroperoxides must first be present in the system before they can decompose into secondary products. However, the AV formation from day 3 is increasing as more hydroperoxides is decomposing.

The change in the pH of the emulsions was also studied for 7 days. As Figure 4-5 shows, the pH of emulsions set at pH 7.3 decreased over 7 days but samples adjusted to pH 3 stayed constant throughout. Van Ruth *et al.*, (1999) reported that the decrease in pH might be due to the formation of several acids, such as acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, and pentanoic as a result of hydroperoxide decomposition (*241*). These acids are also formed at pH 3, but under such acidic condition no pH changes can be detected because at this pH more acid is required to alter the pH.

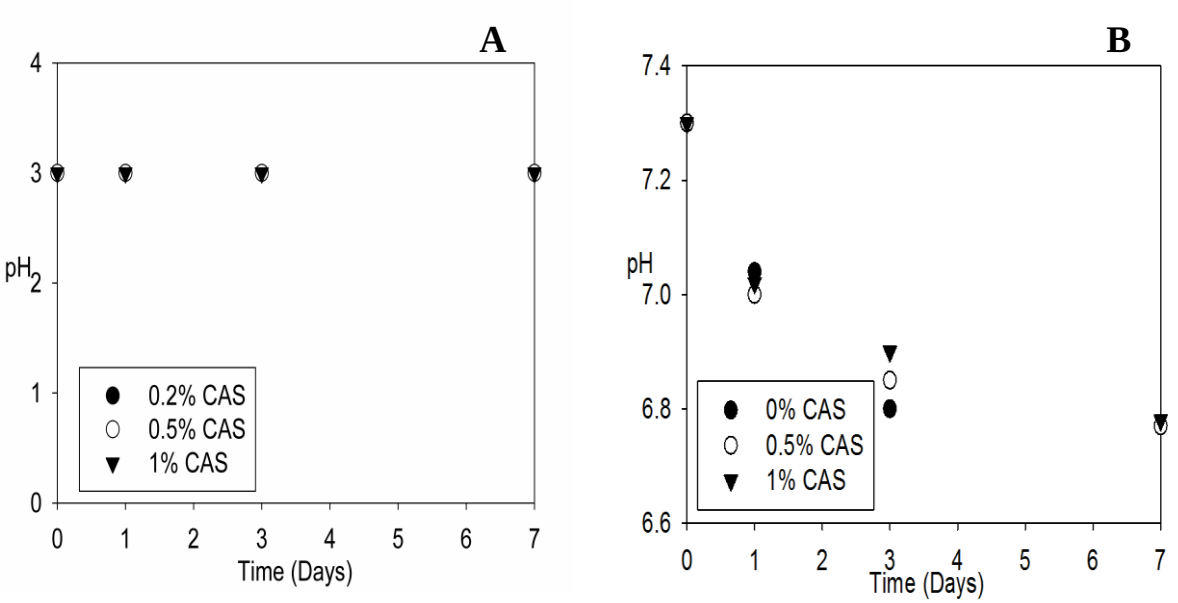


Figure 4- 5: Change in pH of different concentrations of CAS in continuous phase of 20% oil-in-water emulsions stabilised with 0.6% Tween 20 over 7 days (A) Initially pH 3. (B) Initially pH 7.3. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

In order to determine how effective continuous phase CAS is in reduction of lipid oxidation, the 20% oil-in-water emulsions stabilised with 0.6% Tween 20 (0% CAS in the continuous phase) at pH of 3 and 7 and their lipid oxidation rate were measured for 7 days (Figure 4-6).

The hydroperoxide formation for emulsion stabilised with 0.6% Tween 20 and containing no 0% CAS was increased from ~ 1.8 to ~ 28.2 in 7 days (Figure 4-6). While hydroperoxide generated for emulsions stabilised with 0.6% Tween 20 and contain 1% CAS was increased from ~1.0 to ~7.6 (Figure 4-5). This could be due the antioxidant ability of CAS in the continuous phase. As shown in Figure 4-7, the hydroperoxide formation is increased as the pH decreased from 7.3 to 3. Iron solubility is increased at pH 3 which enhanced its ability to react with hydroperoxides at the droplets' interfaces (*159*). Thus, at pH 3 more pro-oxidants are available in the system which leads to a higher rate of oxidation. Cho *et al*., (2002) reported that the concentration of iron in the continuous phase is higher at pH 3 compared with pH 7 in corn oil-in-water emulsions (*209*). Therefore, these results proposed that, the presence of CAS protein in the continuous phase is an effective approach to inhibit lipid oxidation due to the antioxidant ability of CAS to prevent metal ions-hydroperoxides interaction.

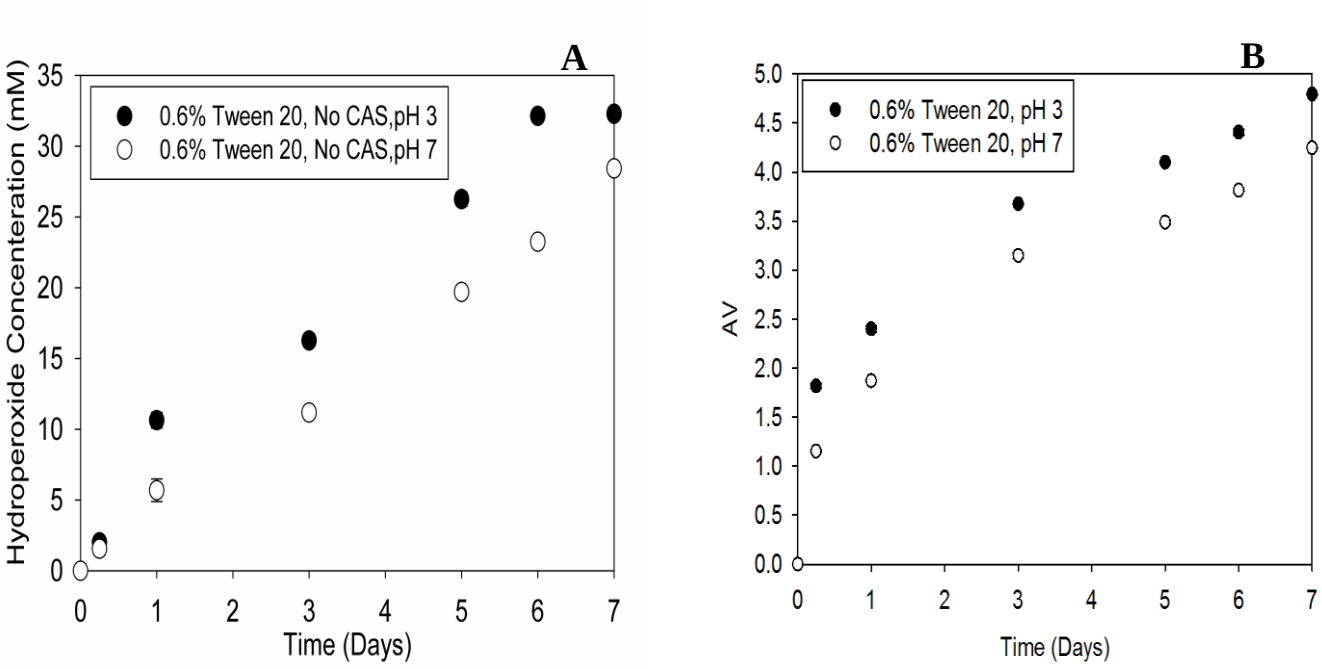


Figure 4- 6: Lipid oxidation of 20% sunflower oil-in-water emulsions stabilised with 0.6% Tween 20 and containing no CAS protein at pH 3 and 7 (A) Primary lipid oxidation product (PV) and (B) Secondary lipid oxidation products (AV). All samples held at 40˚C. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

4.4 Pickering Emulsions

Results obtained in section 4.2 indicate that the thickness of interfacial layer around emulsion droplets play a crucial role in reducing or controlling lipid oxidation by partitioning pro-oxidants in the continuous phase from oil droplets. The thickness of the interface depends on several factors including the actual structure and size of the absorbed species. In addition to proteins, colloidal particles have also been shown to create a thick layer around droplets (*52*). Therefore, in this section the ability of silica particles (as a model system) at the emulsions interface to influence lipid oxidation stability by manipulating the microstructure of emulsion (building rigid layer around droplets) is investigated.

4.4.1 Silica Particles Stabilised Emulsions

In order to understand the impact of silica particles at the droplet interface on lipid oxidation stability the charge of silica particle was initially measured as a function of pH (as described in section 3.3.4 and 3.3.5) and the obtained data are given in Table 4-3.

As can be seen, at low pH conditions (pH 2 or 3) silica particles are either not charged or slightly charged. However, an increase in the pH of the system results in negatively charged silica particles. These data are in very good agreement with zeta potential measurements of silica particles dispersions reported elsewhere (*81*).

Table 4- 3: Charge of 0.5% Silica particles dispersion at different pH conditions. The error bars represent at least 3 different measurements on the same sample.

pH	Zata Potential (mV)
2	0±0.02
3	-2.7±0.7
5	-14.3±1.1
7	-32.05±0.3

As mentioned earlier (chapter 2), the physical properties of emulsions such as droplet size, emulsifiers structure and charge can alter lipid oxidation rate. Therefore, the physical stability of emulsions stabilised solely by silica particles was investigated as a function of silica concentration. Since silica particles had no charge at pH 2 and carried negatively charged at pH 7 these two pHs were selected in order to study the effect of charge on physicochemical stability of emulsions. The droplets size was measured (in triplicates) immediately after emulsification, also after 21 and 40 days. The obtained data are plotted in Figure 4-7.

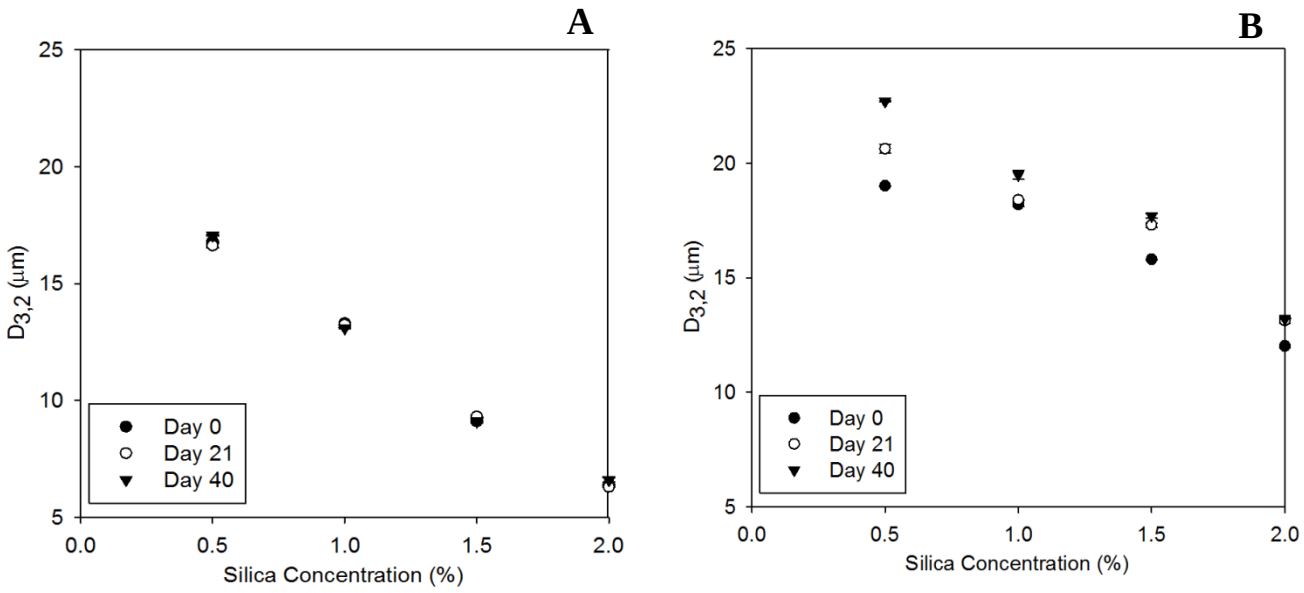


Figure 4- 7: Droplet size of 20% oil-in-water emulsions stabilised with 0.5% to 2% silica particles at (A) pH 2 and (B) pH 7. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

Figure 4-7 shows that emulsions stabilised at pH 2 were stable for 40 days and their mean droplets size remained unchanged within the period of investigation. The mean droplets size of emulsions prepared at pH 7 increased over time indicating coalescence phenomena occurred. Pichot *et al.,* (2009) suggested that uncharged silica particles (pH<4) could arrange "densely packed" multilayer structures at the oil−water interface due to the absence of charge at these conditions. However, charged silica particles (pH>4) were more likely to "spread out" at oil-water interface due to repulsive force between particles. They argued that the occurrence of "densely packed" structure at the oil-water interface would seem to be a less probable event (*81*). It appears that the pH of the system or the charge of particles could influence the arrangement of particles at the interface. Therefore, at pH 2 there are no electrostatic repulsive forces acting between particles, which potentially lead to the formation of multilayer particle structure at oil-water interface. As a result, emulsions stabilised at pH 2 were stable for 40 days and did not show any increase in droplet size over time.

4.4.2 Oxidative Stability of Silica Stabilised Emulsions

The oxidative stability of oil-in-water emulsions stabilised by silica particles was investigated for 1 week. 20% oil-in-water emulsions at both pH 2 and 7 were prepared with 0.5%, 1% and 2% silica particles, as the only emulsifier species. Emulsions prepared with high concentration of silica particles had much smaller droplet sizes than those stabilised with low concentration of silica particles (Table 4-3). The surface area could impact the oxidative stability. Hence, in order to eliminate the effect of surface area on lipid oxidation, the hydroperoxide formation divided by the total surface area of the droplets (as described in section 4.2) is plotted in Figure 4-8.

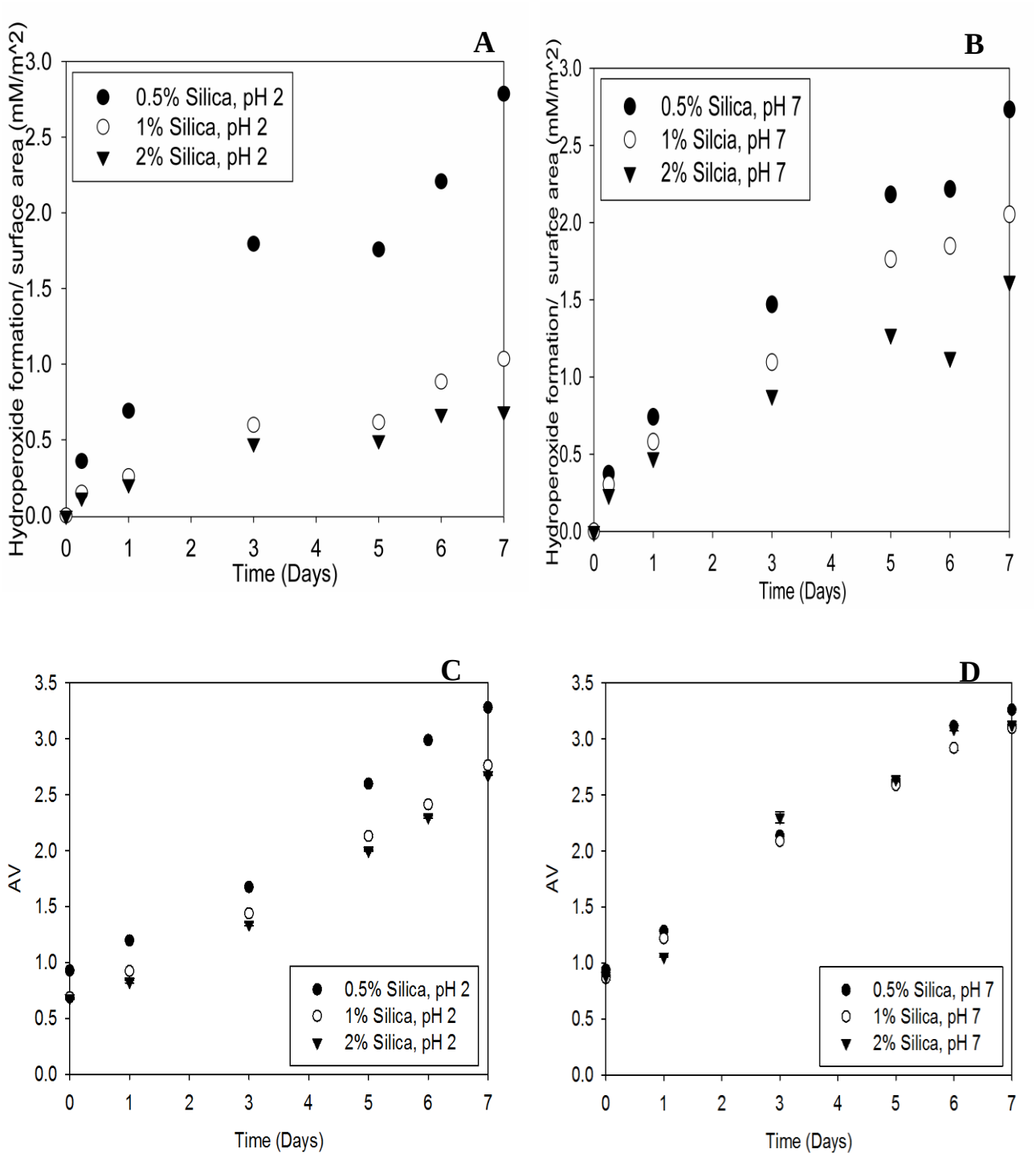


Figure 4- 8: (A&B) Formation of hydroperoxide normalized to total oil droplets surface area over 7 days at pH 2 and pH 7 respectively.). (D & C) Formation of secondary products at pH 2 and pH 7 respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

As Figure 4-8 shows the lipid oxidation rate is reduced as the silica particles concentration increased regardless of pH of the system. This could be due to the presence of particles at droplet interface. At low (0.5%) silica particle concentration, it is less likely to fully cover the droplets interface. While the silica particles increase there might be sufficiently enough particles available in the system to fully cove the droplets interface. This could lead to partitioning of hydroperoxides at droplets interface from pro-oxidants in the continuous phase which results in the reduction of hydroperoxide-metal ions interaction.

The decrease of lipid oxidation rate is more significant at pH 2 than at pH 7 as silica particles concentration increased. As shown in Table 4-3, at pH 7 silica particles are negatively charged and at pH 2 silica particles are uncharged. R.Pichot *et al.,* (2009) reported that silica particles formed a more "packed arrangement" at the droplets interfaces at low pH when they carried no electric charge. Consequently, the presence of electro-repulsive forces between particles at higher pH could be a preventive factor to the formation of a "packed" interfacial structure around droplets (*81*). Therefore, it could be possible that when uncharged particles are located at droplets interface, they can be more effective in forming a protective layer around droplets, which can result in the reduction of hydroperoxides-metal ions reactions.

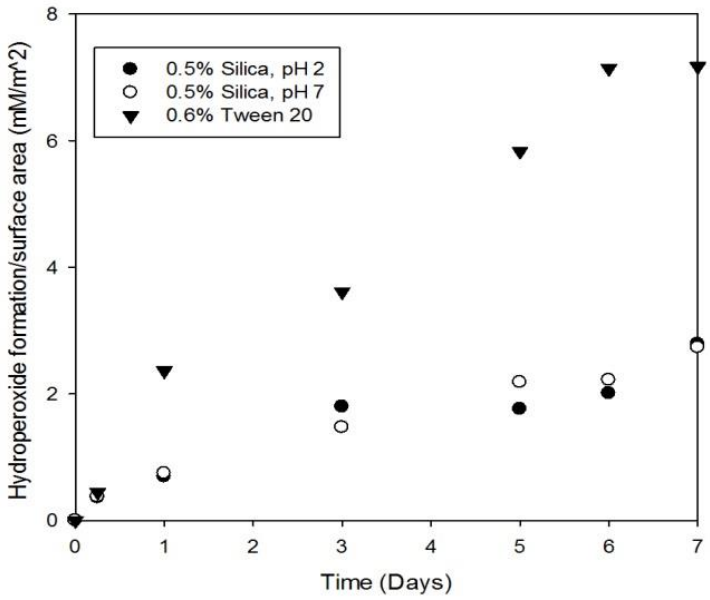


Figure 4- 9: Formation of hydroperoxide normalized to total oil droplets surface area over 7 days. 20% oil-in-water emulsions stabilised with either silica particles or Tween 20. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

As Figure 4-9 shows, silica particles significantly decrease the lipid oxidation rate compared with the emulsions stabilised by surfactant (Tween 20). The hydroperoxide formation/surface area of 0.6% Tween 20 stabilised emulsions increased from 0.4 to 7.1 mM/m^2 over 7 days. However, the hydroperoxide formation/surface area of 0.5% silica particles stabilised emulsions (at pH 2) increased from 0.3 to 2.7 mM/m^2 over. Hence, Pickering particles (silica) reduced lipid oxidation rate up to 40% compared with small molecular surfactant (Tween 20). These results suggest that presence of particles at droplet interface can influence the lipid oxidation rate by holding metal ions away from surface. This could be due to the ability of silica particles to provide a thicker interfacial layer around droplets compared to Tween 20 surfactant. This thick interface can inhibit interactions between pro-oxidants and the hydroperoxide at droplets. Emulsion droplets are surrounded by silica particles which not only prevent the droplets from coalescing but also may protect lipids from oxidation.

4.5 Conclusion

The effect of physical characteristics of emulsions such as droplet size, oil-phase fraction, emulsifier type and concentration on coalescence and oxidative stability were investigated.

The emulsifier type plays an important role in the lipid oxidation stability; CAS reduced the oxidation rate compared with Tween 20 due to its antioxidants ability to chelate pro-oxidants through its phosphoseryl groups. Moreover, it has been shown that CAS in the continuous phase seems to be able to reduce the lipid oxidation rate at pH > pI through chelating pro-oxidants.

It has been shown that emulsion stability depends on oil volume fraction; at high oil volume fraction (30%) the collision frequency among oil droplets is likely to be enhanced and subsequently the rate of coalescence increased which results in an increase in the droplets size. Increasing the oil phase fraction from 5% to 30%, results in the decrease of the hydroperoxide and secondary products formation due to the increase of pro-oxidants per surface area (higher number of metal ions generated per droplet at low oil content).

The particles (silica) at the interface (Pickering particles) were able to reduce the lipid oxidation rate. This has been interpreted due to the thickness of the interfacial layer around droplets thus separating the metal ions from the surface of droplets. Therefore, these results suggested that the present of Pickering particles at droplets interface is effective in reducing lipid oxidation.

Chapter 5:Effect of Food-Grade Particles on the Physical and Oxidative Stability of Oil-in-Water Emulsions

Particle-stabilised (Pickering) emulsions have attracted considerable attention in the past few years because of their remarkable stability against coalescence and their high stability against changes to processing conditions (pH, salt concentration, temperature, etc.). Despite the potential of these systems, applications in food industry are still limited by the absence of suitable "food-grade" Pickering particles.

As discussed in Chapter 4, engineering the interfacial layer of oil-in-water emulsion droplets to produce thick interface (to physically separate the metal ions from the surface of droplets) is an effective method for controlling lipid oxidation. It was shown that the lipid oxidative stability of emulsions stabilised with silica particles improved by up to 50% compared with the emulsions stabilised by surfactant (Tween 20). Since silica particles are not "label friendly", in order to benefit from the unique properties of Pickering emulsions in the food industry, suitable food-grade particles must be used. For this reason, in this chapter,

food-grade particles (Microcrystalline Cellulose (MCC) and Modified Starch (MS)) were used to stabilise oil-in-water emulsions.

The aim of this chapter is to evaluate the effects of selected food-grade particles on the physical and oxidative stability of oil-in-water emulsions. The properties of food-grade particles, such as charge and size are initially reported. The ability of these particles to stabilise oil-in-water emulsions are then discussed as a function of various parameters, such as pH and particles concentration. Lastly, the oxidative stability of oil-in-water emulsions stabilised by food-grade particles and produced under a range of different processing conditions are described.

5.1 Characteristics of Food-Grade Particles

In order to gain a better understanding of the role of particles in the stabilisation of oil-in-water emulsions, the properties of particles such as, size and charge were studied.

5.1.1 Particle Size

As described in section 3.2.3, two different approaches were considered in order to disperse the food-grade particles into the aqueous phase: high shear mixer (Silverson) and "low shear mixing" (magnetic bar). The results from high shear mixer processing indicated that the particles size of MCC and MS dispersions decreased as the dispersion time increased (Table 5-1). During short dispersion time, large particle aggregates were not broken down. This was due to the relatively low mechanical energy input provided to the system. Hence, the dispersion time has to be long enough to break down large particle aggregates. It has been reported that the pro-oxidative transition metals may originate from the raw material itself or from equipment used during processing (*5*). Therefore, the gentle mixing (magnetic bar) method was chosen to disperse the food-grade particles (MCC or MS) into the aqueous phase throughout this study.

Table 5- 1: The particle sizes of 0.5% food-grade particles dispersed in water with either magnetic bar or high shear mixer. The pH of solutions is not adjusted. The error bars represent at least 3 different measurements on the same sample.

	Particle size (nm)			
Particle Type	**Magnetic Bar (45 min)**	**High Shear Mixer (1 min)**	**High Shear Mixer (3 min)**	**High Shear Mixer (5 min)**
MCC	415.3±1.5	1716.9±4.2	1005.4±3.0	442.6±2.2
MS	120.5±3.2	905.9±2.8	303.7±1.3	135.6±8.3

5.1.2 Particles Charge

It has been well established that surface charge of droplets has a major impact on the physiochemical stability of emulsions (*242*). In order to understand the charge of particles at various pH conditions, the zeta potential of 0.5% of particle aqueous dispersions at different pH values was measured and the obtained data is given in Figure 5-1.

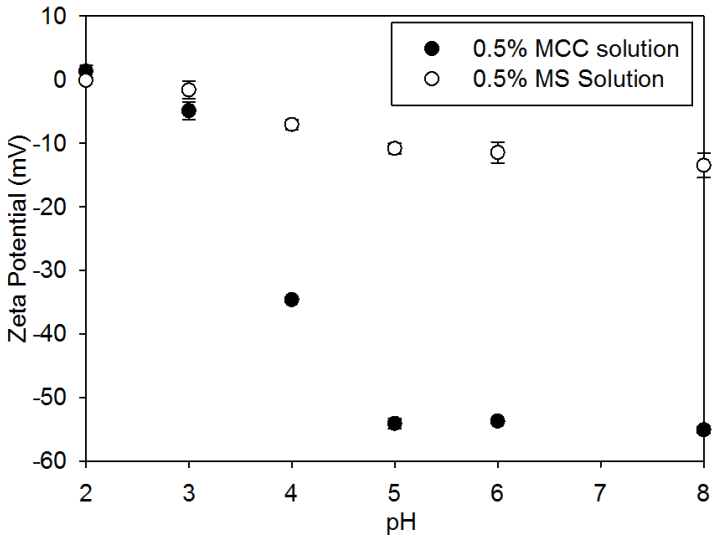


Figure 5- 1: The zeta potential of 0.5% MCC solution and 0.5% MS solution at different pH conditions. Data points represent mean values (n =3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

It was observed that at pH 2, the zeta potential of both MCC and MS particles was nearly zero. An increase in the pH of the system caused an increase in the magnitude of the negative charge of particles (regardless of particle types), due to deprotonation of some of the carboxyl groups in their molecular structure (*243*). The zeta potentials of MCC and MS particles at pH 8 were ~ -55 mV and -14 mV respectively. The MCC particle had a much higher negative charge than the MS particles at all pH values, which could be due to their differences in chemical structure. The linear charge density of the MCC particles might be

higher than the MS particles. Hence, the surface charge of the MCC particles was more negative than MS particles

Optical observations revealed that at pH 2, the particle dispersion became unstable (sedimentation) and large agglomerates of particles were formed in the suspension. However, by increasing the pH of the system, the stability of MCC and MS particles dispersion increased. As can be seen in Figure 5-1, at low pH (pH 2) particles carried no charge; hence, there are no electrostatic repulsive forces between them. Subsequently, particles tend to settle rapidly. While, pH increases the surface of particles becomes negatively charged. Consequently, the electrostatic repulsion forces between particles prevent agglomeration. These results are consistent with data reported elsewhere (*244*).

5.2 Food-Grade Particles Stabilised Emulsions

5.2.1 Effect of Particle Concentration on Physical Stability

A series of preliminary experiments were carried out to determine the minimum concentration of food-grade particles required to be able to produce and stabilise emulsions. 20% oil-in-water emulsions were prepared with different concentrations of either MCC or MS particles (from 0.1% to 2.5%) and their droplet size stability was monitored for 40 days.

Figure 5-2 shows the droplet size distributions of emulsions stabilised with either 0.1% to 2.5% MCC or MS particles. At low MCC or MS particles (0.1% and 0.5%) the size distribution of the emulsion was bimodal and broad. This could be due to the fact that at low particle concentration, the emulsion droplets are less stable and are therefore more likely to coalesce, thus leading to the formation of many different sizes of droplets (mainly large droplets 55.2±0.1 μm). Further increase of food-grade particle concentration (regardless of particle type) leads to a decrease in the width of droplet size distributions and the bimodal

behaviour is no longer present. There is no significant difference between the droplet size distributions when particles concentrations ≥ 1.5%.

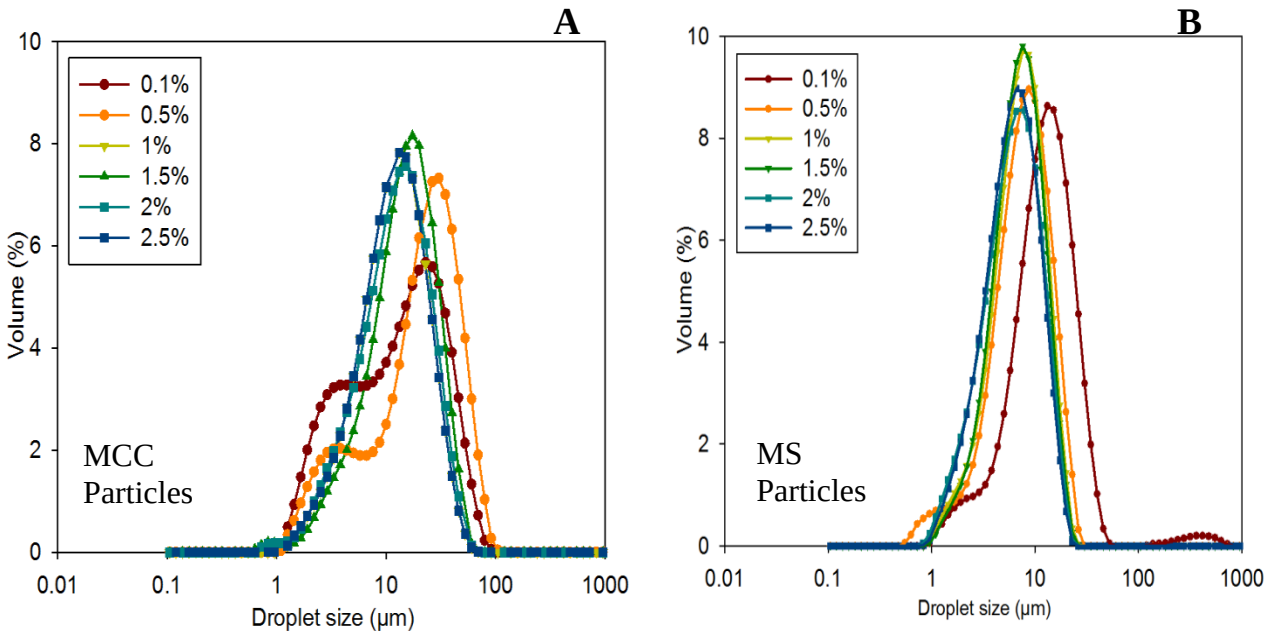


Figure 5- 2: Droplets size distribution of 20% oil-in-water emulsions stabilised with 0.1% to 2.5% (immediately after formation). (A) MCC particles and (B) MS particles. The pH of emulsions is not adjusted. Data points represent mean values (n= 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

The concentration of particles is considered to be a key factor affecting the stability of Pickering emulsions. The droplet size of emulsions in the presence of low MCC particle concentrations (0.1%) increased from ~14.4 µm to ~ 30.7 µm in 7 days. It is suggested that rapid coalescence occurred for emulsions stabilised with low MCC particles concentration. Additionally, visual observation revealed that phase separation occurred for emulsions stabilised with low concentrations of MCC particles (≤ 0.5%) within a short period of time (after 10 days). This can be as a result of insufficient amount of particles available to fully cover the surface of oil droplets and prevent newly formed droplets from coalescing. Similar behaviour was observed for emulsions stabilised with 0.1% MS particles. This emulsion was not stable against coalescence and fully phase separated occurred eventually.

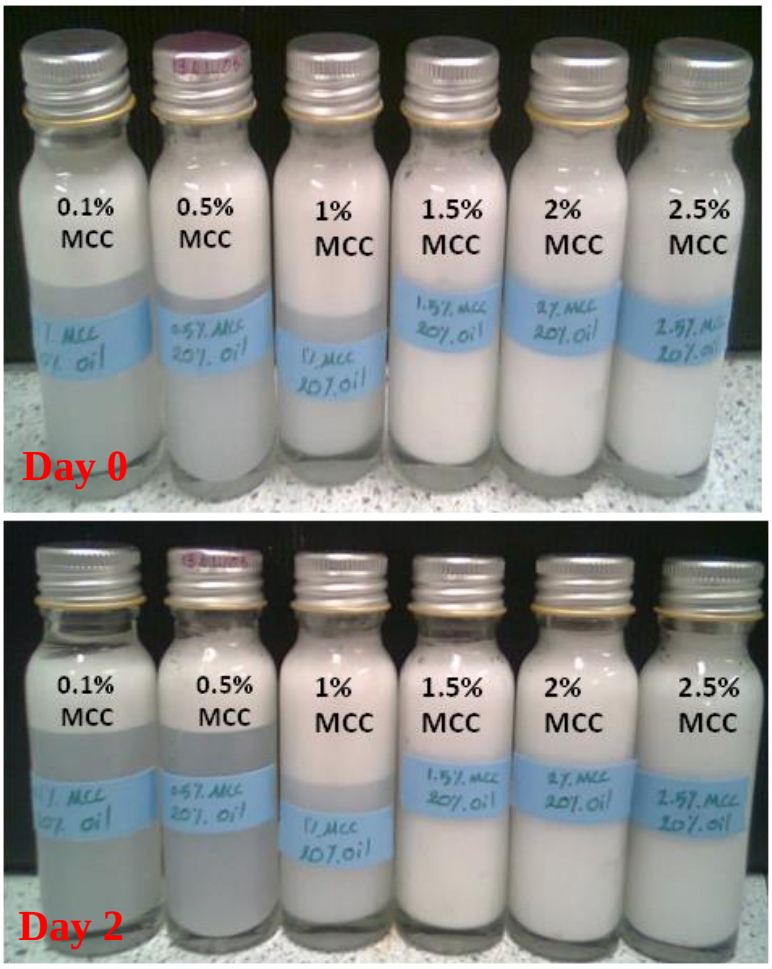
Further increase the particle concentration resulted in a reduction of the droplet sizes and enhancement of the emulsions' stability (regardless of particle type). For instance the droplet size of emulsion stabilised with MS particles was reduced from ~15 μm to ~5 μm as MS particles increased from 0.1% to 2.5%. Furthermore, the droplet size of the emulsion stabilised with 2.5% MS particles did not change over time (40 days), indicating that emulsion droplets were stable against coalescence. By adding more food-grade particles into the system, MCC and MS particles probably migrate to the droplets' interface much faster. Therefore, during emulsification process the rate of particles adsorption to oil-water interface increases (the rate of surface coverage increases), which as a result the protective layer around droplets could be formed faster. Hence, emulsion droplets are more stable against aggregation or coalescence. As can be seen in Table 5-2, further increase in the particle concentration from 1.5% to 2.5% had no effect on the droplets size, indicating that 1% is adequate to produce a stable emulsion.

The droplet size of emulsions stabilised with MS particles were smaller compared to those stabilised with MCC particles. This could be attributed to the differences between their particle sizes. As mentioned in section 5.1.1, MCC particles are larger than MS particles. Thus, their diffusion to the interface is much slower than MS particles. A number of researchers have also reported the influence of particle size on the emulsion droplets size. For instance, Binks *et al.*, (2001) reported the average emulsion droplet diameters initially increased from 35 μm to 75 μm by increasing particle diameter 0.81 and 2.7 μm, (*66*).

Table 5- 2: Droplet size of emulsions stabilised with different concentrations of MCC or MS particles. The pH of emulsions was not adjusted. The error bars represent at least 3 different measurements on the same sample.

		$D_{3,2}$ (μm)		
Particle Concentration (%)	**Particle Type**	**Day 0**	**Day 21**	**Day 40**
0.1	MCC	14.4±0.4	----	----
0.5		11.5±0.2	----	----
1		10.4±0.01	10.4±0.1	10.3±0.06
1.5		10.1±0.03	10.1±0.02	10.1±0.2
2		8.9±0.01	8.8±0.2	8.7±0.3
2.5		10.1±1.0	10.3±0.07	10.3±0.03
0.1	MS	14.5±0.9	22.1±0.9	----
0.5		8.1±0.1	7.8±0.09	9.4±0.4
1		5.2±0.6	6.3±1.8	6.4±0.4
1.5		5.1±0.02	5.5±0.07	5.9±0.1
2		6.4±0.06	6.6±0.1	7.7±0.3
2.5		5.1±0.01	5.0±0.02	6.2±03

The creaming stability of emulsions as a function of particle concentration (MCC or MS particle) was also studied for 2 days. Figure 5-3 and Figure 5-4 show images of the creaming behaviour and creaming index of emulsions stabilised with various concentrations of MCC or MS respectively.



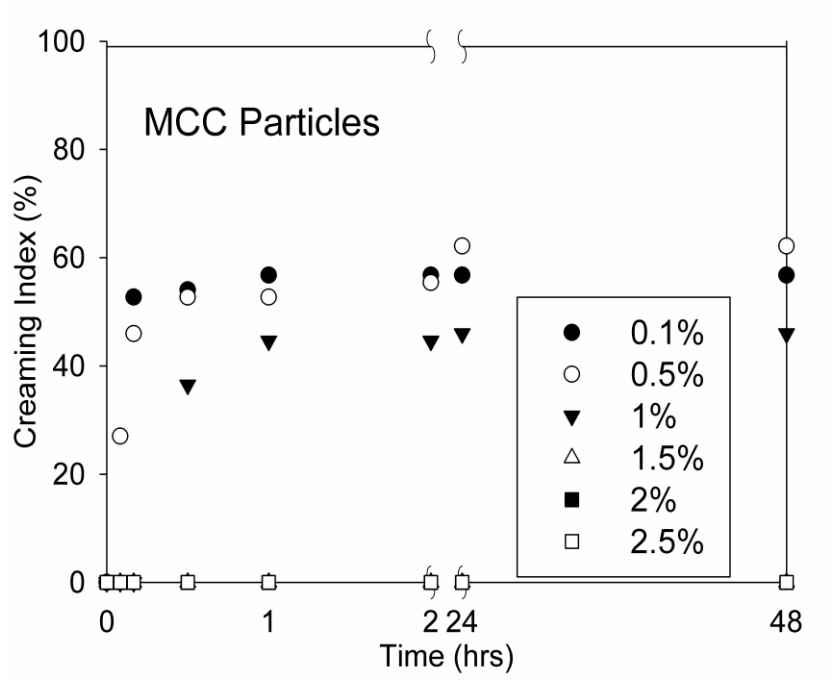
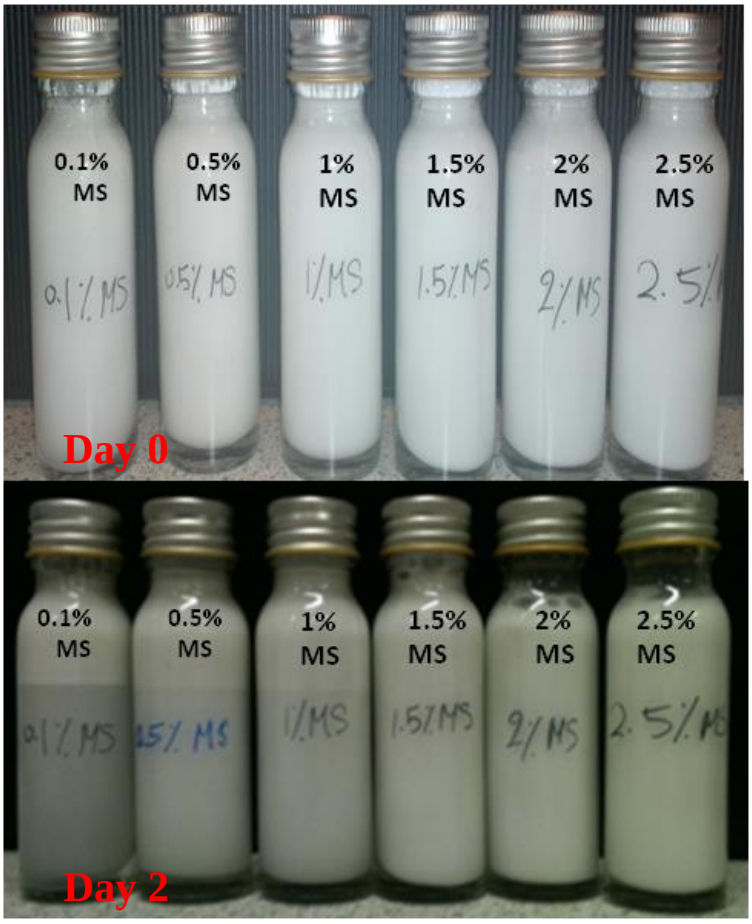


Figure 5- 3: Creaming stability of 20% oil-in-water emulsions stabilised with different concentrations of MCC particles (0.1% to 2.5%). The pH of emulsions was not adjusted. Data points represent mean values (n=3) ± standard deviation. Where not visible error bars are smaller than symbols.



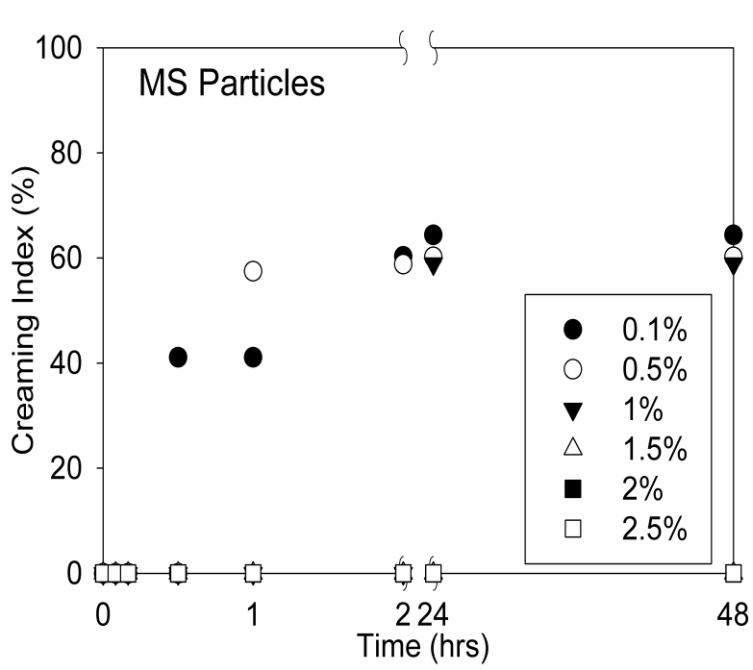


Figure 5- 4: Creaming stability of 20% oil-in-water emulsions stabilised with different concentrations of MS particles (0.1% to 2.5%). The pH of emulsions was not adjusted. Data points represent mean values (n=3)± standard deviation. Where not visible error bars are smaller than symbols.

Optical observation indicated that at low particle concentrations (0.1% and 0.5%), emulsions creamed immediately after emulsification. However, no creaming was observed for emulsions stabilised with high particles concentrations (≥1.5%) (Figure 5-3 and Figure 5-4). The creaming stability increased with increasing particle concentration from 0.1% to 1.5%, but increasing the particle concentration above 1.5% had no further significant effect on creaming stability (regardless of particle type).

As previously discussed (chapter 2), gravitational separation (creaming) in the emulsions depends on the density contrast between the oil and aqueous phases (based on Stokes law equation). The creaming rate could decrease as the concentration of particles increased by minimizing the density contrast between the droplets and the aqueous phase and reducing the size of emulsion droplets. These can explain why no creaming was observed for emulsions stabilised with high concentrations of particles *(245).*

5.2.2 Effect of Charge of Particles on Coalescence and Creaming Stability

As mentioned in section 5.1.1 the zeta potential (charge) of both MCC and MS particles depends strongly on the pH of the system. In order to understand the impact of charge of particles on physical stability of emulsions, 20% oil-in-water emulsions stabilised with "food-grade" particles (either MCC or MS particles) at different pH conditions were prepared. Results shown in section 5.2.1 indicated that a 1.5% particle concentration (in both cases) was sufficient to produce stable emulsions. Thus, this concentration was chosen for both MCC and MS particles to study the effect of particle's charge on emulsion stability. The droplet size distribution and stability of 20% oil-in-water emulsions stabilised with either 1.5% MCC or MS as a function of pH (2, 4, 6 and 8) was monitored for 40 days. The obtained data are given in Figure 5-5 and Table 5-4 respectively.

As mentioned in section 5.1.2, visual observation revealed that at pH 2 (zeta potential is nearly zero), MCC dispersions became cloudy which could indicate particle aggregate. As a result under this pH condition, MCC particles could no longer stabilise emulsions. This can be attributed to the absence of electrostatic repulsion between the oil droplets, which leads to flocculation. This observation is in agreement with a previous study by Yaginuma *et al*., (2006), who reported that the MCC particles become relatively unstable and aggregate into large floccs at low pH values due to the decrease in the electrostatic repulsion force between them (*246*). Therefore, no droplet size distribution data are available for MCC particles stabilised emulsions under this pH condition. As described earlier (Chapter 2), in Pickering emulsions, solid particles diffuse to the interface and remain there in order to form rigid structure that delay the coalescence of emulsion droplets. Moreover, charged particles at the interface can also induce a degree of electrostatic repulsion, which will further enhance the stability of Pickering emulsion.

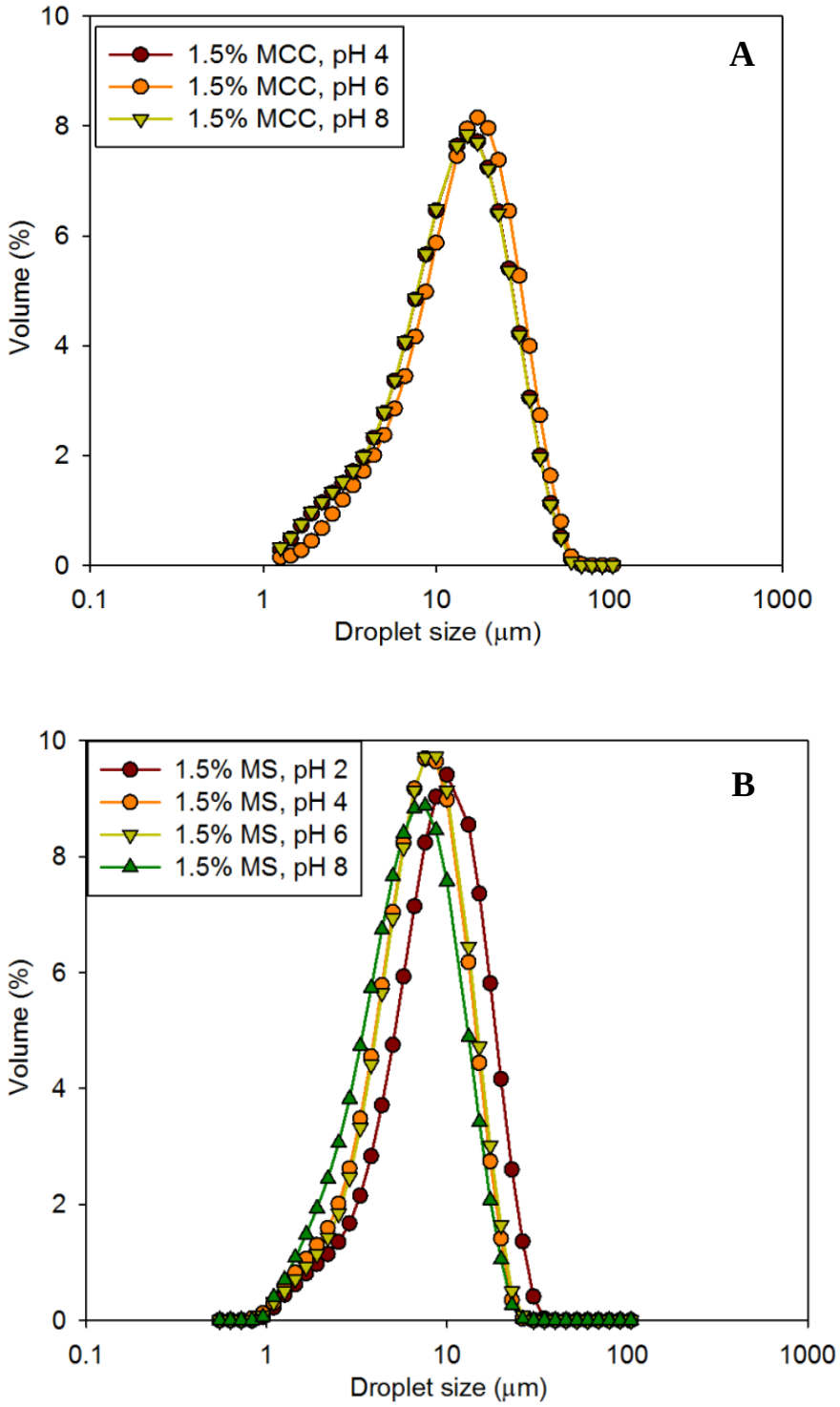


Figure 5- 5: Droplets size distribution of 20% oil-in-water emulsions stabilised with 1.5% food-grade particles as a function of pH (immediately after preparation). (A) MCC particles and (B) MS particles. Data points represent mean values (n= 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

Table 5- 3: Droplet size of oil-in-water Pickering emulsions containing 1.5% either MCC or MS particles at different pH conditions. The error bars represent at least 3 different measurements on the same sample.

		$D_{3,2}$ (μm)		
pH	**Particle Type**	**Day 0**	**Day 21**	**Day 40**
2	MCC	---	---	---
4		9.2±0.02	9.2±0.05	9.4±0.10
6		9.6±0.01	9.4±0.40	9.6±0.10
8		8.9±0.10	9.1±0.20	9.1±0.20
2	MS	5.5±0.20	11.6±1.10	----
4		5.7±0.02	5.4±0.05	5.9±0.17
6		5.2±0.07	6.2±0.80	6.4±1.7
8		4.7±0.80	5.0±0.03	5.4±0.20

As can be seen in Figure 5-5 increasing the pH of the system had no significant effect on droplet size distribution. At pH ≥ 4, the droplet size distribution of the emulsions was monomodal and narrow. Furthermore, as Table 5-3 shows there is no significant difference between the droplet size of emulsions stabilised with 1.5% MCC particles at various pH conditions (pH 4, 6 and 8).

Similar behaviour was observed for emulsions stabilised with MS particles at pH 2, which progressively become unstable (Table 5-3). The droplet size of these emulsions significantly increased from 5.5±0.2 μm to 11.6±1 μm after 20 days which suggests coalescence occurred. The zeta potential of the MS particles was practically zero at pH 2 (Figure 5-1), which indicates that the MS particles are not charged. Hence, there is no

electrostatic repulsive force on the droplet surface to keep the droplets apart and hinder coalescence.

As shown in Table 5-3, the MS stabilised emulsions showed 40 days stability against coalescence when the pH of the system is high (pH≥4) (regardless of the particle type). Since emulsion droplets are covered with an ionic food-grade particles (negatively charged) at pH≥4, they carry similar charges, hence, these charged droplets repel each other due to electrostatic interaction, which prevents droplet aggregation or coalescence.

The creaming stability of emulsions stabilised with food-grade particles as a function of pH was studied. No creaming was observed for emulsions at high pH (pH≥4) (regardless of particle type). However, 1.5% MS stabilised emulsions at pH 2 creamed immediately after formation. This can be attributed to uncharged particles forming aggregates, which no longer reside at the oil-water interface. Hence, these large aggregates of particles have the tendency to settle out of the system.

In order to visualise the structure of the interfacial layer around droplets, Cryo-SEM was used. Figure 5-6 and Figure 5-7 show series of SEM micrographs of droplets stabilised by MCC and MS particles respectively. It appears that food-grade particles (either MCC or MS particles) are located at the droplets' interfaces. In addition, It is seems that nonadsorbed particles are also located in the continuous phase. Thus, the long-term stability (40 days) of emulsions stabilised with food-grade particles against coalescence could be due to the arrangement of particles at the droplet interface and the existence of particles flocs in the continuous phase.

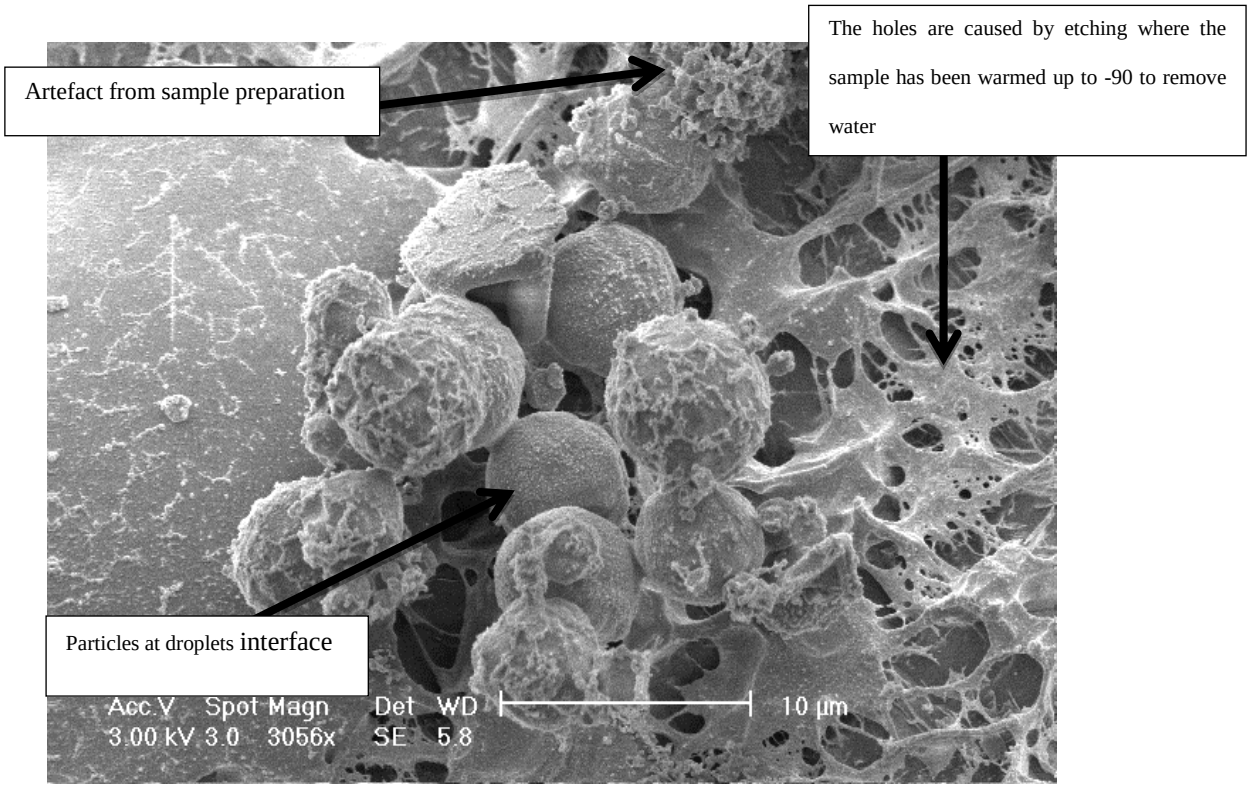


Figure 5- 6: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by 1.5% MCC particle.

The structure of the interfacial layer around droplets stabilised with MCC and MS particles at different pH conditions (pH 4 and 6) was also viewed with Cryo-SEM. There was no significant difference between the structures of the emulsion droplets at different pH values (regardless of particles type).

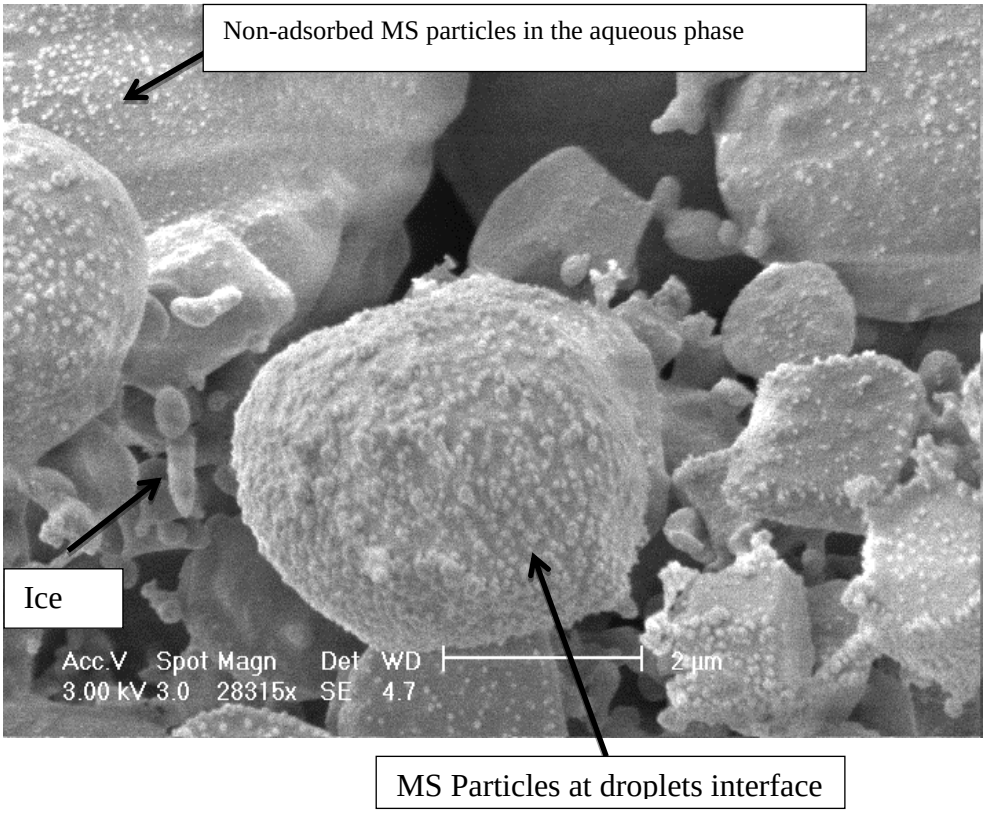


Figure 5- 7: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by 1.5% MS particles.

5.3 Oxidative Stability of Food-Grade Particles Stabilised Emulsions

In order to determine the ability of food-grade particles to inhibit lipid oxidation rate, 20% oil-in-water emulsions were prepared with different concentrations of food-grade particles (0.5% to 2.5%) at two different pH conditions (pH 4 and 6) and their lipid oxidation rates were measured for 7 days. As mentioned in section 5.2.2, emulsions at pH 2 are unstable but emulsions at pH ≥4 are stable. Moreover, emulsions prepared at pH ≥6 had similar droplet sizes (regardless of particle types). Consequently, pH values of 4 and 6 were chosen to study the oxidative stability. All emulsion samples were kept at 40 °C immediately after emulsion formation, for 7 days to accelerate the rate of lipid oxidation. The primary and secondary lipid oxidation products were measured using the PV and p-AV method as

described in section 3.4. Many studies have been conducted to examine the relationship between oxidative stability and droplet size (interfacial area) of oil-in-water emulsions (*247*) (*182*). As mentioned in chapter 4, there are different arguments presented in the literature regarding the impact of droplet size on oxidative stability. Thus, in order to eliminate of the effect of droplet size on lipid oxidation the hydroperoxide formation divided by the total surface area of the droplets (as described in section 4.2).

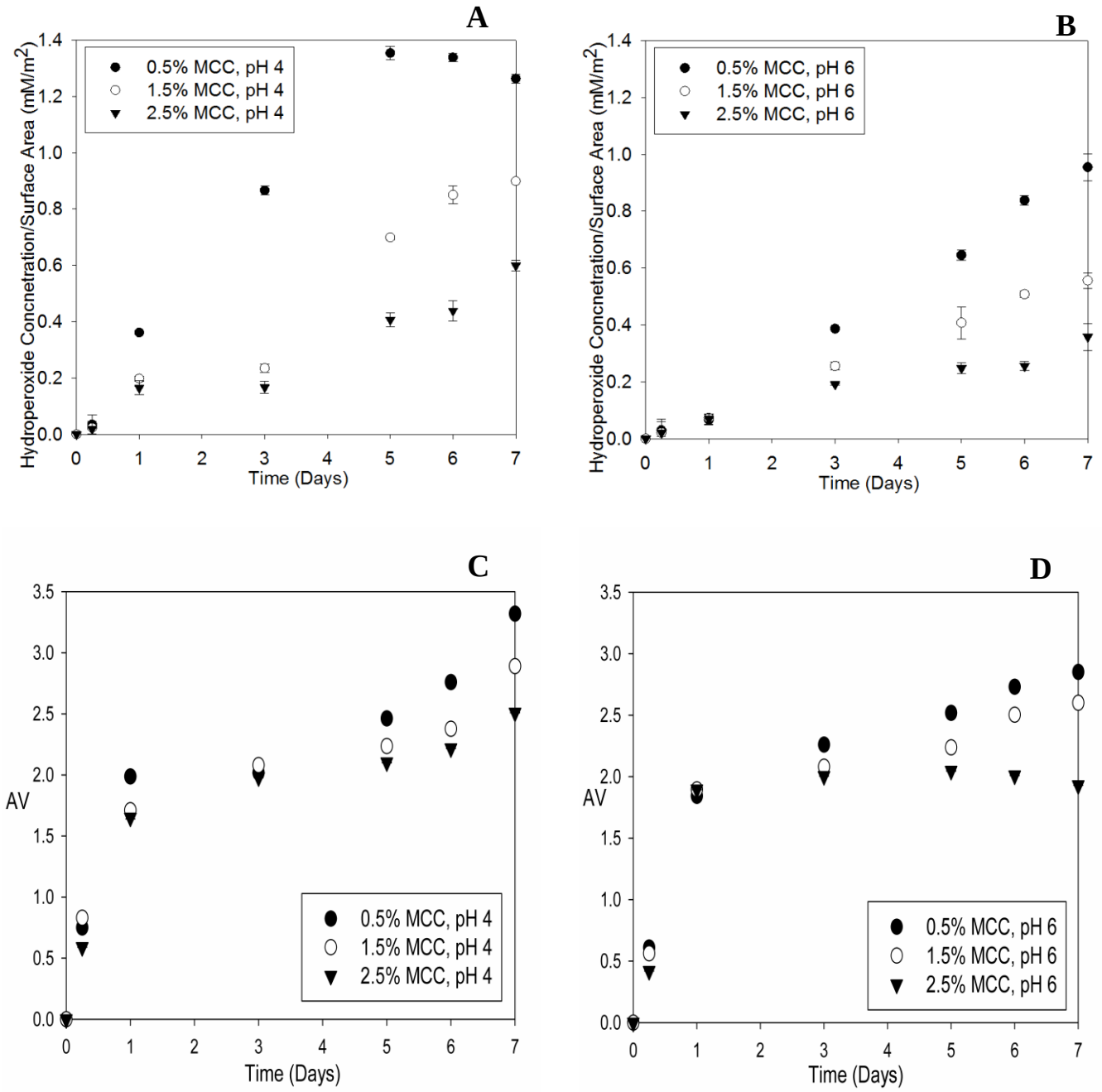


Figure 5- 8: Effects of MCC concentration on lipid oxidation rate of 20% oil-in-water emulsions. Formation of lipid hydroperoxide /surface area in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MCC at pH 4 (A) and pH 6 (B). Formation of secondary products in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MCC at pH 4 (C) and pH 6 (D) respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

As shown in Figure 5-8, lipid oxidation occurred in the oil-in-water emulsion (Figure 5-8), with a lag phase of lipid hydroperoxides and AV formation of 1 day. By progressively increasing the MCC concentration from 0.5% to 2.5%, a decrease in the rate of lipid oxidation (both primary and secondary products) at both pH 4 and 6 values was

observed. The reaction rate between of AV formation is slower than hydroperoxides formation. Unlike hydroperoxides, aldehydes (secondary lipid oxidation product (AV)) do not decompose rapidly. This is because lipid hydroperoxides must first be formed before they decompose into secondary products. Oza and Frank (1986) reported that the MCC particles are able to form physically stable emulsions due to their arrangement at the droplet interface (*87*). The presence of MCC particles at the droplets interfaces was confirmed in the SEM micrograph (Figure 5-6). Therefore, the data presented here clearly suggest that MCC particles at the droplets interfaces are able to increase the lipid oxidation stability by preventing close contact between pro-oxidants in the continuous phase and hydroperoxides located at the droplet interface. As a result, the pro-oxidants-hydroperoxides interactions inhibited. Probably at low particle concentration (0.5%) there are not enough particles available in the system to fully cover the droplets interface. Therefore, it is possible that hydroperoxides at droplets interface are more accessible to pro-oxidants.

By increasing the concentration of MCC particles in the system, not just the transport of colloidal particles to the oil-water interface would be further facilitated, but the amount of non-adsorbed MCC particles present in the aqueous phase of the emulsions would also increase. This can result in the formation of a MCC network in the aqueous phase. These MCC networks in the continuous phase are negatively charged (Figure 5-1), which could suggest that the non-adsorbed MCC particles in the continuous phase could hinder the lipid oxidation rate by binding to positively charged pro-oxidants in the continuous phase, thus further promoting a reduction in the rate of lipid oxidation. Figure 5-8 shows the lipid oxidation rate of MCC particles stabilised emulsion at pH 6 was lower than those prepared at pH 4. At pH 6 the MCC particles are more negatively charged than at pH 4 (Figure 5-1), therefore, under these conditions particles are more effective in binding with positively charged pro-oxidants.

Figure 5-9 shows the lipid oxidation rate of emulsions stabilised by 0.5%-1.5% MS particles at both pH 4 and 6. Similarly to emulsions stabilised with MCC particles, the lipid oxidation rate is higher for emulsions stabilised with low particle concentration (0.5%) regardless of the pH of the system. The presence of MS particles at droplets interface may influence the lipid oxidation rate by holding metal ions away from the surface which reduces interaction between hydroperoxides (located at droplet interface) and pro-oxidants in continuous phase.

There is no significant difference between lipid oxidation rate of emulsions stabilised by MS particles at pH 4, and those prepared at pH 6. This is perhaps due to the small change in the zeta potential (charge) of MS particles induced by increasing the pH of the system form pH 4 to 6. It was previously shown (Figure 5-1) that at pH 4 and 6 the surface charges of the MS particles are -7.1± 0.8 and -11.5±1.6 respectively which suggests that the surface charge of MS particles is relatively similar under these pH conditions.

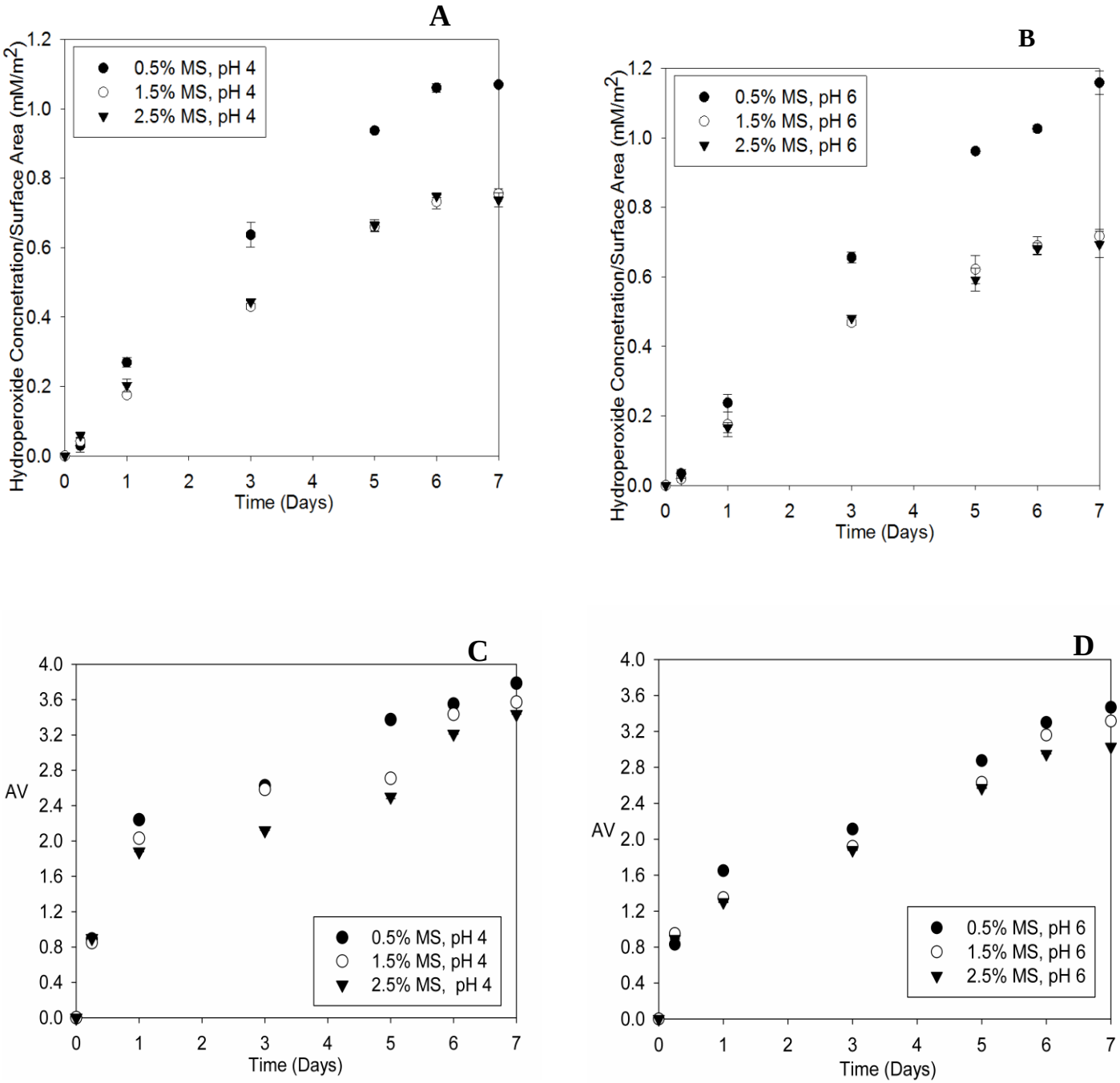


Figure 5- 9: Effects of MS particles concentration on lipid oxidation rate of 20% oil-in-water emulsions. (A&B) Formation of lipid hydroperoxide/ surface area in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MS particles at pH 4 and pH 6 respectively pH 6 (C & D) Formation of secondary products in 20% sunflower oil-in-water emulsions stabilised with 0.5%-2.5% MS particles at pH 4 and pH 6 respectively. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

In order to compare the oxidative stability of emulsions stabilised with food-grade particles to those stabilised with Tween 20, the hydroperoxide formation/surface area for each emulsion was plotted in Figure 5-10.

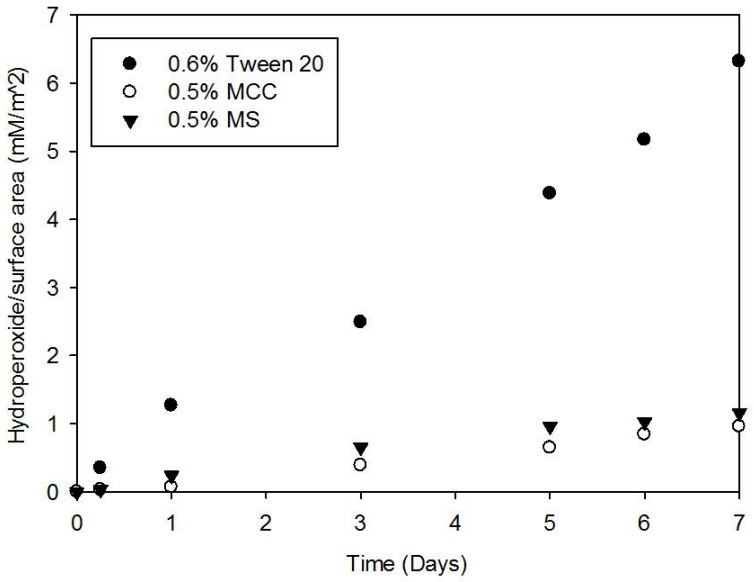


Figure 5-10: Formation of hydroperoxide normalised to total oil droplets surface area for 20% oil-in-water emulsions.

Figure 5-10 shows that both food-grade particles (MCC and MS) appear to reduce the rate of lipid oxidation to a much greater extent than emulsion stabilised with Tween 20. For instance, the hydroperoxide formation/surface area for 0.5% MS particles stabilised emulsions increased from 0.1 to 1.1 mM/m^2. However, the hydroperoxide/surface area of those stabilised with 0.5% Tween 20 increased from 0.3 to 6.3 mM/m^2. It appears that food-grade particles could reduce lipid oxidation up to 80%. This might be due to the ability of particles to transfer to oil-water interface and providing much thicker interfacial layer which could leads to inhibit pro-oxidants-hydroperoxides interactions.

As shown in Figure 5-10, MCC particles are very similar with MS particles in reducing lipid oxidation. Moreover, they both showed good stability against coalescence for

40 days. Thus, based on these results, not just food-grade particles could produce stable emulsions but also could significantly reduce lipid oxidation.

5.4 Conclusions

The work carried out in this chapter showed that, by using food-grade Pickering particles to stabilise emulsion, a significant reduction in lipid oxidation can be achieved. This was attributed to existence of particles at droplets interface which protected the hydroperoxides at oil droplets from interacting with metal ions in the continuous phase. This is simply a microstructure design which results in a decrease in the rate of lipid oxidation.

Both the aqueous dispersions of food-grade particles (MCC and MS) and the physicochemical stability of oil-in-water emulsions in the presence of these particles (MCC or MS) under different processing conditions were investigated. Both MCC and MS particles in an aqueous phase had no ionic charge at pH 2, hence they aggregated into large floccs and became unstable. However, food-grade particles solution became more stable as the pH increased regardless of particle type. The magnitude of the negative charge of both particles increases as the pH increased

Both MCC and MS particles provided stable emulsions (up to 40 days) due to the existence of particles around droplets interface. Emulsion droplet size decreased by increasing particle concentration, probably as a result of faster adsorption of particles at the droplet interface which reduces the risk of re-coalescence during the emulsification process.

Emulsion stability depended on the pH of the aqueous dispersion. Food-grade Pickering emulsions were unstable at low pH (pH 2), however, as the pH of the system increased (pH≥4), emulsions showed much greater stability against coalescence (for up to 40 days). This has been interpreted as the food-grade particles are uncharged at pH 2 and there is

no electrostatic repulsive force acting between the droplets. As a result, the droplets tend to coalesce. However, an increase in the pH of the system results in negatively charged food-grade particles being present at the interface, which increases the electrostatic repulsion force between the droplets.

Food-grade particles stabilised emulsions were also found to be stable against lipid oxidation due to presence of particles around droplets, which hinders interaction between reactive species that promote lipid oxidation. Moreover, non-adsorbed negatively charged particles in the continuous phase could also contribute to the lower lipid oxidation rate, by binding positively charge metal-ions.

Chapter 6:Physical and Oxidative Stability of Oil-in-Water Emulsions Stabilised by Both Food-Grade Particles and Low Molecular Weight Surfactant

Many commercial emulsion formulations contain a mixture of both surfactants and particles (*57*). Hence it is important to understand the stabilisation mechanism and properties of these emulsions. Much is known about the properties of emulsions stabilised with small molecular surfactants and more recently, those stabilised solely by colloidal particles. However, very little is known about the physicochemical properties of emulsions stabilised by both surfactants and colloidal particles. To extend this area of research, the stabilisation mechanism of 20% oil-in-water emulsions stabilised with both food-grade particles and small molecular surfactant was studied and presented in this chapter.

As shown in chapter 5, MCC and MS food-grade Pickering particles are capable of producing stable emulsions with low lipid oxidation rate. Therefore, in order to prepare

particle-surfactant stabilised emulsions, MCC and MS particles are selected as the colloidal particles and Tween 20 is chosen as a surfactant due to its high HLB value (16.7) which results in the formation of stable oil-in-water emulsions.

The aim of this chapter is to study the effect of particle-surfactant stabilised emulsions on physical and oxidative stability. More specifically, it was investigated whether Tween 20 surfactant impact the packing of particles at the droplets interface? The emulsion stability is discussed as a function of various parameters, such as the Tween 20 concentration, particle type and pH of the system. Furthermore, emulsions in the presence of the both food-grade particles and small molecule surfactant at different processing conditions are compared in terms of their physicochemical stability, to those where only stabilised by either surfactants or colloidal particles.

6.1 Surfactant Stabilised Emulsions

Initially, the physical stability of emulsions stabilised only by the surfactant (Tween 20) was investigated as a function of Tween 20 concentration and pH. The droplet size was measured (in triplicates) immediately after emulsification and also after 21 and 40 days.

Figure 6-1 shows the droplet size stability of 20% oil-in-water emulsions stabilised by 0.1% to 2% Tween 20 at pH 4 and 6. Emulsions stabilised with 0.1% Tween 20 at both pH 4 and 6 were found to be unstable against coalescence and the droplet size of these emulsions increased over time (Figure 6-1). However, emulsions prepared with Tween 20 concentrations of 0.5% and above were stable against coalescence and there were no changes in the droplet size of these emulsions over time. These results are in agreement with what was reported previously (*250, 251*).

It has been reported that increasing the small molecular surfactant concentration leads to decrease in the interfacial tension which allows more droplet break up during emulsification process. Therefore, an increase in Tween 20 concentration may decrease the interfacial tension, thereby facilitating droplet break-up during emulsification. However, this reduction in the interfacial tension with increasing surfactant concentration continues until the interface is saturated with surfactant molecules. Thus, at this point no further decrease in interfacial tension is possible (*250*).

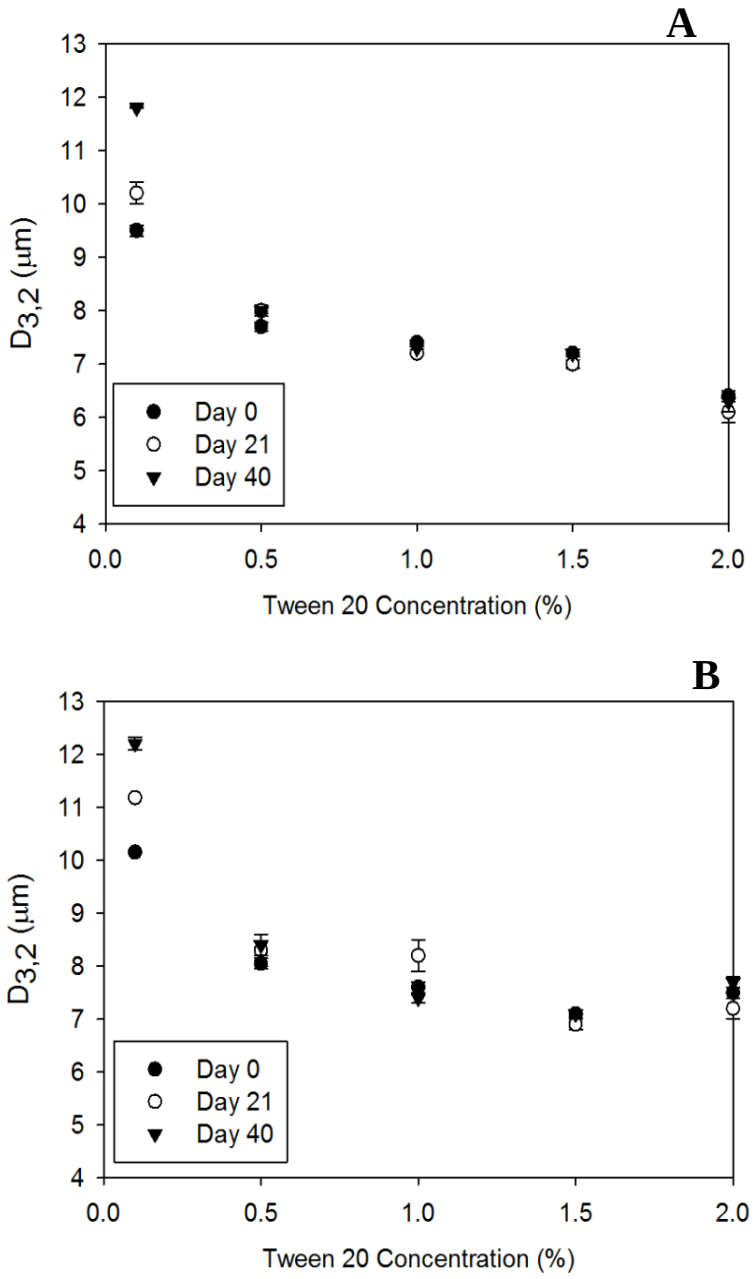


Figure 6- 1: Droplet size stability of 20% oil-in-water emulsion stabilised with 0.1% to 2% Tweeen 20 at (A) at pH 4 and (B) pH 6. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

The creaming stability of 20% oil-in-water emulsion stabilised with different Tween 20 concentrations (0.1% to 2%) at pH 4 and 6 was also investigated. Figure 6-2 shows, the rate of creaming is the fastest at low Tween 20 concentrations (0.1% and 0.5%), and decreases at higher surfactant concentrations (1%, 1.5% and 2%). Moreover, visual

inspection has shown that the continuous phase of emulsions stabilised with high Tween 20 surfactant (>0.5%) became "cloudier". It has been reported that Tween 20 in the aqueous solution forms micelle at concentration above 0.05% (*252*). Therefore, the cloudiness of continuous phase of emulsions with high concentration of Tween 20 could be due to the existence of Tween 20 micelles in the continuous phase.

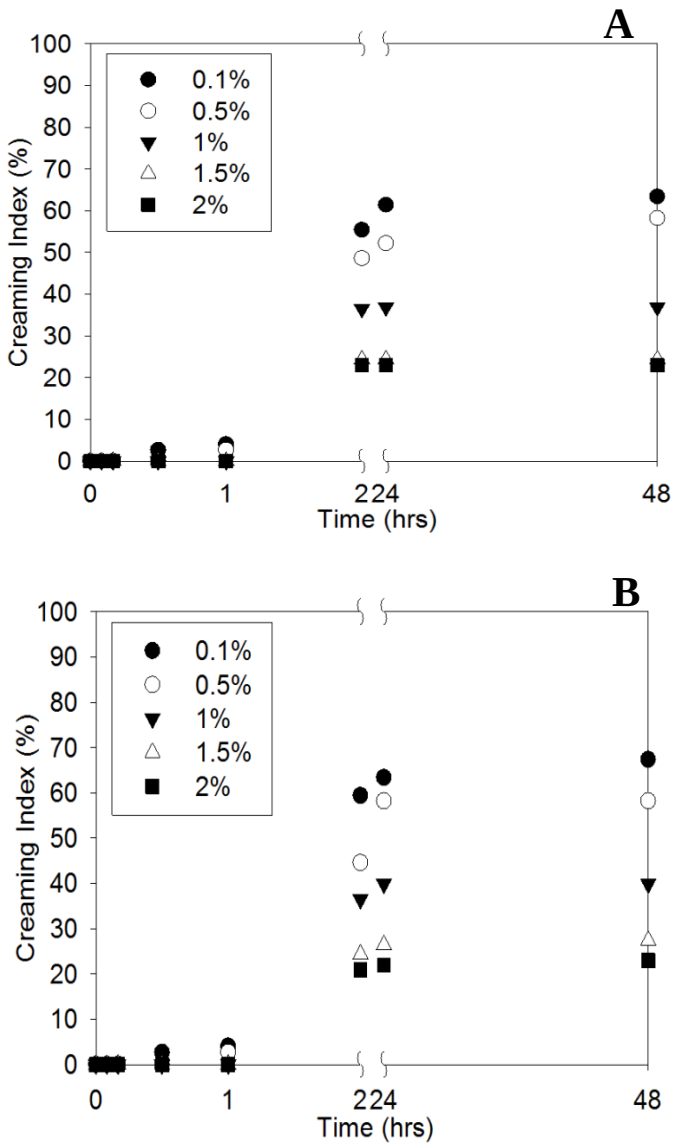


Figure 6- 2: Effects of Tween 20 concentration on the creaming stability of 20% oil-in-water emulsions. Emulsions stabilised with (0.1% to 2%) Tween 20 at (A) pH 4 and (B) pH 6. The error bars represent at least 3 different measurements on the same sample.

6.2 Particles-Surfactant Stabilised Emulsions

6.2.1 Physical Stability

The physical stability of oil-in-water emulsions in the presence of both food-grade particles (MCC or MS) and surfactant (Tween 20) at different pH conditions (pH 4 and 6) was initially investigated. The Tween 20 concentration was varied from 0.1% to 2% while the concentration of food-grade particle (MCC or MS particles) was kept constant at 1.5%.

Figure 6-3 and Figure 6-4 show the droplet size distribution of emulsions prepared with 1.5% food-grade particles and various Tween 20 concentrations at different pH conditions. It can be seen from Figure 6-3 and Figure 6-4 that the droplet size distribution shifted to the lower droplet size as Tween 20 concentration increased regardless of the particle types. The final droplet size of an emulsion is based on break up and re-coalescence mechanism during emulsification process. These results suggest that re-coalescence mechanism could hinder as Tween 20 concentration increased from 0.1% to 2% due to more efficient surface coverage. Moreover, there is no significant difference between the droplet size distributions of emulsions stabilised with both 1.5% MCC particles and various concentrations of Tween 20 at pH 4 and those prepared at pH 6. Similarly, the pH had no significant effect on droplet size distribution of emulsions stabilised with 1.5% MS and different concentrations of Tween 20.

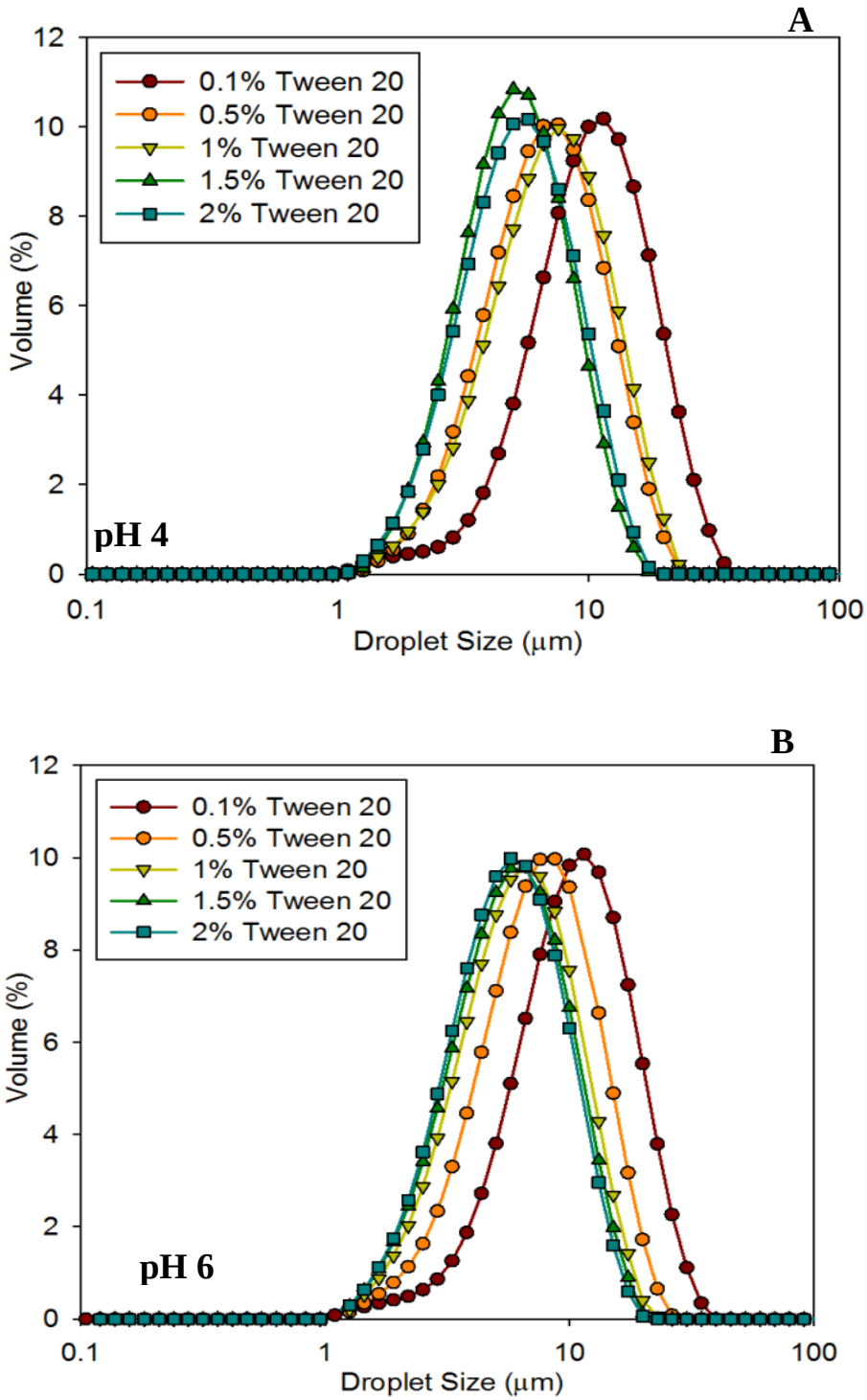


Figure 6- 3: Droplet distribution of 20% oil-in-water emulsions stabilised with both 1.5% MCC particles and different Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6. The error bars represent at least 3 different measurements on the same sample.

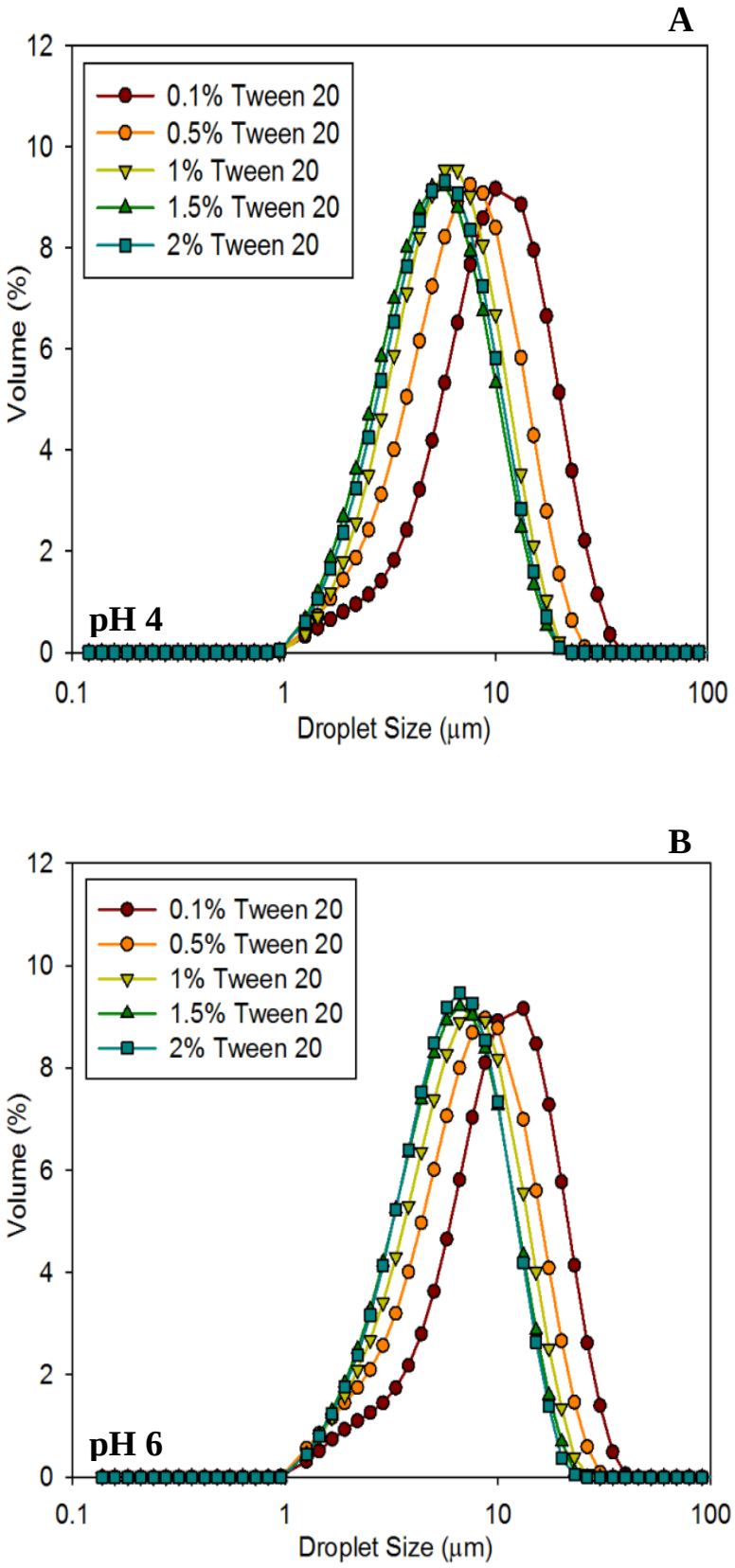


Figure 6- 4: Droplet distribution of 20% oil-in-water emulsions stabilised with both 1.5% MS particles and different Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6.The error bars represent at least 3 different measurements on the same sample.

The stability of droplets against coalescence was also studied for 40 days and the obtained data are given in Table 6-1. As it can be seen, the use of both particles and surfactants to prepare mixed emulsifier systems has resulted in the production of stable emulsions with small droplet size for all processing conditions. As shown in Table 6-1 for emulsions prepared with either 1.5% MCC or 1.5% MS particles, their droplet sizes decreased with increasing Tween 20 concentrations, and a constant droplet size was achieved for Tween 20≥1.5%. Table 6-1 shows, the pH of the system had no significant effect on droplets size, hence Tween 20 concentration was the only parameter that has influence on the emulsion droplet size. In all cases, no oil layer was observed at the top of the emulsions for at least 40 days. The most noticeable effect of particle-surfactant stabilised emulsions was the absence of coalescence for all oil-in-water emulsions even after 40 days. This is in contrast to the instability which occurs in emulsions solely stabilised with low concentrations of Tween 20 surfactant (section 6.2) or food-grade particles (section 5-2). This is in agreement with previously reported results for mixed-emulsifier emulsions (*81*).

As mentioned in chapter 2 the role of the low molecular surfactant is to assemble quickly at the formed interfaces. Hence initially Tween 20 surfactant adsorbs at droplets interface, lowers interfacial tension and reduces the energy needed to break up the large droplets into smaller ones (enhance break up during emulsification process). This should provide enough time for particles to migrate to the interface of newly formed droplets and cover the interface. As a result the newly formed droplets are more stable against coalescence during emulsification process. Wang *et al*., (2010) reported the long-term stability of emulsions was poor with Span 80 (surfactant) or Laponite particles alone, however, emulsion stability remarkably improved by the combination of Laponite particles and Span 80 surfactant. They found that the droplet size of emulsions stabilised with both Laponite particles and Span 80 surfactant gradually decreased as the Span 80 concentration increased.

They argued that both Laponite particle and Span 80 surfactant stayed at the oil-water interface and resisted emulsion coalescence (*77*). It has been reported that surfactant can adsorb onto particle surfaces and modify the surface charge and wettability of particles. The interaction of these two components, strongly depends on the characteristics of both the particles (size, shape, charge density, etc.) and surfactant (type, charge, etc.) used. The interaction of surfactant on the particle surface is due to the number of forces such as the electrostatic force, covalent bonding, nonpolar interactions, and hydrophobic interactions *(79-82).* Since the non-ionic surfactant (Tween 20) was used in this study it is possible that the particles and surfactant interacting through hydrophobic forces. Therefore, non-ionic Tween 20 micelles might adsorb to the food-grade particle surface.

Table 6- 1: Mean droplet size ($D_{3,2}$) of 20% oil-in-water emulsions stabilised with both 1.5% food-grade particles (MCC or MS) and various Tween 20 (0.1% to 2%) concentrations at pH 4 and 6. The error bars represent at least 3 different measurements on the same sample.

			$D_{3,2}$ (µm)		
Tween 20 Concentration (%)	**Particle Type**	**pH**	**Day 0**	**Day 21**	**Day 40**
0.1	MCC	4	8.8±0.01	8.90±0.05	8.70±0.01
0.5			6.08±0.03	6.10±0.10	6.10±0.02
1.0			6.2±0.02	6.40±0.01	6.30±0.06
1.5			5.01±0.02	4.90±0.02	5.10±0.07
2.0			4.8±0.01	4.80±0.05	4.70±0.03
0.1	MCC	6	9.02±0.05	8.70±0.10	8.90±0.05
0.5			6.60±0.10	6.50±0.01	6.70±0.01
1.0			5.40±0.03	5.50±0.02	5.60±0.04
1.5			5.20±0.02	5.10±0.07	5.20±0.10
2.0			4.90±0.10	5.01±0.10	5.02±0.03
0.1	MS	4	8.10±0.20	8.20±0.06	8.40±0.30
0.5			5.90±0.10	6.01±0.03	5.90±0.20
1.0			5.1±0.02	5.30±0.08	5.10±0.01
1.5			4.7±0.01	4.70±0.07	4.60±0.10
2.0			4.4±0.03	4.60±0.10	4.50±0.01
0.1	MS	6	7.80±0.20	8.02±0.10	8.10±0.20
0.5			6.20±0.03	6.10±0.10	6.20±0.08
1			5.70±0.10	5.60±0.07	5.60±0.03
1.5			5.30±0.04	5.10±0.09	5.20±0.10
2			5.20±0.10	5.01±0.05	5.20±0.02

In order to compare the droplet sizes of particle-surfactant stabilised emulsions (mixed-emulsifier system) with emulsions stabilised with particles or surfactant only (solo emulsifier system), the droplet size of each system as a function of emulsifier concentration is plotted in Figure 6-5.

Figure 6-5 shows the droplet sizes of emulsions stabilised with both surfactant and particles are much smaller than those stabilised by either Tween 20 surfactant or food-grade particles. As previously discussed, surfactants are able to rapidly cover the oil interfaces, reduce interfacial tension (promote break up stage) and make small droplets. While particles assemble at the interfacial layer and form a densely packed layer around oil droplets thus, prevents small freshly formed droplets from coalescing. During emulsification, the Tween 20 surfactant adsorbs to the surface of freshly formed droplets and reduces the interfacial tension which facilitates the further breakdown of the droplets. Hence, at this stage small droplets are formed which are temporarily stable against coalescence. At the same time, less mobile particles move to the droplets' interface and cover the oil droplets surfaces which protect those small droplets from further coalescence *(253).* Therefore, particles-surfactant emulsions are able to produce long-term stable emulsion with smaller droplet sizes than solo emulsifier emulsions (samples stabilised by either particles or surfactant alone). It is suggested that the reason for the observed stability of the emulsions is the existence of both food-grade particles and Tween 20 surfactant located at the droplets' interface. Therefore, the mixed emulsifier systems lead to synergistic stabilisation.

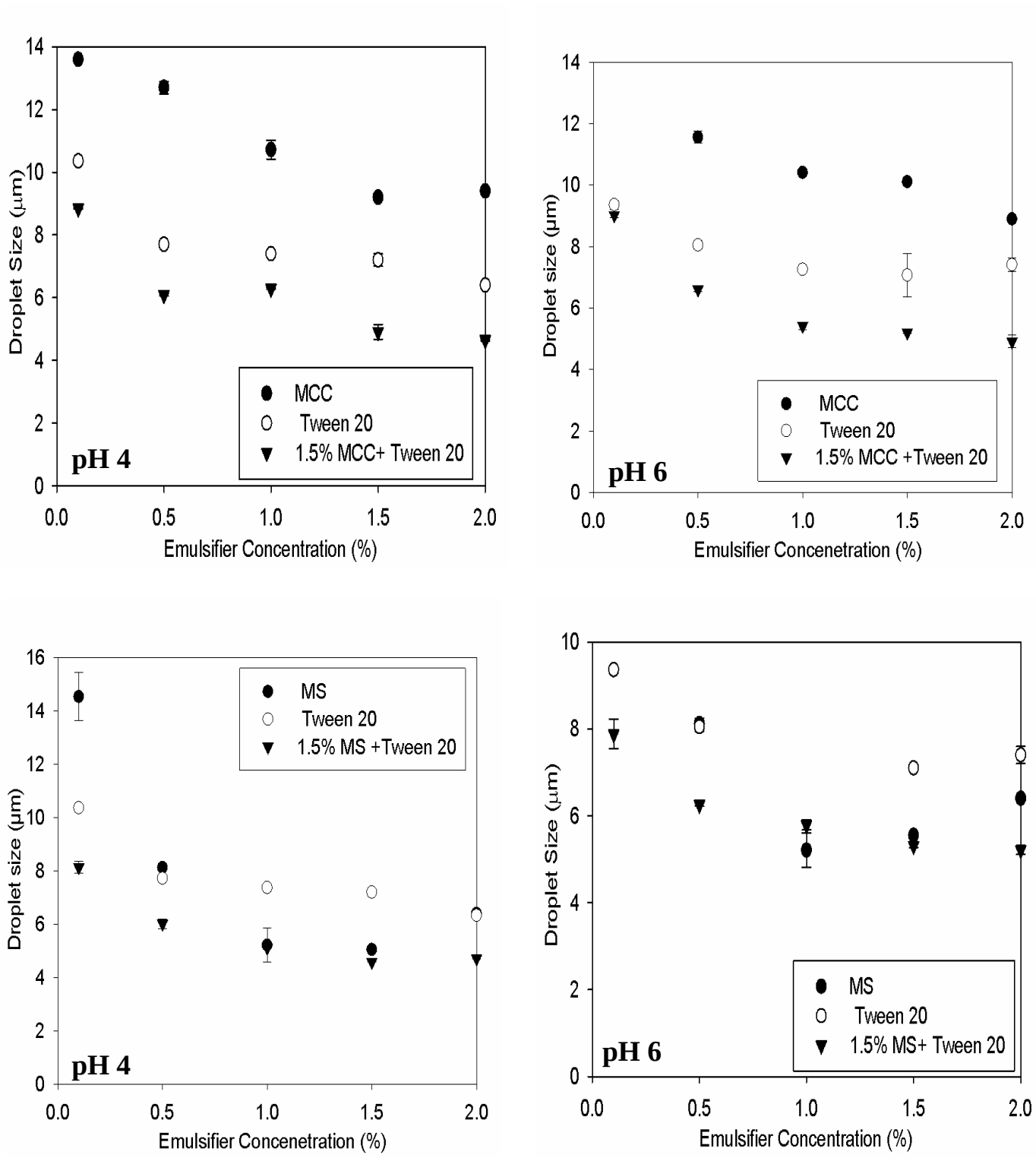


Figure 6- 5: Average emulsion droplet size as a function of emulsifier concentrations and pHs. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

The existence of particles at droplet interface was confirmed in SEM micrographs of emulsions containing 1.5% food-grade particles (MCC or MS) and 2% Tween 20 surfactant (Figure 6-6).

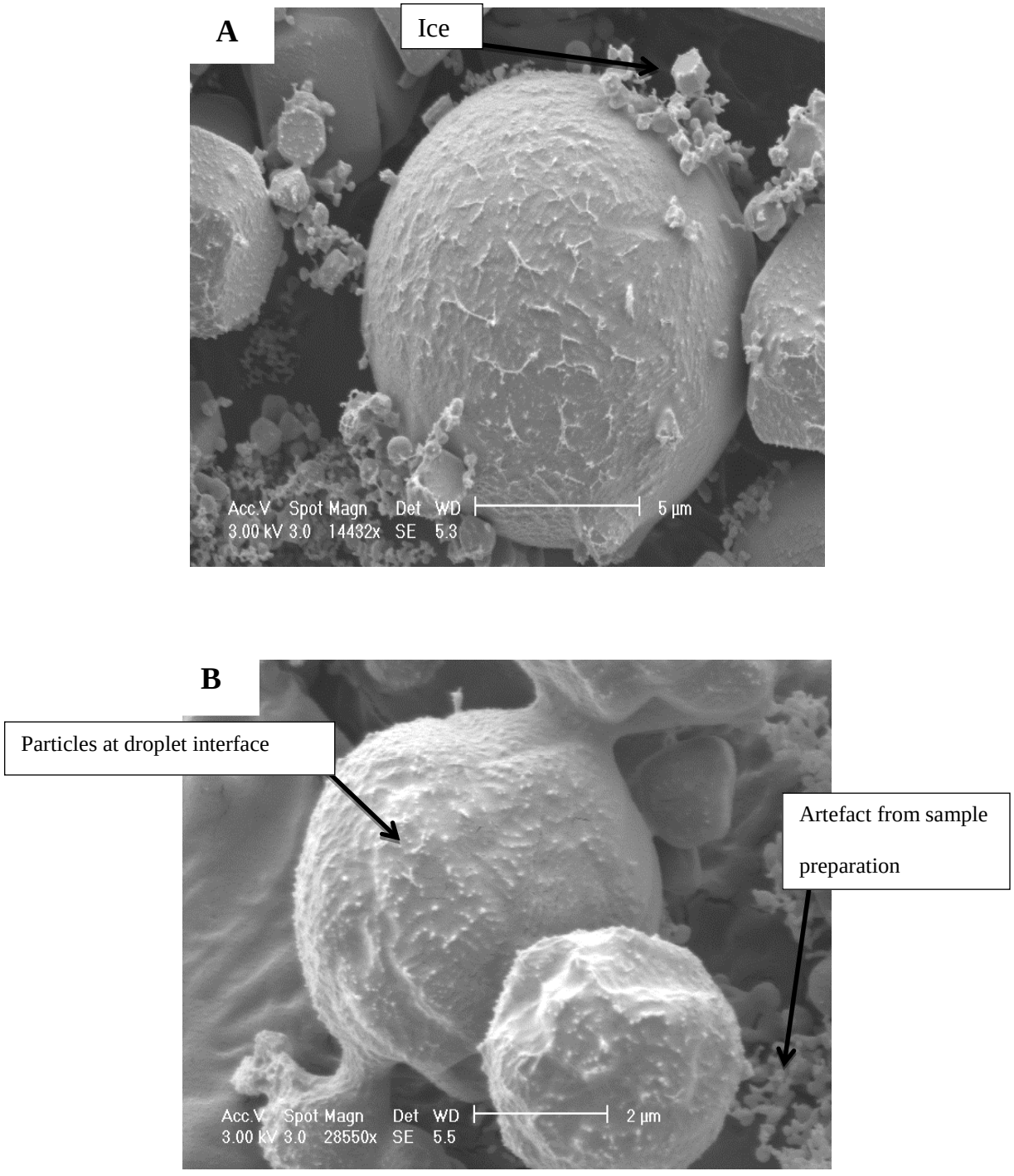


Figure 6- 6: SEM micrographs of 20% oil-in-water emulsion droplets stabilised by (A) 1.5% MCC particles and 2% Tween 20 surfactant at pH 4 (B) 1.5% MS particles and 2% Tween 20 surfactant at pH 4.

Figure 6-6 shows that particles are still present at the droplet interface even when the concentration of surfactant (Tween 20) was higher than the concentration of particles (regardless of particle type and pH of system). It appears that increasing the Tween 20 concentration from 0.5% to 2% did not completely remove all particles from the droplet interface. These observations are in good agreement with data from droplet size of emulsions stabilised with both food-grade particles and Tween 20 surfactant (Table 6-1). Pichot *et al.*, (2010) reported that the stability of mixed-emulsifier system (1% silica particles and various Tween 60 concentrations) depends on the surfactant concentration. They found that the droplet size of mixed-emulsifier systems initially decreased at low Tween 60 and increased for intermediate Tween 60 concentration and argued that this complex behaviour was due to the reduction of numbers of particles at the interface. Hence, further increase in Tween 60 concentrations led to the removal of all particles at the droplets' interface and stability was only provided by Tween 60 surfactant at droplet interface *(253).* However, the results obtained in this chapter indicate that the particles remained at the droplets' interface, even at high Tween 20 concentration. Moreover, emulsions stabilised with both particles and Tween 20 showed long term stability against coalescence regardless of Tween 20 concentrations (Table 6-1). It has been reported that, the particle attachment energy and contact angle determine how difficult it is to remove the particles from the oil-water interface (*57*). These results could suggest that the particle attachment energy of MCC and MS particles is higher than silica particle, thus more energy needed to remove all food-grade particles from the oil-water interface (*252*). SEM images were obtained for emulsions solely stabilised by food-grade particles (MCC or MS) revealed that the particles formed a close-packed (mono) layer around the oil droplets (Section 5.5.2). However, SEM images for emulsions stabilised with food-grade particles and various concentration of surfactant did not show the formation of a densely packed structure around droplets interface (Figure 6-6). This could be due to the

fact that in the mixed-emulsifier systems (particle-surfactant stabilised emulsions) both food-grade particles and Tween 20 surfactant existed at interface. Thus, number of food-grade particles at droplets interface reduced (Tween 20 surfactant removed number of particles from interface) thereby closely packed structure was not formed around droplets interface. Probably, the unadsorbed food-grade particles were present in the continuous phase.

The creaming stability of particle-surfactant stabilised emulsions was also investigated. No creaming was observed in emulsions containing 1.5% MCC particles and various Tween 20 concentrations (irrespective of the pH) (Figure 6-7).

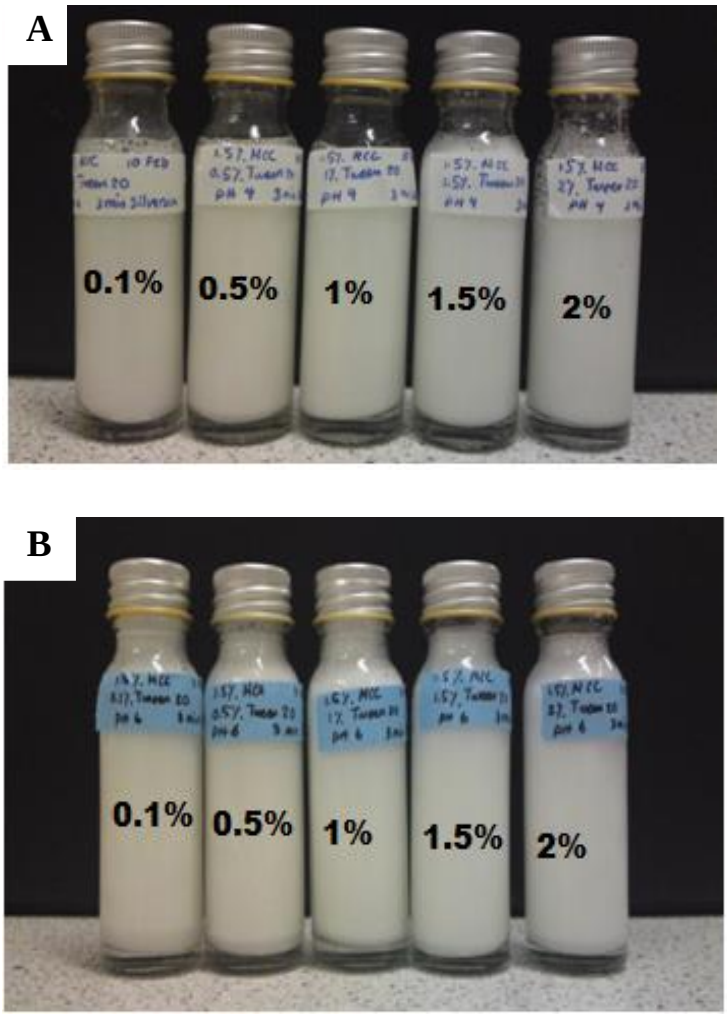


Figure 6- 7: Effect of increasing Tween 20 concentrations on the cream layer of 20% oil-in-water emulsions stabilised with 1.5% MCC particles and various Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6 after 3 days.

However, visual observation of oil-in-water emulsions stabilised by both 1.5% MS particles and various Tween 20 concentrations revealed that increasing Tween 20 concentration resulted in an increase in the cream layer (Figure 6-8).

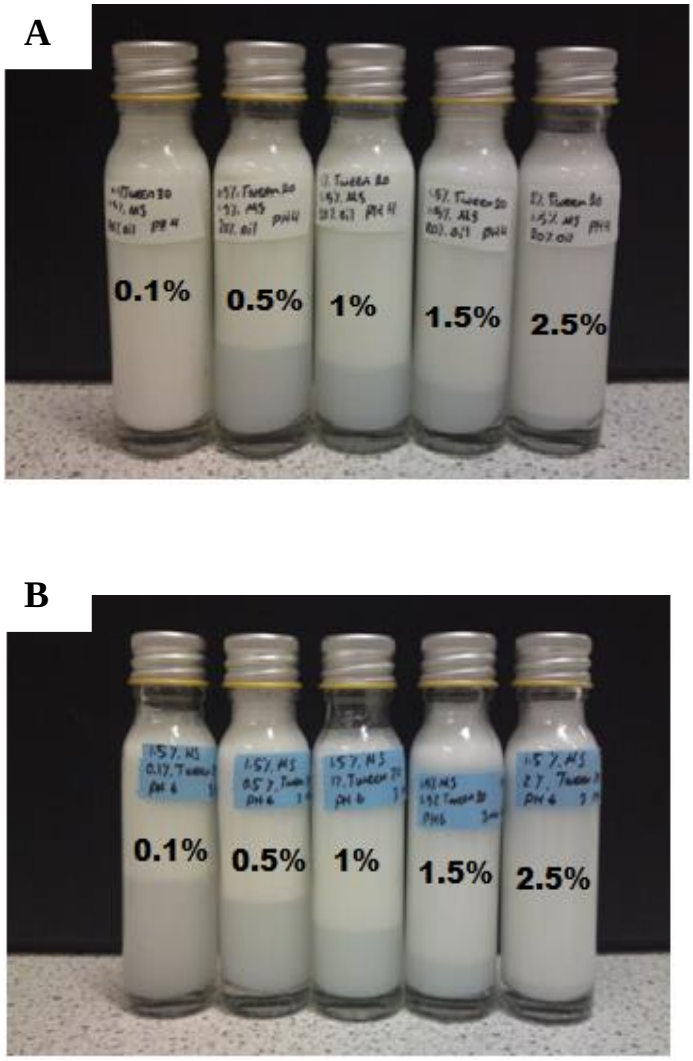


Figure 6- 8: Effect of increasing Tween 20 concentrations on the cream layer of 20% oil-in-water emulsions stabilised with 1.5% MS particles and various Tween 20 concentrations (0.1% to 2%) at (A) pH 4 and (B) pH 6 after 3 days.

6.2.2 Oxidative Stability

The rate of lipid oxidation was measured in the emulsions containing both surfactant (from 0.5% to 2% Tween 20) and food-grade particles (1.5% MCC or MS) in order to investigate the effect of particle-surfactant stabilised emulsions on the lipid oxidation rate. As

described in section 3.4 all samples were kept at 40 °C immediately after emulsion formation for 7 days to accelerate oxidation. Many research groups have studied the effect of droplets size or surface area on the lipid oxidation rate (*5, 254*). In order to eliminate the effect of droplets size on lipid oxidation, the hydroperoxide formation, divided by the total surface area as described in chapter 4 (section 4.2).

Figure 6-9 and Figure 6-10 show that increasing the Tween 20 surfactant concentration from 0.5% to 2% results in a decrease in the rate of lipid oxidation regardless of the particle types used.

The critical micelle concentration (CMC) of Tween 20 surfactant is very low (2×10^{-5}M) (*255*), which means high concentration of surfactants in the system are not associated with the emulsion's droplet interface therefore, would likely form micelles in the continuous phase. Hence, increasing Tween 20 concentration increased Tween 20 micelles in the continuous phase. It is well known that surfactant micelles could impact the physical location of metal ions in oil-in-water emulsions (*8*). Nuchi *et al*., (2002) reported that surfactant micelles solubilised lipid hydroperoxides out of emulsion's droplets which could decrease free radicals in the lipid droplets due to the hydroperoxide decomposition (*210*). Therefore, surfactant micelles may increase the partitioning of lipid hydroperoxides out of emulsion' droplets. Che *et al*., (2002) also argued that surfactant micelles inhibited lipid oxidation through their ability to solubilise iron and remove it from emulsion's droplets (*209*). Consequently, surfactant micelles could alter the physical location and pro-oxidant activity of iron in oil-in-water emulsions. As a result, lipid oxidation rate decreased as the hydroperoxide-pro-oxidants interaction reduced.

By comparing Figure 6-9 with Figure 6-10 it can be seen that emulsions prepared with both MCC particles and Tween 20 showed a slightly lower lipid oxidation rate in 7 days compared with those stabilised with both MS particles and Tween 20. As mentioned in

chapter 5, MCC and MS particles are different from each other in terms of charge, size and chemical structure. As previously discussed, MS particles are less negatively charged than MCC particles (Figure 5-1). Therefore, the unadsorbed MCC particles in the continuous phase can be more effective in binding with positively charged metal ions through their higher negative charge. This could explain why lipid oxidation rate is slightly lower for emulsions prepared with MCC particles and Tween 20 compared with those stabilised with MS particles and Tween 20.

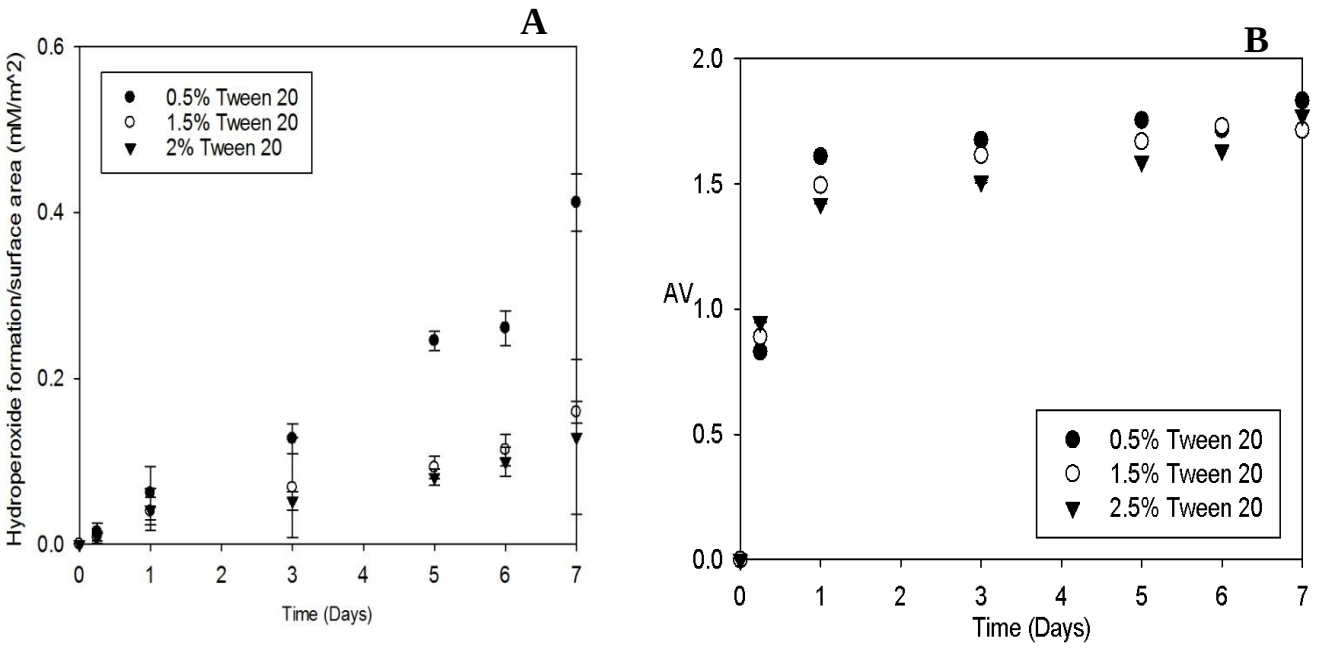


Figure 6- 9: Effects of mixed-emulsifier system on lipid oxidation rate of 20% oil-in-water emulsions stabilised with both 1.5% MCC particles and (0.5% to 2% Tween 20): (A) Formation of hydroperoxide/surface area (B) Secondary lipid oxidation products. The pH of emulsions was not adjusted. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

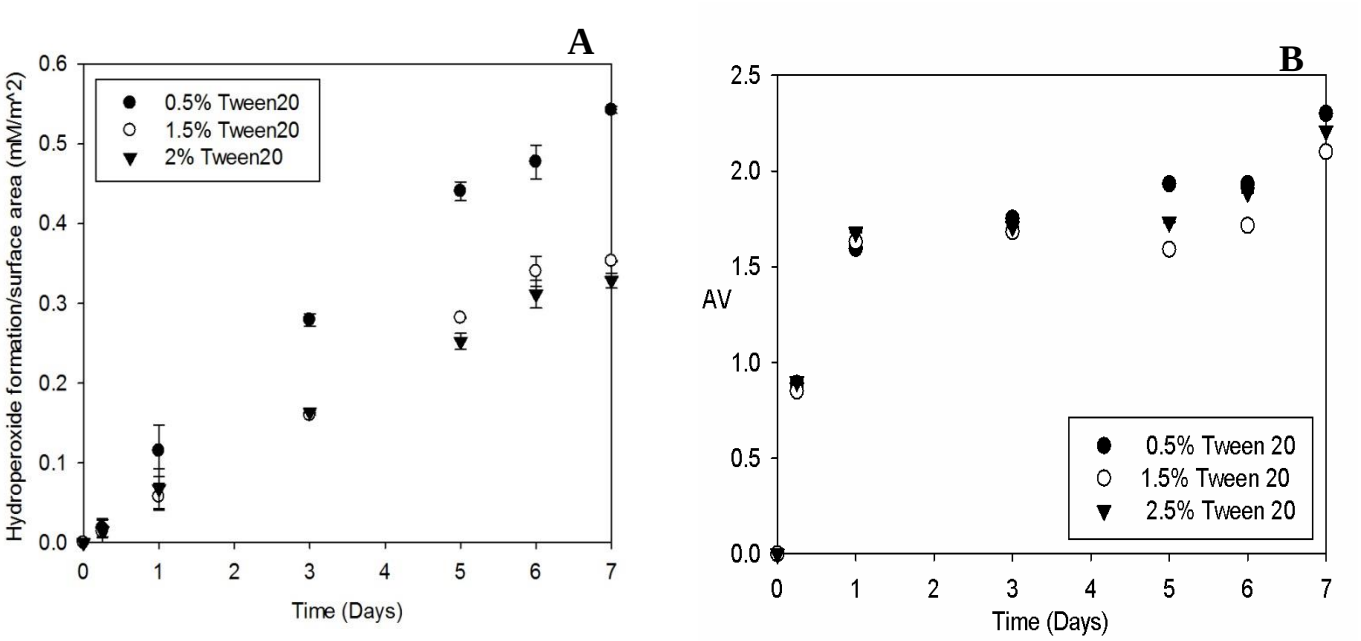


Figure 6- 10: Effects of mixed-emulsifier system on lipid oxidation rate of 20% oil-in-water emulsions stabilised with both 1.5% MS particles and (0% to 2% Tween 20): (A) Formation of hydroperoxides/surface area and (B) Secondary lipid oxidation products. The pH of emulsions was not adjusted. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

In order to find out how effective is particle-surfactant stabilised emulsions in enhancing oxidative stability, the lipid oxidation rate of emulsions stabilised by both particles and Tween 20 was compared to the lipid oxidation rate of emulsions stabilised with either particles or Tween 20 alone (Figure 6-11). It can be seen that the oxidative stability of emulsions decreases in the order of particles-Tween 20 stabilised emulsions > food-grade particles stabilised emulsions > Tween 20 stabilised emulsions. As mentioned earlier, the droplets interfacial layer thickness has a great impact on lipid oxidation rates. Increasing the thickness of interfacial layer can hinder the interaction between pro-oxidants and hydroperoxides. Food-grade particles can form thicker layer around the oil droplets compared to those stabilised with Tween 20. The presence of particles at droplets interface may reduce the lipid oxidation rate by preventing hydroperoxides-metal ions interactions. Therefore, the lipid oxidation rate of emulsions stabilised by particles is much slower than those stabilised by surfactant only. For emulsions stabilised by both particles and Tween 20 not only the

particles at the droplets interface enhanced the oxidative stability by holding metal ions away from the surface but also the Tween 20 micelles in the continuous phase can reduce the lipid oxidation by reducing the pro-oxidant (metal ions) activity due to their ability to solubilise ions and remove them from emulsion's droplet. Moreover, unadsorbed particles in the continuous phase can act as effective chelating agents due to their negative charge. As a result the lipid oxidation rate was much slower for emulsions stabilised with both particles and surfactant compared with those stabilised by either Tween 20 or particles (solo emulsifier).

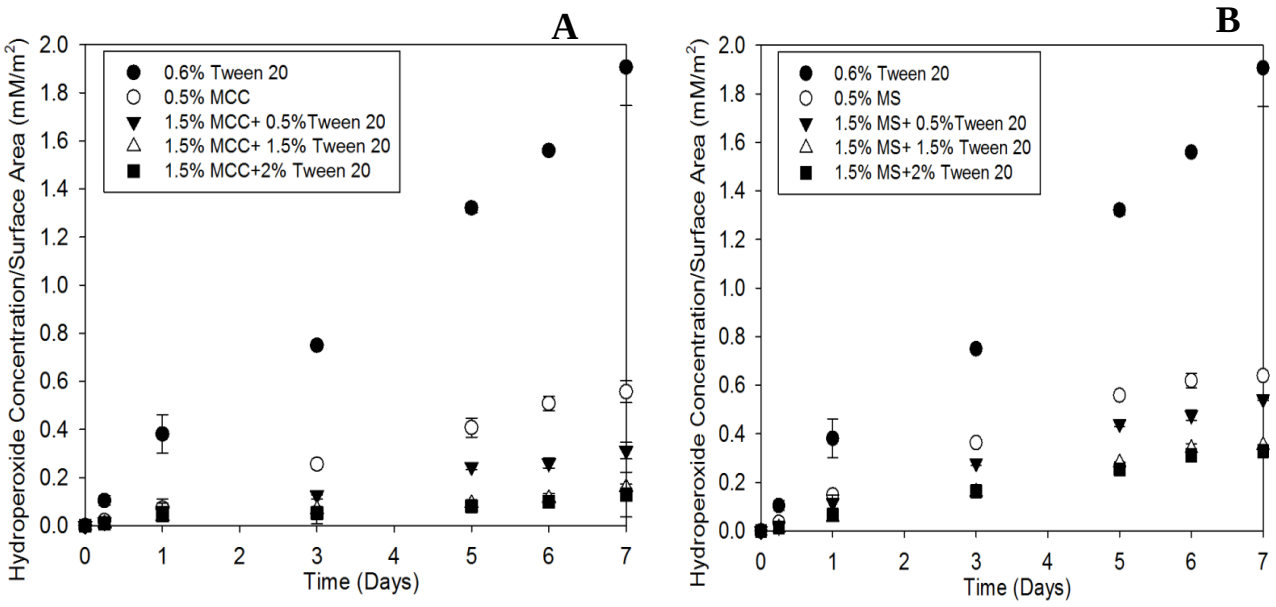


Figure 6- 11: Formation of hydroperoxide normalised to total oil droplets surface area for emulsions stabilised with both 1.5% particles and (0.5 to 2%) Tween 20, emulsions stabilised by either 0.6% Tween 20 alone and emulsions stabilised with 0.6%. The error bars represent at least 3 different measurements on the same sample.

6.3 Conclusions

In this chapter, coalescence and lipid oxidation phenomena in oil-in-water emulsions stabilised with both surfactant (Tween 20) and food-grade particles (MCC or MS) have been investigated.

Particles-surfactant stabilised emulsions showed long term stability against coalescence regardless of particles type and surfactant concentration. It was observed that the droplets size of emulsions stabilised with both particle and surfactant were much smaller than droplets size of emulsions stabilised by either surfactants or particles only. This is because; during emulsification process surfactant (Tween 20) can rapidly adsorb at droplet interface, reduce interfacial tension, form small droplets and induce further droplet break-up. Consequently, this provides enough time for MCC or MS particles to cover the droplets interface to protect small newly formed droplets from coalescence. It was proposed that the great stability of the emulsions was due to the existence of both food-grade particles and Tween 20 surfactant at the droplets' interface.

Lipid oxidation rate in emulsions stabilised by both food-grade particles and Tween 20 was much slower than emulsions solely stabilised with particles or surfactant. This could potentially be due to the ability of particles at droplets interface to separate pro-oxidants from the surface of droplets and also the presence of Tween 20 micelles in the continuous phase which could alter the physical location and pro-oxidants activity of ions in oil-in-water emulsions. Furthermore, unadsorbed negatively charged food-grade particles in the aqueous phase seem to be able to reduce the lipid oxidation through binding with positively charged pro-oxidants.

Chapter 7:Physical and Oxidative Stability of Oil-in-Water Emulsion Stabilised with Fat Crystals

An alternative approach to manipulate the emulsions' interfacial microstructure is to use fat crystals to produce a "perfect crystal shell" around each droplet. The presence of fat crystal "shell" at droplets interface can potentially inhibit lipid oxidation rate by preventing the transition metals from coming into close contact with oil droplets.

The fat crystal particles can form a solid-like network in the continuous phase (network stabilisation) or adsorb at the oil-water interface (Pickering stabilisation). Triglyceride crystals (found in fully saturated fats) are able to form a solid-like network in the system. However, saturated monoglycerides (MG) are able to stabilise by a Pickering mechanism, which each droplet is protected by an adsorbed layer of fat crystals (*21*). Consequently, in this chapter saturated monoglycerides was used to stabilise oil-in-water emulsion.

The main objective of this chapter is to determine whether fat crystal "shells" (monoglycerides crystals) at the droplets' interface are capable of increasing the physical and oxidative stability of the emulsion. Initially, melting and crystallisation points of different

concentration of MG are studied using Differential Scanning Calorimetry (DSC). Then 20% oil-in-water emulsions stabilised with different concentrations of MG at different processing conditions and their stability against coalescence are discussed as a function of various parameters, such as temperature of the system, emulsifier concentration, pump rate and shaft speed of scraped surface heat exchanger ("A" unit) and pin stirrer ("C" unit). The ability of different concentrations of Xanthan Gum (XG) in the continuous phase to improve the stability of emulsions against coalescence is also reported. Finally, the lipid oxidation rates of emulsions stabilised with different concentrations of fat crystals are reported and compared with the lipid oxidation rate of emulsions stabilised with other types of particles or surfactant.

7.1 Characterisation of MG Fat Crystal

7.1.1 Determination of the Thermal Properties of MG

In order to determine the melting and crystallisation behaviour of the different concentrations of MG in sunflower oil, DSC was used to monitor the heating-cooling thermogram of different concentrations of MG in the oil phase (Figure 7-1). As described in section 3.3.8, all samples were heated up from 0 to 100 º C at 10 ºC/min. The onset, peak and end temperatures are given in Table 7-1.

As shown in Figure 7-1, the samples have a broad melting and crystallisation peaks, which are the result of impurities contained within the monoglyceride. The melting and crystallisation temperature shift to a higher temperature as the MG concentration increases (Table 7-1), which could be due to the increased concentration of crystalline material within the oil *(98).* For the sample with the highest MG concentration (25% MG), complete melting of crystals occurred above ~73 ºC. Conversely, for lower concentration of MG (5%), complete melting of crystals occurred above ~ 41 ºC. This could be due to the fact that higher crystal concentrations result in stronger crystal interactions. Therefore, more energy is required to overcome these interactions before melting commences, which explains the shift to higher melting temperatures with increasing MG concentration (*111*). Research groups have reported that the melting point of different triglycerides depends on the concentration of fat. They have shown that melting peak shifted to higher temperatures as the concentration of crystal increased as a result of more energy (heat) needed to overcome crystal interactions. They explained that higher crystal concentration resulted in stronger crystal interactions (*21, 256*).

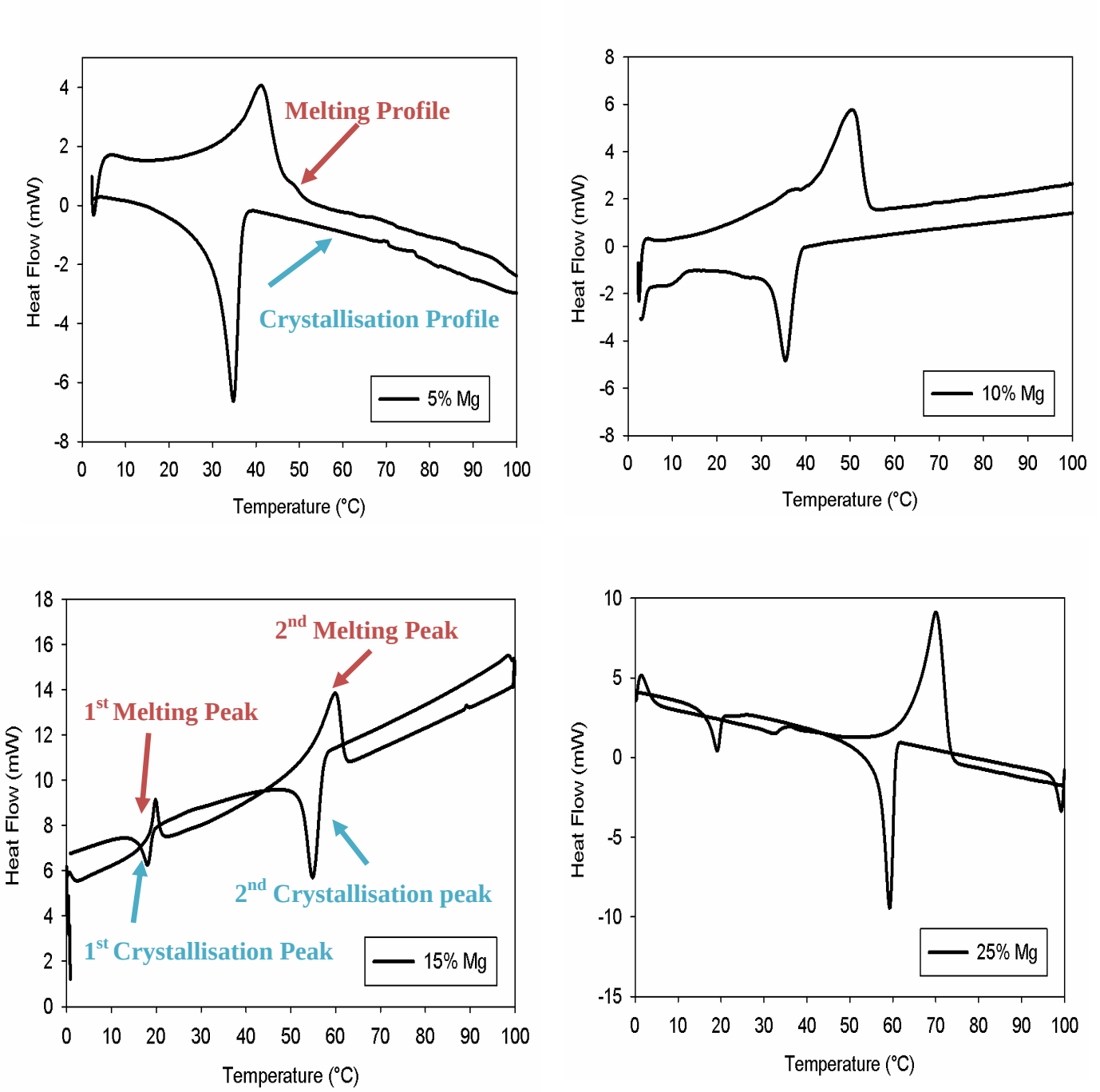


Figure 7- 1: DSC curves of different concentrations of MG. The Samples were heated at 10 ºC/min from 0 to 100 ºC.

As Figure 7-1 shows, at high MG concentrations (15% and 25%) the melting profile had two peaks, however, only a single peak appeared for low MG concentration. As mentioned in chapter 2, monoglycerides are polymorphic and can exist in various polymorphic forms, namely α, β´ and β depending on temperature conditions (*257*).

However, it has been reported that commercial distilled monoglycerides (MG) can only exists in the α, and β–polymorphic form and do not form any β´-crystal polymorphs (*258*). Since distilled monoglyceride was used in this study, therefore, β´ polymorphs did not form. It is known that α-crystals are least stable, least densely packed and have low melting point However, β-crystals have highest melting point and are more compact, denser crystal structure than α-crystals (*259*). Therefore, the two peaks in melting curves of high concentration of MG probably correspond to a polymorphic transition from a less stable (α-crystal) to a more stable polymorphic form (β-crystal).

Table 7- 1: The melting properties of different concentrations of MG. The Samples were heated at 10 ºC/min from 0 to 100 ºC.

MG Concentration (%)	T_{Onset} (ºC)	T_{Peak} (ºC)	T_{End} (ºC)
5	31	41	46
10	42	50	54
15	54	59	62
25	64	70	73

7.2 Emulsion Stability

As discussed in chapter 3, scraped surface heat exchanger (“A” unit) and pin stirrer (“C” unit) were used in order to prepare MG stabilised emulsions. There are many parameters that can influence the properties of emulsions (droplet size stability) produced using “A” and “C” unit such as, shaft speeds of each unit, pump rate and temperature of each unit. Therefore, a series of preliminary experiments were carried out to identify the processing conditions (shaft rate, throughout rate, etc.) that yield the smallest and stable droplets.

7.2.1 Effect of Shaft Speed

The effect of the shaft rate of both "A" (scraped surface heat exchanger) and "C" (pin stirrer) units on droplet size of 20% oil-in-water emulsions stabilised with 2% MG were studied; the data is shown in Figure 7-2.

The "A" unit shaft speed was kept constant at either 1320 rpm or 800 rpm, while the "C" unit speed was varied from 650 rpm, 800 rpm and 1200 rpm. Figure 7-2 shows that increasing the shaft speed of the "A" unit leads to a reduction in the droplets size. Research groups have shown that shear can enhance nucleation stage, accelerate polymorphic transition and cause break of crystals, leading to smaller crystals (*97*, *260*). High shaft speeds cause high shear rates which promotes nucleation (both primary and secondary) and crystallisation. High shear speeds enhance primary nucleation by providing mechanical energy to overcome the energy barrier for nucleation. In addition, high shear rate supplies a good dispersion of the oil phase, the nuclei and crystals, which promotes secondary nucleation. A higher rotational speed provides more scraping actions per unit time (high crystallisation rate) and hence is more effective to inhibit the layer growth between two scarping sections, which leads to the formation of small crystal (*261*).

Rousseau (2000) reported that larger crystals could not efficiently stabilise emulsions as they could not adsorb to the interface, whereas smaller crystals are likely to provide better coverage at droplet interface, thus more effective in stabilising emulsions (*90*). When the "A" unit shaft is at maximum speed much smaller droplets were made and then passed to the "C" unit. Thereby, as the shaft speed of the "C" unit increases, the droplets size decreases (*25*). This can be due to the further increase in the "C" unit shaft speed which could prevent formation of larger crystal networks. Norton *et al* (2012) describes the importance of the shear experienced during the margarine line (A&C unit) on the size of fat crystals

produced. They showed that high shear in the "C" unit resulted in the formation of "shell" around the droplets. The authors argued that this was due to the formation of smaller crystals and the enhanced migration of these crystals to the O/W interface. They also showed that with no stirring in the "C" unit the crystals aggregate, and so are no longer available for Pickering (*262*). Therefore, it was decided to select "A" and "C" unit shaft speeds at 1320 rpm and 1200 rpm respectively in order to produce the smallest droplets size.

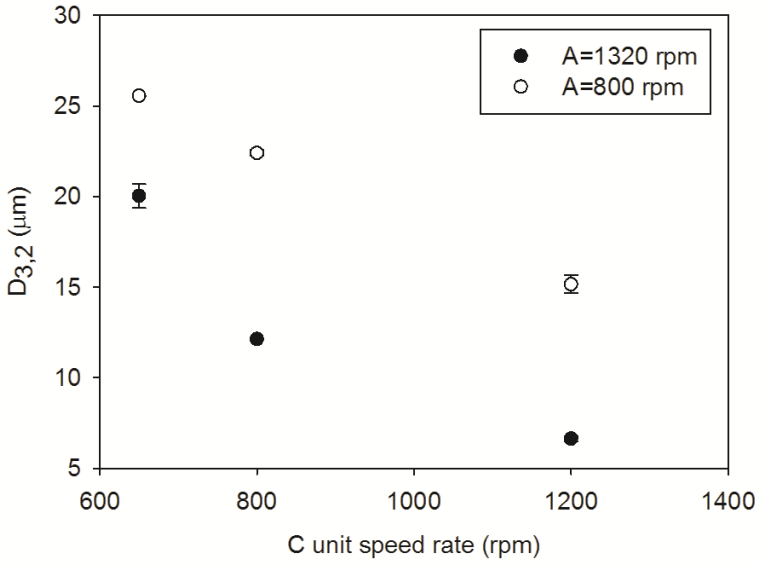


Figure 7- 2: Effect of shafts speeds on droplets size of 20% oil-in-water emulsions stabilised with 2.5% MG. The error bars represent at least 3 different measurements on the same sample.

7.2.2 Effect of Throughput Rate

The effect of pump rate on the droplet size of 2% MG stabilised 20% oil-in-water emulsions was also studied. The speed that the pre-emulsion was passed through the scraped-surface tube ("A" unit) and pin-worker system ("C" unit) could impact the droplet size of final emulsion. Table 7-2 shows that the droplet size increased from ~ 6 μm to ~ 48 μm as the pump rate was increased from 25 to 125 ml/min. This could be due to the fact that at the lower pump rate, samples were passing through the units at a much slower rate. This created

enough time for the fat crystals to form and adsorb at the surface of the droplets, which could result in the formation of small and stable droplets. This data shows that the resistance time is important in droplet size, due to the time for the crystals to get to the droplet interface. Hence, 25 ml/min was the optimum pump rate to form small droplets.

Table 7- 2: Effect of pump rate on droplet size of 20% oil-in-water emulsions stabilised with 2% Mg. The error bars represent at least 3 different measurements on the same sample.

Pump Rate (ml/min)	$D_{3,2}$ (µm)
25	6.1±1.1
75	13.2±0.7
125	46.7±0.8

7.2.3 Effect of Fat Crystal Concentration

The influence of MG concentration (from 0.5% to 2.5%) on droplet size stability was also studied. Results shown above indicated that, when pump rate was 25 ml/min and shafts speeds of "A" and "C" units were at 1320 and 1200 rpm respectively, the emulsions had the smallest droplets size. Hence, these settings were selected to prepare all samples.

The "A" and "C" units' jacket temperatures were manipulated from 35 to 50 ºC for all samples. These jacket temperatures were selected to start fat crystallisation in the "A" unit and control the crystallisation in the "C" unit. The exit temperature was measured for both "A" and "C" units (Table 7-3). Samples were collected from both "A" and "C" units and their stability against coalescence were studied for 21 days. At least 3 different samples were produced for each formulation and each measurement was repeated at least three times.

Table 7- 3: Details of experimental set up for perpetration of 20% oil-in-water emulsions stabilised with different concentrations of MG (0.5 to 2.5%). The error bars represent at least 3 different measurements on the same sample.

Jacket Temperature (ºC)	Exist Temperature (ºC)
35	34
40	38
45	43
50	47

None of "A" unit emulsions were stable against coalescence and visual observation revealed that phase separation occurred for these emulsions after 3 days. This could be due to the short residence time in the "A" unit. The main functions of "A" unit are to promote rapid cooling, crystal nucleation and start crystallisation. Moreover, Rosenthal (1999) reported that the residence time in the scraped surface heat exchanger is too short for the formation of more stable polymorphic form crystals but long enough to form metastable polymorphic crystals (*263*).

In order to view the structure of the interfacial layer around droplets, Cryo-SEM was used. Figure 7-3 SEM micrographs of droplets stabilised by 2.5% MG. As shown in Figure 7-3 the structure of "shell" around droplets is broken which causes scattering cracks. It is possible that these "cracks", indicated in Figure 7.3 by arrows, are artefacts that developed due to the method for preparation of the sample (which includes shock-freezing the sample in liquid nitrogen). However, the large sizes of some individual droplets combined with the emulsion instability against coalescence during storage indicate that there is no tight fat crystal network surrounding the droplets to adequately stabilise the emulsion.

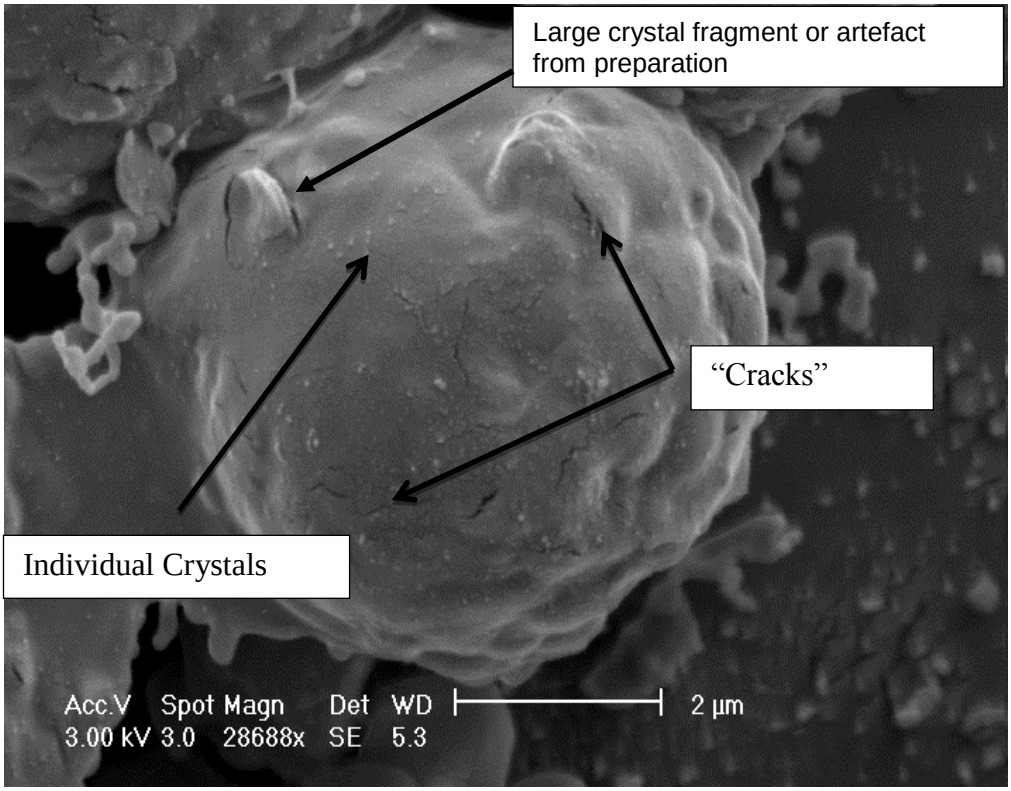


Figure 7- 3: SEM micrograph of an emulsion stabilised by 2.5 % monoglyceride which was collected from "A" unit.

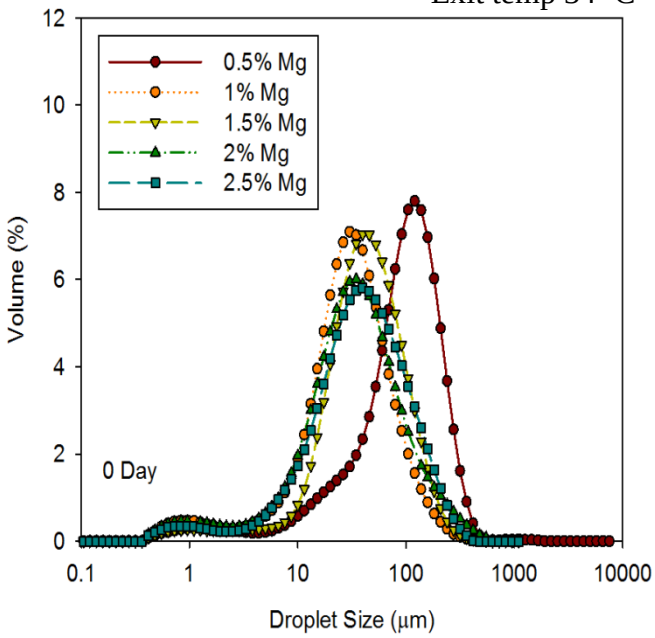
The samples collected from "A" unit had much larger droplet size compared with the samples collected from "C" unit regardless of jacket temperature. The residence time in the "A" unit is short for the formation of stable polymetric crystals (*109*). Thus, the fat crystal networks "shell" formed around droplet are not strong enough to protect droplets from coalescing.

Figure 7-4 shows the changes in the droplet size distributions of emulsions (collected from "C" unit) when stabilised with different concentrations of MG (from 0.5% to 2.5%) with different exit temperatures. Emulsions stabilised with 0.5% MG were unstable against coalescence and complete phase separation occurred after 7 days (confirmed by visual observation). This was probably because there was insufficient amount of fat crystals present in the system to fully cover the droplets interface and produce strong protective "shell"

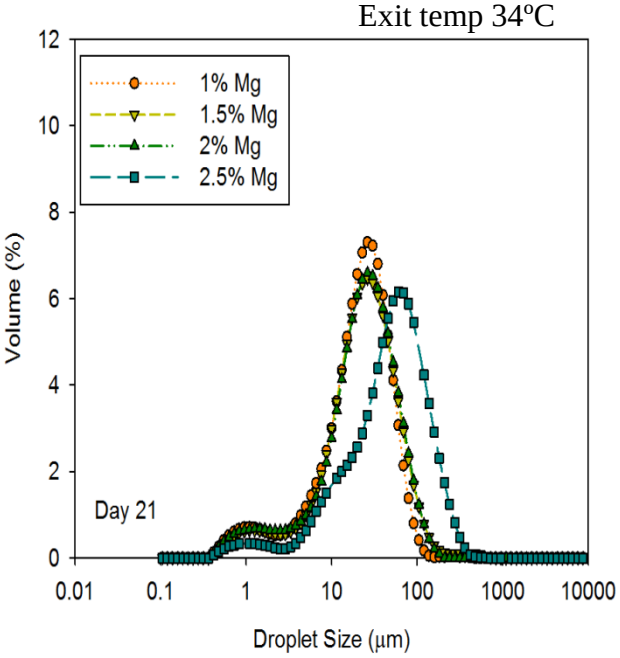
around oil droplets. Hence, coalescence occurred immediately after two droplets came into contact with each other.

As shown in Figure 7-4, the droplet size is dependent on the both concentration and the temperature of the system. At 35 ºC the droplets size ($d_{3,2}$) of 1% MG stabilised emulsions was 8.1±0.03 immediately after emulsification. However, as the temperature was raised to 50 ºC the droplet size dramatically increased to 163.1±3.1. This is because MG crystals were no longer solid at this temperature (all the crystals have been melting). Hence the emulsions become destabilised due to the lack of crystals at the interface to prevent droplets from coalescing (emulsion is no longer Pickering-stabilised), which resulted in the formation of larger droplets. Frasch-Melnik *et al.,* (2010) reported that at 55 ºC emulsions stabilised with MG became destabilised as a result of the fat crystals melting at the droplets' interface (*21*). In contrast, when the MG concentration was high (2.5%) and the temperature was held at 45 ºC the droplets size were much smaller ($d_{3,2}$ is 5.4±0.5) compared with when the temperature was held at 35 ºC ($d_{3,2}$ is 32.2±0.1). Therefore, it is crucial to control temperature in order to allow the fat crystals to sinter together at the droplets interface and thereby produce a "perfect crystal shell" around each droplet.

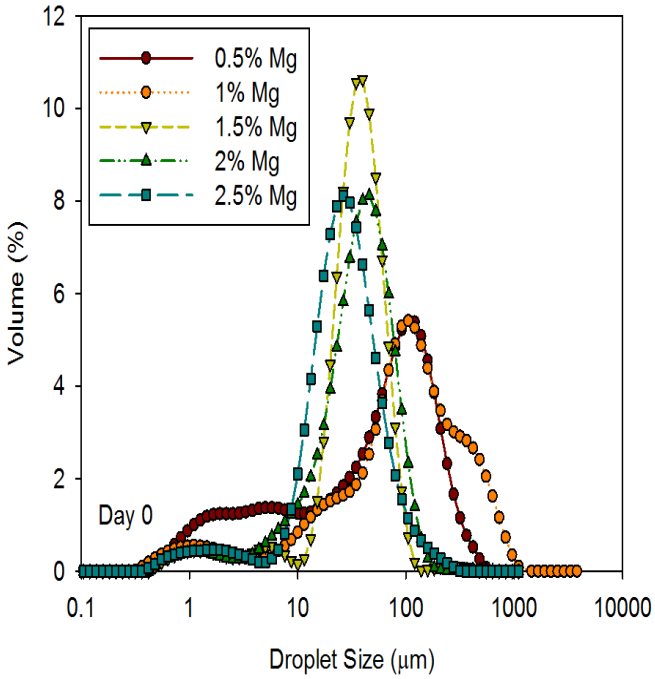
Exit temp 34 ºC



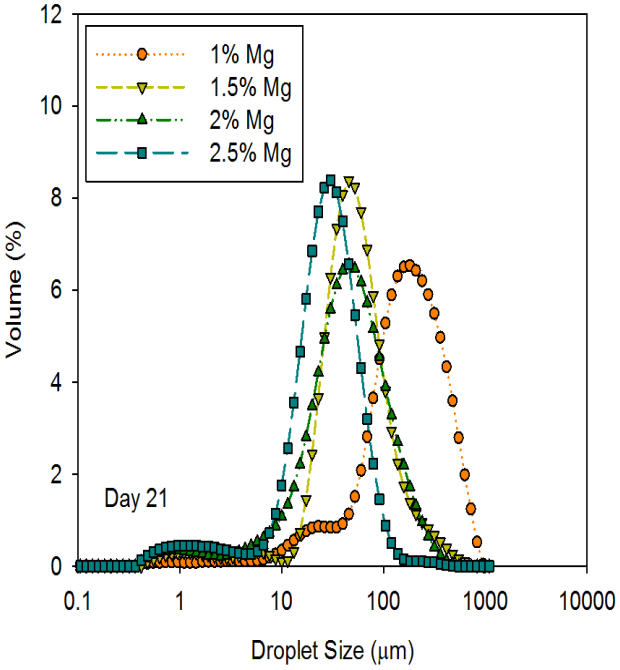
Exit temp 34ºC



Exit temp 38 ºC



Exit temp 38 ºC



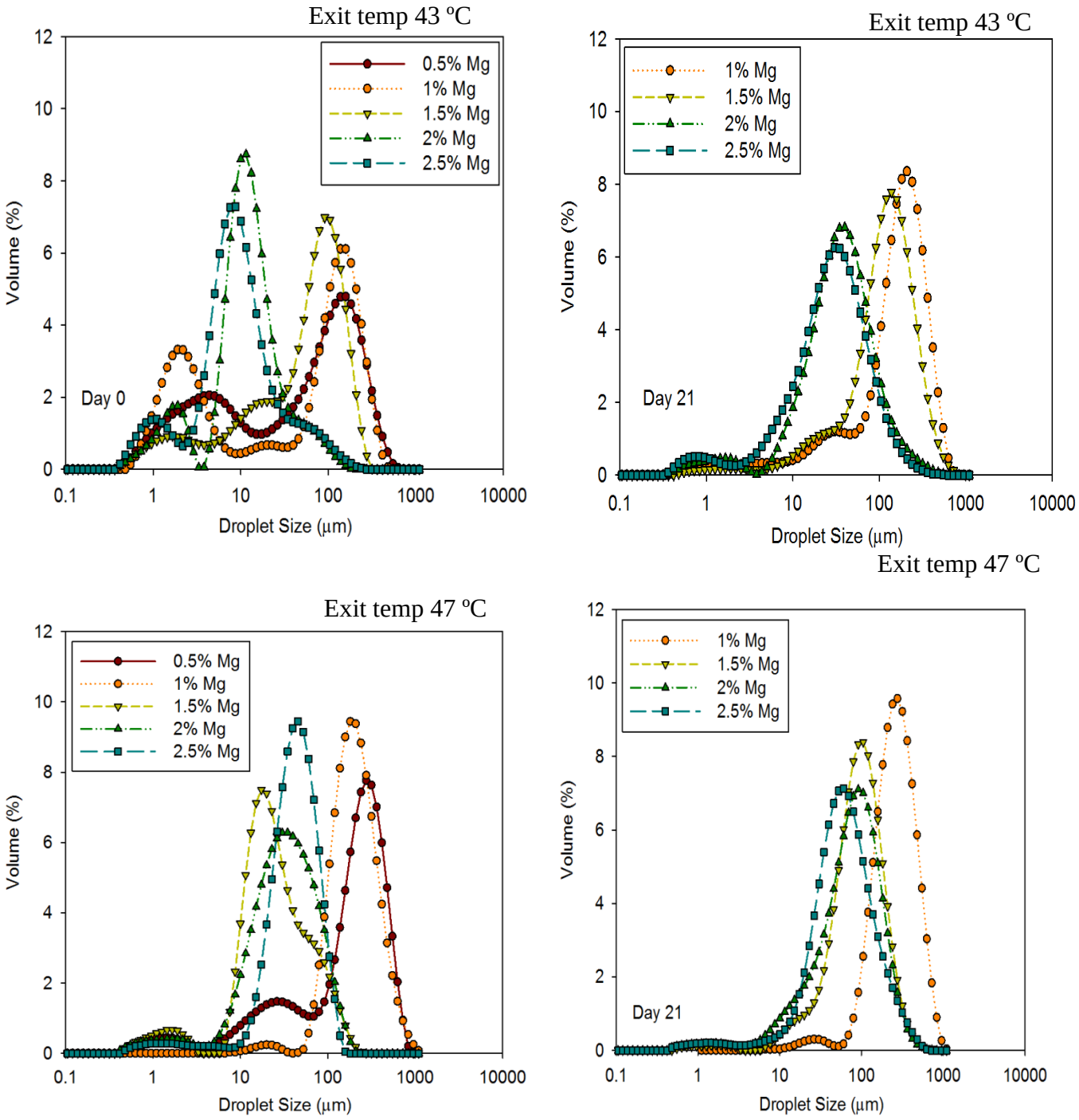


Figure 7- 4: Droplet size distributions of 20% sunflower oil-in-water emulsions stabilised with 0.5 to 2.5% MG-collected from "C" unit. The error bars represent at least 3 different measurements on the same sample.

Figure 7-5 shows the Cryo-SEM and light microscope images taken for a 20% oil-in-water emulsion stabilised by 2.5% MG. In contrast to emulsions collected from "A" unit (Figure 7-3), where crystal cracks were visible on droplets, no individual cracks were visible at the droplet surface in emulsion samples collected from "C" unit. This suggests that the structure of "shells" around oil droplets did not break and much stronger "shell" was formed around oil droplets compared with samples collected from "A" unit. This could be due to the fact that the resistance time in the "C" was longer than "A" unit therefore more stable polymorphic form crystals could be formed. However, as can be seen in Figure 7-5A, the droplets interfaces are rough and look as if total sintering has not occurred. Large crystal fragments also exist on the droplet interface which suggests that crystals failed to form continuous sintered crystalline networks around the oil droplets. Hence, rough "shell" around droplets cannot completely prevent the droplets from coalescence. Furthermore, Figure 7.5B shows a light microscope image of 2.5% Mg stabilised emulsion which was collected from "C" unit immediately after emulsification. Large oil droplets in the continuous phase may represent coalescence phenomena. Le Re´ve´rend *et al.*, (2011) also reported that the microstructure of the droplets stabilised by wax crystals were rough as a result of crystals which did not stick together at the droplet interface (sinter). Hence, the emulsions had a poor stability due to the weak interface (*264*).

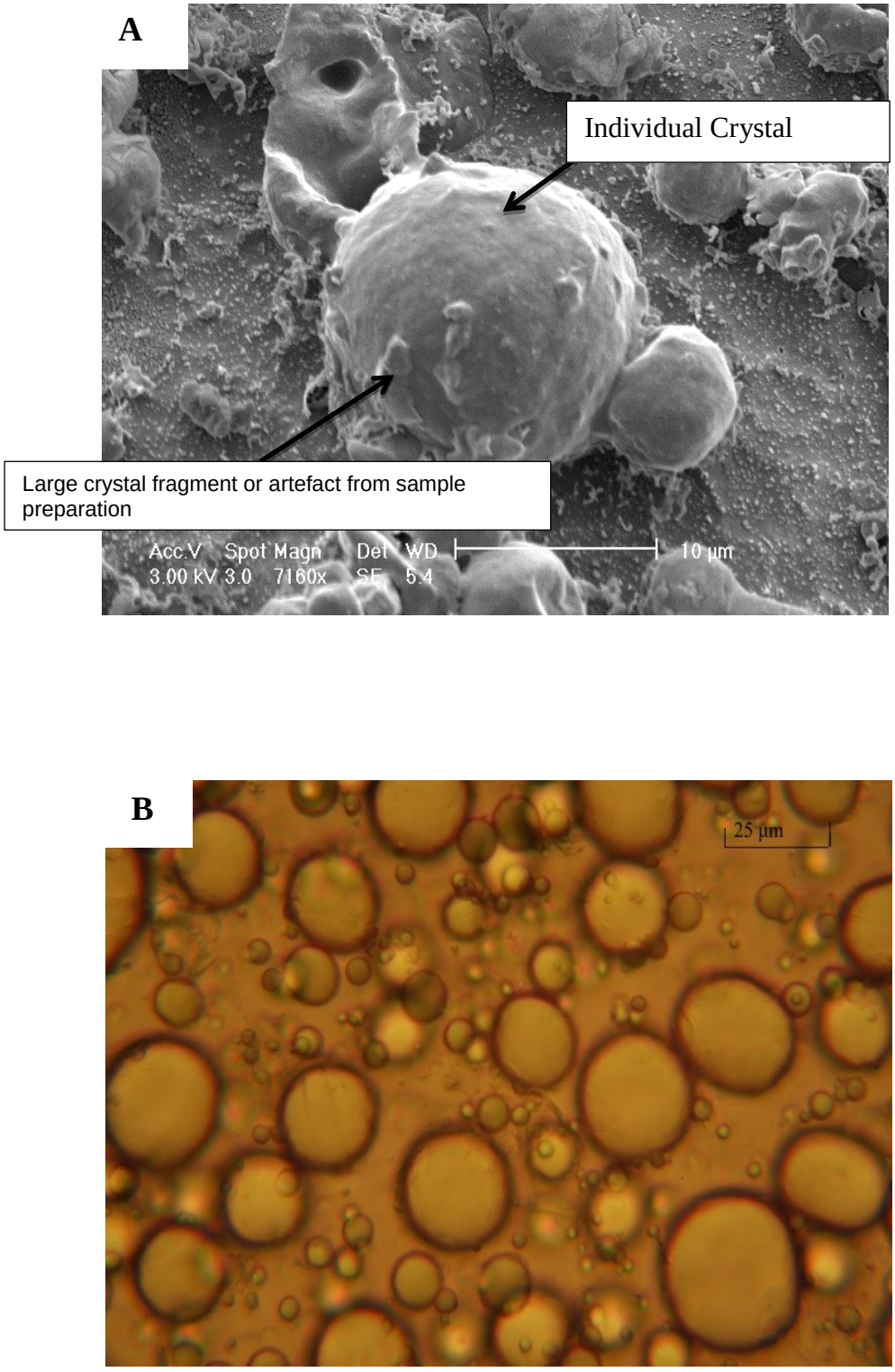
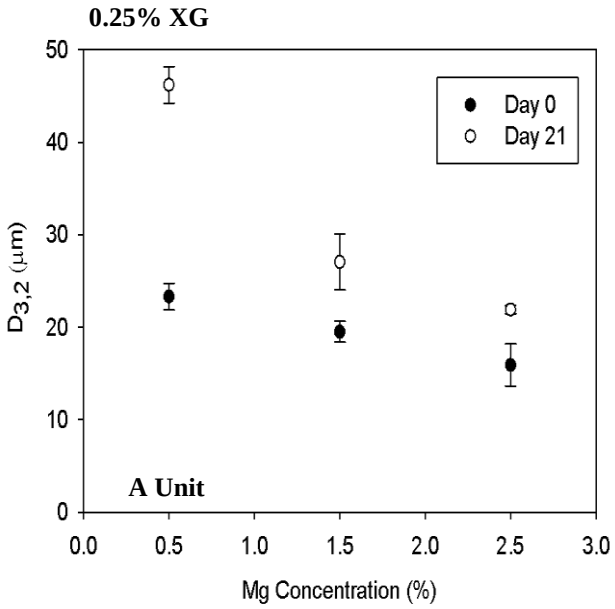


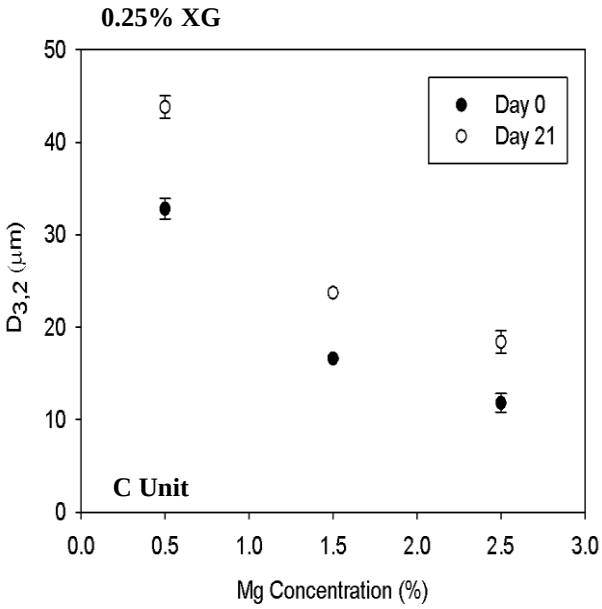
Figure 7- 5: (A) Cryo-SEM image of 20% oil-in-water emulsion stabilised with 2.5% MG. (B) light microscope image of 20% oil-in-water emulsion stabilised with 2.5% MG. Sample was collected from "C" unit.

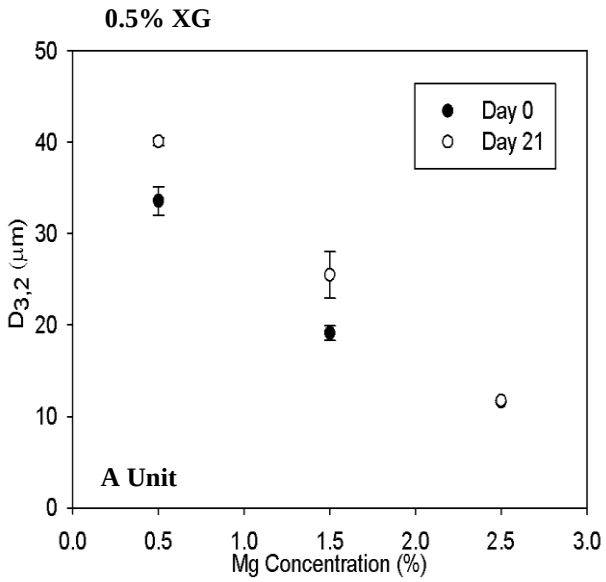
7.2.4 Effect of Thickening Agent

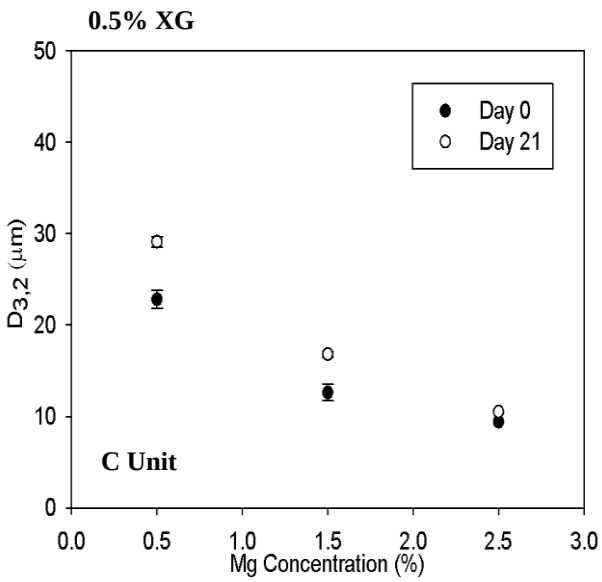
The free movement of large droplets in the continuous phase might be one of the reasons of poor stability of emulsions stabilised with MG. It is well known that thickening agents have a great ability to enhance the stability of emulsions by increasing the viscosity of the continuous phase (restrict the free movement of droplets in the continuous phase). Therefore, Xanthan gum (XG) was used as a thickening agent to investigate whether XG could improve the physical stability of emulsions by preventing the free movement of the droplets. The effect of using various concentration of XG (0.25%, 0.5% and 1%), present in the continuous phase of emulsions (20% oil) stabilised by different concentrations of MG (0.5%, 1.5% and 2.5%) on droplet size stability was studied for 21 days.

The results shown above (section 7.2.3) indicate that the temperature of the system must be carefully controlled, as it has significant effect on stabilising role of the crystals at the interface. Consequently, to prepare emulsions with low MG concentrations, the temperature of the system was set at 35 ºC and for emulsions with high MG concentration the temperature was held at 45 ºC. Samples were collected from both “A” and “C” units and their droplets size stability was monitored for 21 days (Figure 7-6).


0.25% XG
Day 0
Day 21
A Unit
D3,2 (μm)
Mg Concentration (%)


0.25% XG
Day 0
Day 21
C Unit
D3,2 (μm)
Mg Concentration (%)


0.5% XG
Day 0
Day 21
A Unit
D3,2 (μm)
Mg Concentration (%)


0.5% XG
Day 0
Day 21
C Unit
D3,2 (μm)
Mg Concentration (%)

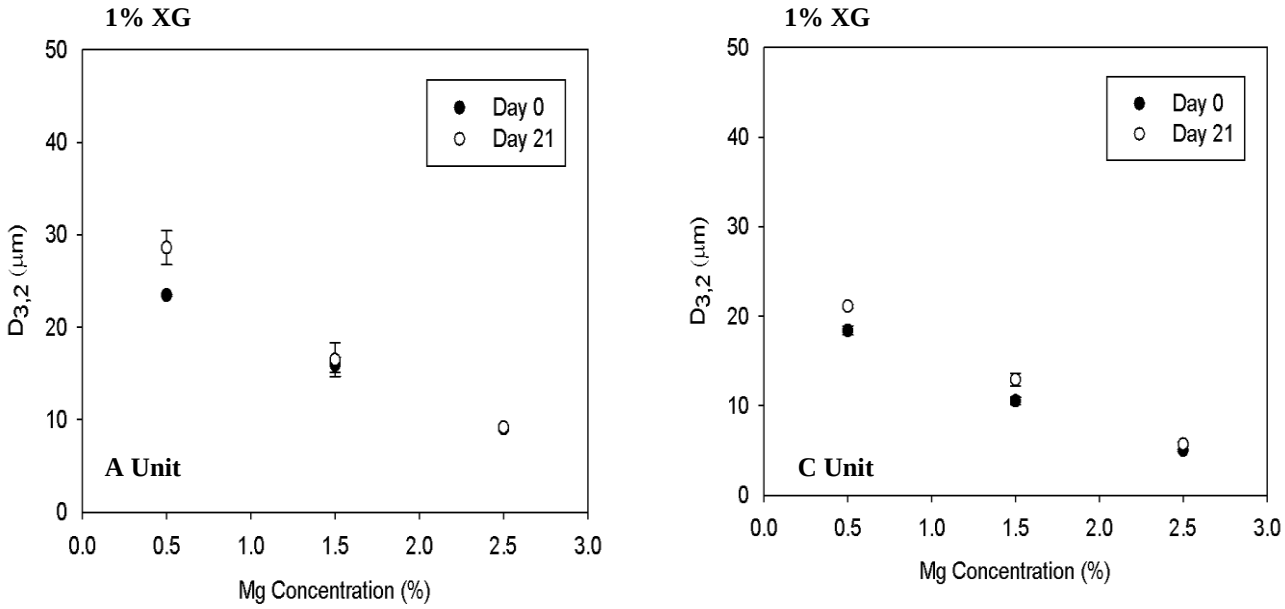


Figure 7- 6: Effect of different concentrations of XG in the continuous phase of 20% sunflower oil-in-water emulsions stabilised with different concentrations of MG (0.5-2.5%) on droplet size. Samples were collected from "A" unit (Left column), and "C" Unit (Right Column). Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

As mentioned earlier (section 7.2.3), all samples collected from "A" unit had very large droplet size and eventually became destabilised. However, the presence of XG in the continuous phase decreased the droplets size to a greater extent and prevented phase separation from occurring. Adding XG into the system enhanced the viscosity of the continuous phase, which could sufficiently retard the collision rate between the fat crystals. In addition, no creaming was observed for all emulsions containing XG regardless of XG concentration.

Emulsions containing low MG concentration (collected either from "A" or "C" unit) showed an increase in the average droplet size after 21 day regardless of XG concentration (Figure 7-6). On the other hand, emulsions containing high MG and XG concentrations did not show any increase in the average droplet size for the same time period (Figure 7-6). It is suggested that the reason for poor droplets size stability is that the fat crystals did not fully

cover the droplets interface and failed to produce a protective "shell" around the oil droplets. Therefore, when two crystalline droplets come close together, coalescence may happen when the film between two droplets is ruptured by protruding crystal (*139*). It is proposed that the presence of sufficient amount of XG in the continuous phase could arrest droplets movement and subsequently prevent coalescence. Figure 7-6 shows droplets sizes of emulsions from "C" unit which are smaller compared with emulsions from the "A" unit. This is because the fat crystals in the "C" unit are under more shear, which results in the breakage of large droplets into smaller ones. Additionally, high shear rate could promote crystallisation rate which leads to formation of smaller crystals. Therefore, these small crystals could stick together (sinter) at the interface and form a thick "shell" around the droplets (*265*).

In order to view the structure of the interfacial layer around droplets, Cryo-SEM was used. Figure 7-7 shows the SEM and light microscope micrographs of 2.5% MG stabilised emulsions containing 0.25% XG collected from the "A" unit (Figure 7-7 A&B) and "C" unit (Figure 7-7 C&D). It can be seen that, at low XG concentration the surface of emulsion droplets seems to be made up of crystalline fragments that do not form a continuous layer. As a result these emulsions showed poor droplet size stability over time.

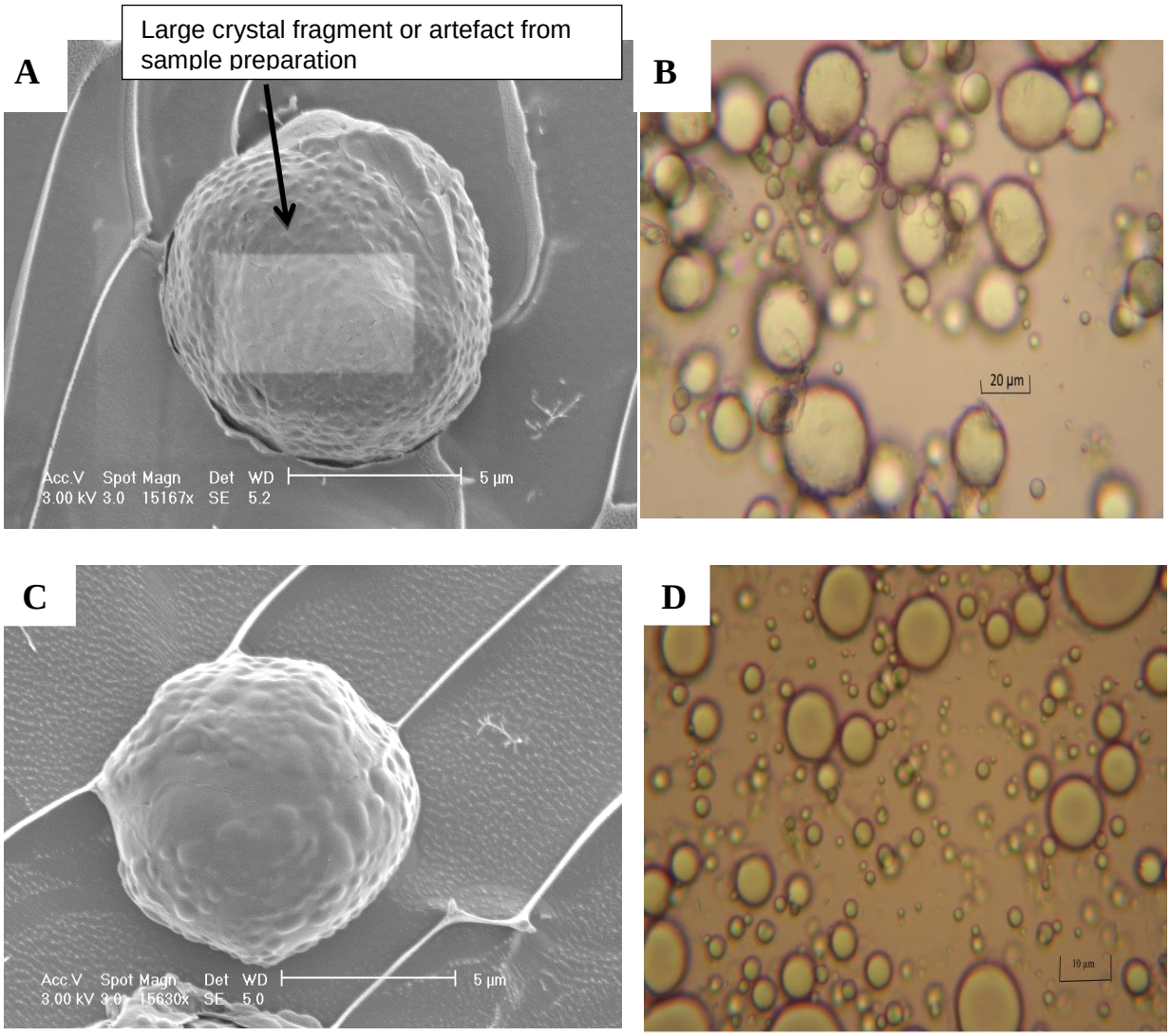


Figure 7- 7: 20% oil-in water emulsions stabilised with 2.5% MG containing 0.25% XG: (A& B) Cryo-SEM image and Light microscope image for samples from "A" unit respectively. (C&D) Cryo-SEM image and Light microscope image for samples from "C" unit respectively.

Figure 7-8 shows a smooth crystalline "shell" formed around the droplets interface as XG concentration increases to 1% (for both "A" and "C" units). These "shells" are similar to those already described in margarine (*266*) and chocolate (*267*). It is suggested that the sub-micron crystals have probably formed and sintered around each droplets. Johansson and Bergenstahl (1995) reported that small crystal nuclei can aggregate in floccs due to mutual adhesion and close proximity between these floccs, allowing the formation of solid bridge (sintering) and forming strong network ("shell") around droplets (*106*).

Fine crystals (sub-micron) can be produced as a result of high shear and fast cooling in the scraped surface heat exchanger ("A" and "C" unit). Crystallisation is promoted as a result of rapid cooling and coincidence shear accelerates the crystallisation by distributing the crystals. Therefore, several small crystallites are formed in the system. The viscosity of the system was increased by addition of more XG which efficiently lowered down the collision frequency between crystallites. This restricted the growth of crystals which led to formation of smooth shell (*101*). Thus, adding sufficient amount of XG into the system appears to be a successful approach to build a smooth "shell" around droplets which leads to the formation of stable emulsions with small droplet sizes.

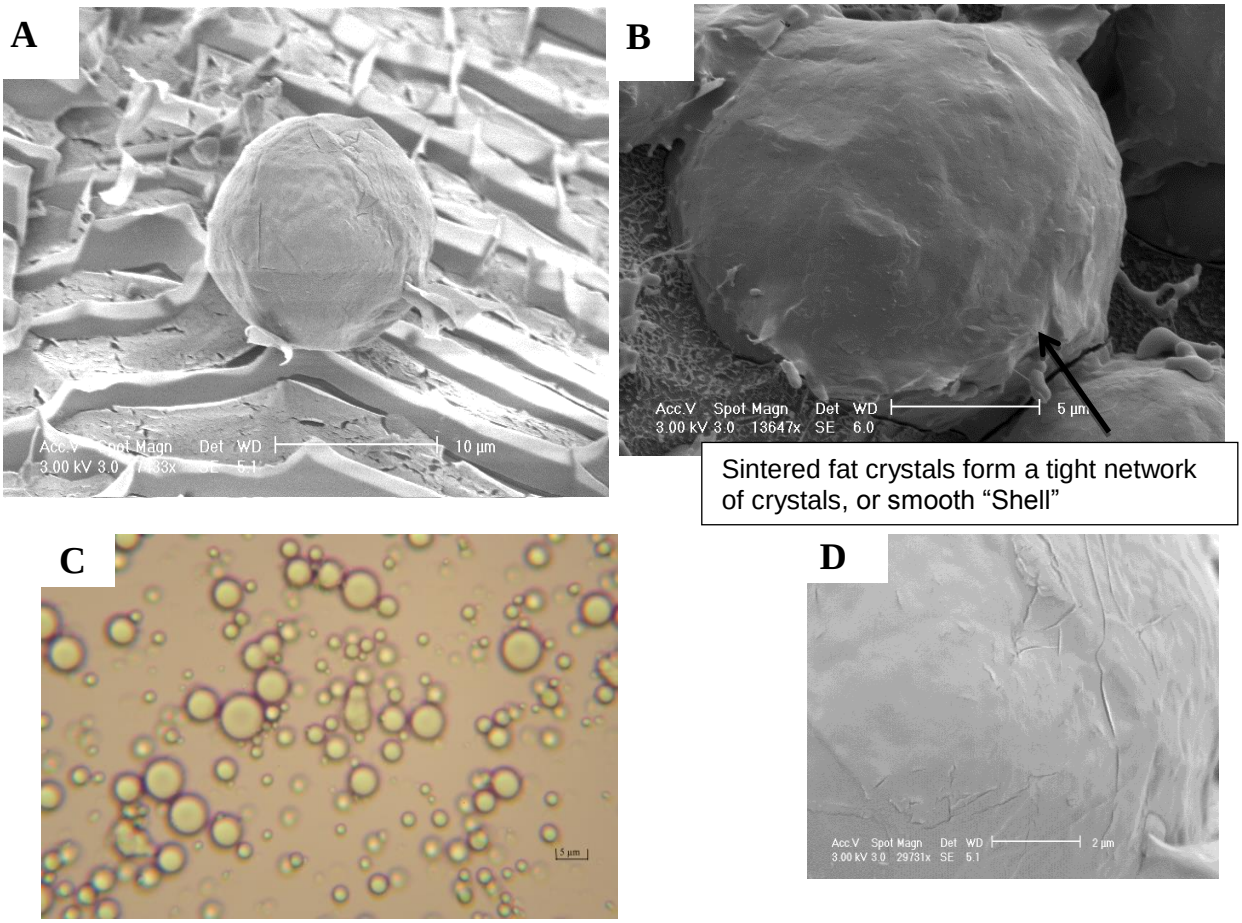


Figure 7- 8: Cryo-SEM image of 20% oil-in water emulsions stabilised with 2.5% MG containing 1% XG (A) Samples were collected from "A" unit. (B) Samples were collected from "C" unit. (C) Light microscope image for samples from "C" unit. (D) Image D is magnification of image B.

The rheological behaviour of emulsions stabilised with 2.5% MG in the presence of different concentrations of XG was studied. Figure 7-9 shows all samples had shear thinning behaviour regardless of XG concentrations. The apparent viscosity at a given shear rate increased progressively with an increase in XG concentration from 0.25% to 1%. Figure 7-9 shows that samples collected from the "A" unit are more viscous compared with samples from "C" unit. As mentioned earlier samples collected from "C" unit are under higher shear conditions compared with samples from the "A" unit. This can be explained by the fact that all samples have shear thinning properties. Thus, as shear rate increased, viscosity decreased.

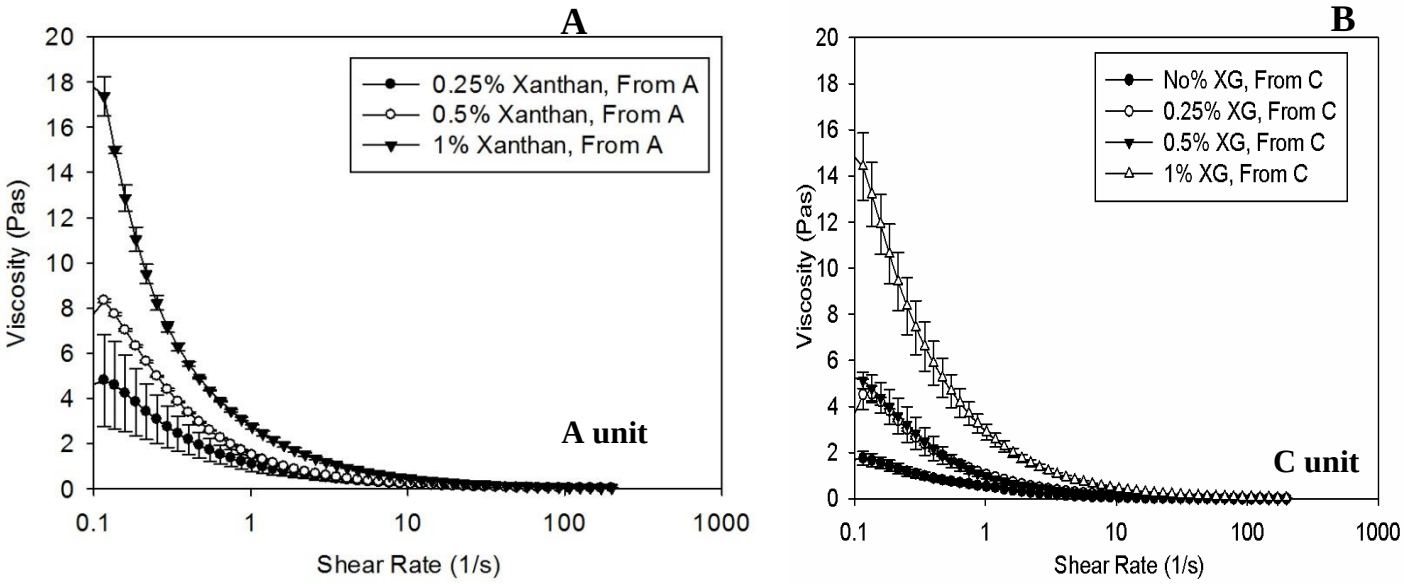


Figure 7-9: Effect of XG concentration on the flow curves of 20% oil-in water emulsions stabilised with 2.5% Mg (A) Samples were collected from "A" unit. (B) Samples were collected from "C" unit. The error bars represent at least 3 different measurements on the same sample.

7.3 Oxidative Stability

7.3.1 Effect of Fat crystals on Oxidative Stability

Results shown above clearly demonstrate that sufficient amount of XG (1%) in the continuous phase is effective in reducing droplets size and improving the physical stability by restricting the collision frequency between crystals. Moreover, Cryo-SEM micrograph of

microstructure of these samples confirmed the existence of smooth fat crystal "shells" around the droplets (Figure 7-8). Thus, the oxidative stability of emulsions stabilised with different concentrations of MG (0.5%, 1.5% and 2.5%) in the presence of 1% XG (collected from either "A" or "C" unit) was studied for 7 days to investigate the ability of fat crystal "shells" to hinder the lipid oxidation rate in the emulsion.

As reported in previous chapters (chapter 4-6), emulsion samples were kept at 40 °C immediately after emulsion formation, for 7 days to accelerate the rate of lipid oxidation. Since the existence of fat crystals at the droplets interface is dependent on the temperature of the system, in this study 0.07 mM ferrous was added to the system in order to accelerate the lipid oxidation rate instead of keeping the samples at a temperature of 40 °C. As described in section 3.4 the primary lipid oxidation product was measured using the PV method and secondary products were measured using the p-AV method. Many research groups showed that surface area (droplet size) can impact lipid oxidation rate (*8, 190,247 ,271*). Hence, in order to eliminate the effect of droplets size on lipid oxidation, the hydroperoxide formation divided by the total surface area as described in section 4.4.2.

Figure 7-10 and Figure 7-11 show that increasing the MG concentration progressively from 0.5% to 2.5% results in a decrease in the rate of lipid oxidation (for both "A" and "C" units). As mentioned in section 7.2.3, emulsions stabilised with low MG concentration had poor stability against coalescence. At low concentration there is not enough fat crystal available in the system to fully cover the droplets interface, thus, fat crystal network around droplets are not significantly strong to prevent film rupture. For this reason, the crystal network ("shell") around each droplet is not effective enough to prevent hydroperoxide-metal ions interactions. Therefore, emulsions containing low MG concentration showed higher rate of lipid oxidation compared to emulsions stabilised with high concentration of MG. These results suggest that if the concentration of crystals in the

system is adequately enough, fat crystals can sinter at interface and form smooth and strong "shells" around droplets. Thus, the existence of sintered fat crystal network around oil droplets could prevent pro-oxidants (in the continuous phase) come into direct contact with the hydroperoxides (at the droplet interface). Moreover, Rousseau and Hodge *et al.*, (2005) argued that a higher number of crystals leads to the formation of more rigid networks around droplets (*268*). Therefore, as the MG concentration increased, the pro-oxidants-hydroperoxides interactions decreased due to the presence of tighter barriers at the lipid droplet interface.

In addition to fat crystals "shell", the presence of XG in the system can be another effective parameter used to hinder lipid oxidation. Morris and Foster (1993) reported that XG has a cellulose backbone with a trisaccharide side chain at the C-3 atom. The terminal mannose residues are 4, 6- pyruvated, which can chelate pro-oxidant metal ions to inhibit lipid oxidation (*269*). Hence, as reported in many studies, XG can chelate metals between two side chains with a pyruvate residue (*248*) (*270*). Shimada *et al*., (1994) also reported that in addition to the structure of XG and its Fe^{2+} binding activity, XG can also scavenge free radicals through its negative charge (*248*).

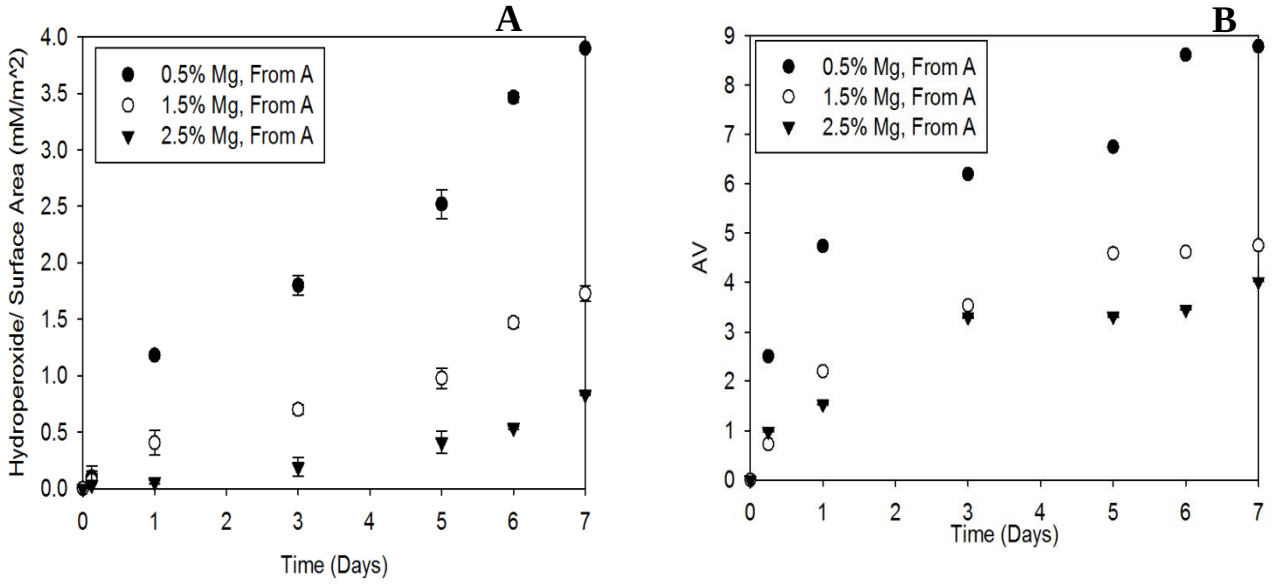


Figure 7-10: Lipid oxidation rate of 20% oil-in-water emulsions stabilised with (0.5%, 1.5% and 2.5%) MG and containing 1% XG. Samples were collected from "A" unit (A) Hydroperoxide formation/surface area (B) Secondary lipid oxidation products. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

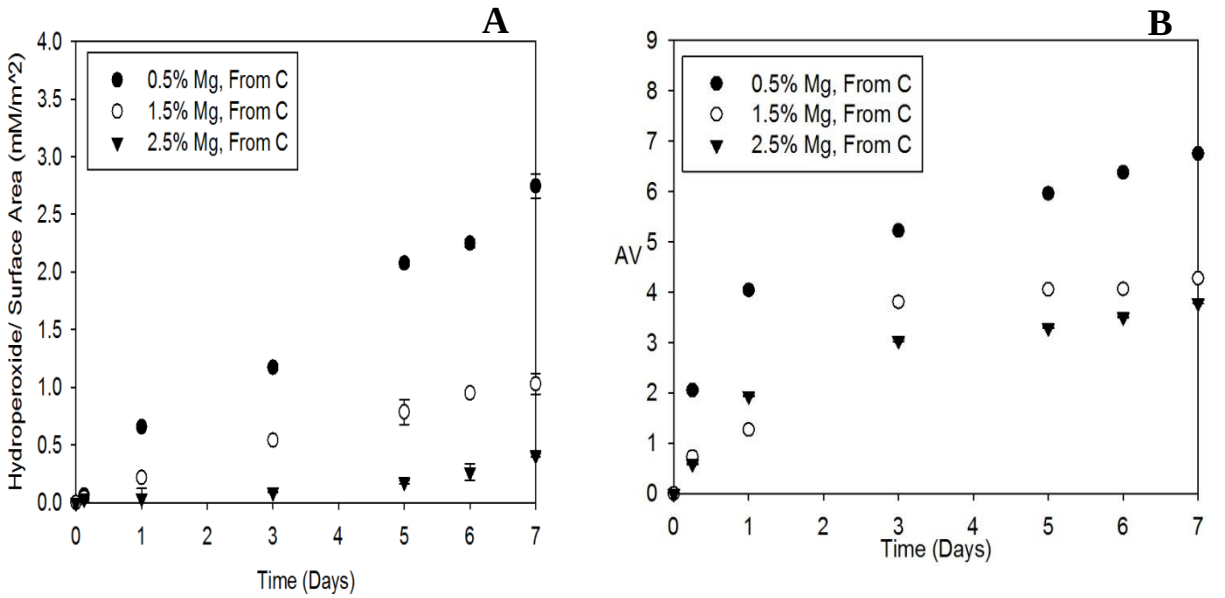


Figure 7-11: Lipid oxidation rate of 20% oil-in-water emulsions stabilised with (0.5%, 1.5% and 2.5%) MG and containing 1% XG. Samples were collected from "C" unit (A) Hydroperoxide formation /surface area (B) Secondary lipid oxidation products. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

7.4 Fat Crystals Versus Food-Grade Particles

In order to evaluate the effectiveness of fat crystal "shells" in improving oxidative stability, the lipid oxidation rates of 20% oil-in-water emulsions stabilised with 2.5% food-grade particles (either Microcrystalline cellulose (MCC) or Modified Starch (MS)) were studied and compared with lipid oxidation rate of emulsions stabilised with 2.5% MG. The lipid oxidation rates of emulsions stabilised with different concentrations of MCC or MS particles were discussed in chapter 5 (section 5.3). However, all emulsions samples were kept at 40 °C to accelerate lipid oxidation. In order to compare the antioxidant ability of MCC or MS with MG, 0.07 mM ferrous was added into the continuous phase of 20% oil-in-water emulsions stabilised with either MCC or MS (Figure 7-12) to keep processing conditions the same for all samples.

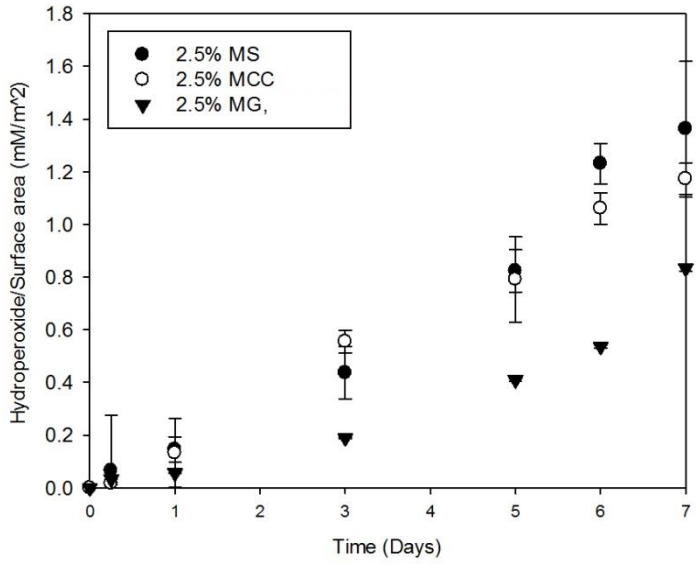


Figure 7-12: Hydroperoxide formation/surface area of 20% oil-in-water emulsions, containing 0.07 mM ferrous. Data points represent mean values (n = 3) ± standard deviation. Where not visible error bars are smaller than symbols. The error bars represent at least 3 different measurements on the same sample.

As Figure 7-12 shows, that the oxidative stability of emulsions stabilised with different types of particles decrease in the order of MG> MCC ≥MS. Particles at the droplets interface reduced lipid oxidation rate due to their ability to migrate at droplet interface and form a structure around the oil droplets which inhibit hydroperoxides-pro-oxidants interactions. As mentioned above, emulsions stabilised with 2.5% MG crystals could inhibit lipid oxidation due to the existence of fat crystals network around droplets interface which results in preventing metal ions from coming in close contact with hydroperoxide. In addition, the presence of 1% XG in the continuous phase of these emulsions could also chelate pro-oxidants (through their structure). Both MCC particles and MS particles showed similar effect on lipid oxidation which is in consistence with results were obtained in Chapter 5.

7.5 Conclusion

In this chapter it has been shown that the presence of fat crystals at droplet interface (Pickering mechanism) and addition of sufficient amount of XG in the aqueous phase can provide good stability against coalescence and lipid oxidation. It is suggested that this is because tight fat crystal network ("shells") was formed around oil droplets as a result of sub-micron fat crystals sintered at interface. These "shell" crystals inhibited coalescence and prevented pro-oxidants from close contact with oil droplets.

Emulsions with no XG showed signs of coalescence and phase separation. However, the presence of sufficient amount of XG (≥ 0.5%) in the continuous phase enhanced the physical stability of the emulsions. This has been interpreted as the viscosity of the continuous phase being sufficiently high to lower the frequency of collision between crystallites and immobilise droplets from further interaction. Coalescence stability of emulsion was also found to be dependent on the crystal concentration. At low MG

concentration the oil droplets are not fully covered by fat crystals, hence, the “shells” formed around oil droplets are not significantly strong to prevent film rupture.

Lipid oxidation rate decreased as MG concentrations increased as a result of the higher number of crystals sintered at droplet interface which separated the metal ions from the surface of droplets. Moreover, the presence of XG in the continuous phase reduced further lipid oxidation due to its ability to chelate metals. Consequently, fat crystals were able to build the “perfect shell” around oil droplets if sufficient amount of XG existed in the continuous phase which not only improved the droplet size stability but also enhanced the oxidative stability.

Chapter 8:Conclusions

8.1 Microstructural Design to Reduce Lipid Oxidation

The physical and oxidative stability of oil-in-water emulsions in the presence of different types of emulsifiers, such as protein (CAS) and small molecule surfactant (Tween 20) at different processing conditions were studied. Both Tween 20 and CAS were able to provide stable emulsions. Emulsions stabilised with CAS appeared to have larger droplet size compared to those stabilised with Tween 20. This was explained in terms of the adsorption kinetics. Generally, small molecule surfactant (Tween 20) adsorb much faster than macromolecules such as proteins (*25*). Therefore, surfactants induced further droplet break-up by rapidly adsorbing to newly formed interfaces.

The lipid oxidation rate in emulsions stabilised with CAS was slower than those stabilised with Tween 20. This was attributed to the ability of CAS to create a thick interface layer around droplets and their ability to scavenge free radical through their molecular structure. In addition, the presence of negatively charged CAS in the continuous phase of 20% oil-in-water emulsions was effective in inhibiting lipid oxidation. CAS has a great chelating capacity due to its phosphorylated serine residues, hence was able to chelate pro-oxidants. Moreover, negatively charged CAS in the continuous phase could bind to cationic transition metals. Therefore, the impact of continuous phase CAS (positively charged, pH < pI) on lipid oxidation rate was less significant than continuous phase negatively charged proteins (pH > pI).

It was also shown that lipid oxidation could be reduced by using Pickering particles (silica particles as model system). This was attributed to the ability of particles to produce a structure around droplets thus, reducing pro-oxidants-hydroperoxide interactions. Uncharged silica particles were more effective in reducing lipid oxidation than negatively charged particles. It is possible that the existence of electrorepulsive forces between charged particles prevent close arrange of particles at droplet interface.

8.2 Food-Grade Particles Stabilised Emulsions

The stability of emulsions stabilised with food-grade particles (MCC and MS) was found to be affected by concentration and the pH of the system. It was shown that emulsions stabilised with low concentration (<0.5%) of food-grade particles were unstable (rapid coalescence occurred), whereas by adding more particles in the system, the rate of surface coverage (i.e. rate of particles' adsorption) of the oil droplets increased, which led to the formation of stable emulsion.

At pH 2, MCC particles became relatively unstable and aggregated into large floccs. Similar behaviour was observed for MS particles. At this pH, food-grade particles were uncharged; hence, they could not repel each other thereby formed aggregates instead (no electrostatic repulsion force between particles). However, both types of particles became more negatively charged as the pH increased (pH≥4). Food-grade Pickering particles enhanced the stability of emulsions at high pH by keeping the oil droplets apart due to high electrostatic repulsive forces on the droplet surface. Moreover, the emulsions that were prepared at pH≥4 had similar droplet sizes (regardless of particle type).

It was demonstrated using food-grade particles (Pickering emulsions) was an effective approach to slow down lipid oxidation. Both MCC and MS particles were able to reduce lipid oxidation rate due to the presence of particles around the oil droplets, thus

preventing the metal ions from close contact with the surface of the droplets. Moreover, nonadsorbed negatively charged particles in the continuous phase could scavenge free radicals and chelate pro-oxidants in the continuous phase, thus further reduction in the rate of lipid oxidation.

8.3 Emulsions Stabilised by Both Food-Grade Particles and Low Molecular Weight Surfactant

The physical stability of oil-in-water emulsion in the presence of both "food grade" particles (MCC or MS) and surfactant (Tween 20) was studied. The concentration of particle was kept constant at 1.5%, while the concentration of Tween 20 was varied from 0.1% to 2%. Emulsions stabilised with both particles and surfactant showed great stability against coalescence and no oil layer was observed at the top of the emulsions for at least 40 days.

The droplet size of emulsions stabilised with both particle and Tween 20 was much smaller than emulsions stabilised with particles only. Initially, Tween 20 surfactant rapidly adsorbed to freshly formed droplets and reduced interfacial tension (induced break up). This allowed enough time for the "food-grade" particles to migrate to the oil–water interface and thereby prevent newly formed small droplets from coalescence. It was shown that increasing the Tween 20 concentration from 0.5% to 2% did not completely remove particles from the droplet interface. Lipid oxidation rate in emulsions stabilised by both "food-grade" particles and Tween 20 was much slower than emulsions solely stabilised with particles or surfactant. This was interpreted as the ability of particles at droplets interface to separate pro-oxidants from the surface of droplets and also due to the presence of Tween 20 micelles in the continuous phase which altered the physical location and pro-oxidants activity of ions in oil-in-water emulsions. Furthermore, nonadsorbed negatively charged "food-grade" particles in

the aqueous phase were likely able to reduce the lipid oxidation through chelating pro-oxidants.

8.4 Emulsions Stabilised by Fat Crystals

Initially, the physical stability of emulsion stabilised with different concentration (0.5% to 2.5%) of monoglyceride was studied. It was shown that none of these emulsions were stable against coalescence and after short time phase separation occurred. It was suggested that the crystalline "shell" structure around droplet was weak to protect droplets from coalescence (total sintering did not occur).

The presence of xanthan gum in the continuous phase of monoglyceride stabilised emulsions increased the stability of emulsions. However, emulsions which contained low monoglycerides or low xanthan gum, were unstable against coalescence. This was probably due to insufficient amount of fat crystals present in the system to fully cover the droplets interface and produce strong protective "shell" around oil droplets. It was suggested that large crystals could not effectively adsorb at the interface, while smaller crystals were more likely to effectively adsorb and accommodate at the interface. Adding xanthan gum into the system enhanced the viscosity of the continuous phase, which sufficiently retarded the collision rate between the fat crystals and subsequently prevented the formation of large crystals. Therefore, sub-micron crystals formed tight sintered crystal network around oil droplets to protect droplets from coalescence. The existence of smooth fat crystal ''shells" around oil droplets was confirmed in SEM micrographs of an emulsion containing 1% xanthan gum.

It was shown that the formation of tight "shells" around oil droplets (as a result of fat crystals sintered at interface) prevent pro-oxidant (in the continuous phase) to come into direct contact with the hydroperoxides (at the droplet interface). Therefore, lipid oxidation was much slower for emulsions containing high concentration of monoglyceride (2.5%) than

those stabilised with low concentration of monoglyceride (0.5%). Moreover, xanthan gum in the continuous phase acted as chelating agents and bonded with positively charged metal ions through its pyruvate residue. Thus, addition of xanthan gum led to further oxidative stability.

8.5 Future Work

In terms of future work recommendations, there are several aspects that need further investigation. These are listed below:

- The ability of "food-grade" particles to control lipid oxidation was confirmed in this study. It would be interesting to use different types of "food-grade" particles (particle size and morphology) to extend the present findings.
- In this study, the concentration of "food-grade" particles was kept constant (1.5%) for preparation of particles-surfactant emulsions. Thereby, the effect of surfactant concentration on the physical and oxidative stability was only studied. It would be interesting to vary the concentration of particles (<1.5%) to gain better understanding on the synergistic stabilisation of oil-in-water emulsions and its impact on oxidative stability. In addition, it was shown that "food-grade" particles carried negatively charged at pH≥4. Further research can be carried out to evaluate the effect of oppositely charged surfactants, polysaccharides or proteins on the synergetic stabilisation of oil-in-water emulsions. For this purpose, various concentrations of CAS (pI<pH) or Chitosan can be used.
- In this study, saturated monoglyceride was used as fat crystal particles. It would be useful to stabilise emulsions with different types of fat crystals such as triglycerides or the mixture of fat crystals (monoglycerides and triglycerides) in order to gain better understanding on the impact of fat crystal "shell" on lipid oxidation phenomena. Furthermore, monoglycerides appeared to produce unstable

emulsions in the absence of xanthan gum. In order to improve the stability of these emulsions, the next step could be using proteins or surfactants.

- The next microstructural approach could be encapsulation of pro-oxidants within the interior aqueous phase of double emulsions (water-in-oil-in-water). Based on this technique pro-oxidants may be prevented from coming into close contact with the oil phase. For this purpose, several suitable surfactants or food-grade particles can be used to produce stable emulsions. Double emulsions also can be stabilised with fat crystals and their ability to hinder lipid oxidation can be compared with those stabilised with MCC or MS particles. Moreover, it would be interesting to produce double emulsions with different emulsification process (membrane) to study the effect of processing conditions on physical and oxidative stability of emulsions.

References

1. P. Scarborough; K. Wickramasinghe; P. Bhatnagar; M. Rayner, *Trends in Coronary Heart Disease 1961-2011*. British Heart Foundation: London, 2011.

2. S. C. Cunnane and B. A. Griffin, Nutrition and Metabolism of Lipids. In *Introduction to Human Nutrition*, M.J.Gibney; S.A.Lanham-New; A.Cassidy; H.H.Vorster Second ed.; John Wiley & Sons: Oxford, 2009.

3. A.P.Simopoulos, Essential Fatty Acids in Health and Chronic Disease. *The American Journal of Clinical Nutrition* **1999,** *70*, 260S-269S.

4. A.P.Simopoulos, The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids. *Biomedicine & Pharmacotherapy* **2002,** *56*, 365-379.

5. D.J.McClements; E.A.Decker, Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of Food Science* **2000,** *65*, 1270-1282.

6. T.Waraho; V.Cardenia; E.A.Decker; D.J.McClements, Lipid Oxidation in Emulsified Food Products. In *Oxidation in Foods and Beverages and Antioxidant Applications: Understanding Mechanisms of Oxidation and Antioxidant Activity*, E.A.Decker; R.J.Elias; D.J.McClements, Eds. Woodhead Publishing: London, 2010; Vol. 1.

7. S.Kiokias; T.H.Varzakas; I.S.Arvanitoyannis; A.E.Labropoulos, Lipid Oxidation and Control of Oxidation. In *Advances in Food Biochemistry*, F.Yildiz, T. L., Ed. CRC Press: New York, 2009.

8. Th.Waraho; D.J.McClements; E.A.Decker, Mechanisms of Lipid Oxidation in Food Dispersions. *Trends in Food Science and Technology* **2011,** *22*, 3-13.

9. E.N.Frankel, Control of oxidation. In *Lipid Oxidation*. The Oily Press: Dundee, 1998.

10. B. Matthaus, Oxidation of Edible Oil. In *Oxidation in Foods and Beverages and Antioxidant Applications*. E.A.Decker; R.J.Elias; D.J.McClements Woodhead Publishing: London, 2010; Vol. 1:Understanding mechanisms of oxidation and antioxidant activity.

11. E.A.Decker, Strategies for Manipulating the Prooxidative/Antioxidative Balance of Foods to Maximize Oxidative Stability. *Trends in Food Science & Technology* **1998,** *9*, 241-248.

12. U.Klinkesorn; P.Sophanodora; P.Chinachoti; D.J.McClements; E.A.Decker, Increasing the Oxidative Stability of Liquid and Dried Tuna Oil-in-Water Emulsions with Electrostatic Layer-by-Layer Deposition Technology. *Journal of Agricultural and Food Chemistry* **2005,** *53*, 4561-4566.

13. D.Guzey; D.J.McClements, Formation, Stability and Properties of Multilayer Emulsions for Application in the Food Industry. *Advances in Colloid and Interface Science* **2006,** *128*, 227-248.

14. W.Herbst; K.Hunger, Emulsion Paint In *Industrial Organic Pigments: Production, Properties, Applications*. Third ed.; Wiley-VCH: Weinheim, 2004.

15. D.G. Dalgleish, Food Emulsion. In *Encyclopedic Handbook of Emulsion Technology*. J.Sjöblom, Marcel Dekker: New York, 2001.

16. D.J.McClements; Lipid Based Emulsion and Emulsification In *Food Lipids: Chemistry, Nutrition, and Biotechnology*. C.C.Akoh and D.B.Min, Third ed.; CRC Press/Taylor & Francis Group: NewYork, 2008.

17. D.G Dalgleish; Structures and Properties of Food Emulsion. In *Food Emulsion*. S.J.Friberg; K.Larsson; J.Sjöblom, Fourth ed.; CRC Press: New York, 2003.

18. E.A.Decker; D.J.McClements, Trends And Developments In *Advances in Food Colloids*. Blackie Academic and Professional: London, 1995.

19. M.Shimizu; Y.Nakane, Encapsulation of Biologically Active Proteins in a Multiple Emulsion. *Bioscience, Biotechnology, and Biochemistry* **1995,** *59*, 492-496.

20. E.Dickinson, Double Emulsions Stabilized by Food Biopolymers. *Food Biophysics* **2011,** *6*, 1-11.

21. S. F. Melnik; F.Spyropoulos; I.T.Norton, Fat-Crystal Stabilised W/O Emulsions for Controlled Salt Release. *Journal of Food Engineering* **2010,** *98*, 437-442.

22. J.E.Norton; I.T.Norton, Designer Colloids—Towards Healthy Everyday Foods. *Soft Matter* **2010,** *6*, 3735-3742.

23. R.Lutz and A. Aserin; Multiple Emulsions Stablised By Biopolymers. In *Multiple Emulsions: Technology and Applications*. A.Aserin, John Wiley & Sons: New Jersy, 2008.

24. V.A.Vaclavik; E.W.Christian, Food Emulsions and Foams In *Essentials of Food Science*. Third ed.; Springer: New York, 2007.

25. D.J.McClements, *Food Emulsions: Principles, Practice, and Techniques*. Second ed.; CRC Press: Florida, 2005.

26. P.M.Gaman; K.B.Sherrington, Food Dispersion. In *The Science of Food*. Butterworth-Heinemann: Boston, 1996; Vol. Fourth.

27. Pieter Walstra; Dispersed sysyem: Basic Considerations. *Food Chemistry*. InO.R.Fennema, Third ed.; Marcel Dekker: New York, 1996.

28. H.G. Bueschelberger, Lecithins. In *Emulsifiers in Food Technology*. R.J.Whitehurst, Wiley-Blackwell: Oxford, 2004.

29. P.Becher, Emulsifying Agents: Classification and Chemistry. In *Emulsions: Theory and Practice*. Reinhold Publishing Corporation: New York, 1965.

30. M.J.Rosen, Emulsification by Surfactants. In *Surfactants and Interfacial Phenomena*. Third ed.; John Willy & Sons: New Jersey, 2004.

31. R.Caenn; H.Darley; G. R. Gray, Surface Chemistry of Drilling Fluids. In *Composition and Properties of Drilling and Completion Fluids*. Sixth ed.; Gulf Professional Publishing: Oxford, 2011.

32. I.Kralova; J.Sjöblom, Surfactants Used in Food Industry: A Review. *Journal of Dispersion Science and Technology* **2009,** *30*. 1363-1383

33. J.W.McBain, Surfactants and Their Properties. In *Colloid and Interface Science*, P.Chosh, Ed. PHI Learning: New Delhi, 2009.

34. W.C.Griffin, Classification of Surface-Active Agents by HLB. *Journal of the Society of Cosmetic Chemists* **1994,** *1*, 311-326.

35. S.E.Friberg and J.Yang, Emulsion Stability. In *Emulsions and Emulsion Stability:Surfactant Science*. J.Sjöblom, Second ed.; CRC Press: Florida, 2006; Vol. 132.

36. Y.Moroi, Surface Active Agents. In *Micelles: Theoretical and Applied Aspects*. Springer: New York, 1992.

37. G.L.Hasenhuettl; R.W.Hartel, Physicochemical Aspects of an Emulsifier Functionality. In *Food Emulsifiers and Their Applications*. Springer: New York, 2008; Vol. Second.

38. D.J.McClements, Protein-Stabilized Emulsions. *Current Opinion in Colloid and Interface Science* **2004,** *9*, 305-313.

39. F.Spyropoulos; E.A.K.Heuer; T.B.Mills; S.Bakalis, Protein-Stabilised Emulsions and Rheological Aspects of Structure and Mouthfeel. In *Practical Food Rheology: An Interpretive Approach*, I.T.Norton; F.Spyropoulos; Ph.Cox, Eds. John Wiley & Sons: Oxford, 2011.

40. E.Dickinson, Hydrocolloids as Emulsifiers and Emulsion Stabilizers. *Food Hydrocolloids* **2009,** *23*, 1473-1482.

41. J.F.Zayas, Emulsifiying Properties of Proteins. In *Functionality of Proteins in Food*. Springer: New York, 1997.

42. A. Fillery-Travis; E. N. C. Mills; P. Wilde, Protein-Lipid Interactions at Interfaces. *Federation of African Societies of Chemistry* **2000,** *51*, 50-55.

43. M.E.Mangino, Protein Interactions in Emulsions: Protein-Lipid Interactions. In *Protein Functionality in Food Systems*. N.S.Hettiarachchy; G.R.Ziegler, Marcel Decker: New York, 1994.

44. D.Lorient; B.Closs; J.L.Courthaudon, Surface Properties of the Bovine Casein Components: Relationships between Structure and Foaming Properties. *Journal of Dairy Research* **1989,** *49*, 495-502.

45. H.Singh, Aspects of Milk-Protein-Stabilised Emulsions. *Food Hydrocolloids* **2011**, 25,1938-1944.

46. P.F.Fox, Milk: An Overview. In *Milk Proteins: From Expression to Food*, A.Thompson; M.Boland; H.Singh, Eds. Academic Press: New York, 2009.

47. V.Raikos, Effect of Heat Treatment on Milk Protein Functionality at Emulsion Interfaces. A Review. *Food Hydrocolloids* **2010,** *24*, 259-264.

48. M.Srinivasan; H.Singh; P.A.Munro, Creaming Stability of Oil-in-Water Emulsions Formed with Sodium and Calcium Caseinates. *Journal of Food Science* **2001,** *66*, 441-446.

49. M.Srinivasan; H.Singh; P.A.Munro, Sodium Caseinate-Stabilized Emulsions: Factors Affecting Coverage and Composition of Surface Proteins. *Journal of Agricultural and Food Chemistry* **1999,** *44*, 3807-3811.

50. E.Dickinson; M.Golding; J.W.Povey, Creaming and Flocculation of Oil-in-Water Emulsions Containing Sodium Caseinate. *Journal of Colloid and Interface Science* **1997,** *185*, 515-529.

51. W.Ramsden, Separation of Solids in the Surface-Layers of Solutions and 'Suspensions' (Observations on Surface-Membranes, Bubbles, Emulsions, and Mechanical Coagulation) - Preliminary Account. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **1903,** *72*, 156-164.

52. S. U. Pickering, Emulsions. *Journal of Chemical Society* **1907,** *91*, 2001-2020.

53. B.R.Midmore, Preparation of a Novel Silica-Stabilized Oil-Water Emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1998,** *132*, 257-265.

54. N.P.Ashby; B.P.Binks, Pickering Emulsions Stabilized by Laponite Clay Particles. *Physical Chemistry Chemical Physics* **2000,** *2*, 5640-5646.

55. K.Golemanov; S.Tcholakova; P.A.Kralchevsky; K.P.Ananthapadmanabhan; A.Lips, Latex Particle-Stabilized Emulsions of Anti-Bancroft Type. *Langmuir* **2006,** *22*, 4968-4977.

56. R.Aveyard; B.P.Binks; J.H.Clint, Emulsions Stabilised Solely by Colloidal Particles. *Advances in Colloid and Interface Science* **2003,** *100-102*, 503-546.

57. S.Melle; M.Lask; G. G. Fuller, Pickering Emulsions with Controllable Stability. *Langmuir* **2005,** *21*, 2158-2162.

58. E.Dickinson, Stabilising Emulsion-Based Colloidal Structures with Mixed Food Ingredients. *Journal of the Science of Food and Agriculture* **2013,** *93*, 710-721.

59. S.Tsuji; H.Kawaguchi, Thermosensitive Pickering Emulsion Stabilized by Poly(N-isopropylacrylamide)-Carrying Particles *Langmuir* **2008,** *24*, 3300-3305.

60. S.Abend; N.Bonnke; U.Gutschner, Stabilization of Emulsions by Heterocoagulation of Clay Minerals and Layered Double Hydroxides. *Colloid and Polymer Science* **1998,** *276*, 730-737.

61. J.Frelichowska; M.A.Bolzinger; Y.Chevalier, Pickering Emulsions with Bare Silica. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2009,** *343*, 70-74.

62. I.Akartuna; An.R.Studart; E.Tervoort; U.T.Gonzenbach; L.J.Gauckler, Stabilization of Oil-in-Water Emulsions by Colloidal Particles Modified with Short Amphiphiles. *Langmuir* **2008,** *24*, 7161-7168.

63. R.J.G Lopetinsky, J.H.Masliyah and Z.Xu, Solids-Stablised Emulsions: A review. In *Colloidal Particles at Liquid Interfaces*. B.P.Binks; T.S.Horozov, Cambridge University Press: Cambridge, 2006.

64. B.P.Binks; J.H.Clint, Solid Wettability from Surface Energy Components: Relevance to Pickering Emulsions *Langmuir* **2002,** *18*, 1270-1273.

65. E.Vignati; R.Piazza, Pickering Emulsions: Interfacial Tension, Colloidal Layer Morphology, and Trapped-Particle Motion. *Langmuir* **2003,** *19*, 6650-6656.

66. B.P.Binks; S.O.Lumsdon, Pickering Emulsions Stabilized by Monodisperse Latex Particles: Effects of Particle Size *Langmuir* **2001,** *17*, 4540-4547.

67. J.H.Schulman; J.Leja, Control of Contact Angles at the Oil-Water-Solid Interfaces. Emulsions Stabilized by Solid Particles ($Baso_4$). *Transactions of the Faraday Society* **1954,** *50*, 598-605.

68. E.Dickinson, Food Emulsions and Foams: Stabilization by Particles. *Current Opinion in Colloids and Interface Science* **2010,** *15*, 40-49.

69. B.P.Binks; S.O.Lumsdon, Influence of Particle Wettability on the Type and Stability of Surfactant-Free Emulsions. *Langmuir* **2000,** *16*, 8622-8631.

70. B.P.Binks, Particles as Surfactants-Similarities and Differences. *Current Opinion in Colloids and Interface Science* **2002,** *7*, 21-41.

71. S.Levine; B.D.Bowen; S.J.Partridge, Stabilization of Emulsions by Fine Particles I. Partitioning of Particles between Continuous Phase and Oil/Water Interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1989,** *38*, 325-343.

72. S.Abend; G.Lagaly, Bentonite and Double Hydroxides as Emulsifying Agents. *Clay Miner* **2001,** *36*, 557-570.

73. B.P.Binks; J.H.Clint; G.Mackenzie; C.Simcock; C.P.Whitby, Naturally Occurring Spore Particles at Planar Fluid Interfaces and in Emulsions. *Langmuir* **2005,** *21*, 8161-8167.

74. T.S.Horozov; B.P.Binks, Particle-Stabilized Emulsions: A Bilayer or a Bridging Monolayer? *Angewandte Chemie International Edition* **2006,** *45*, 773-776.

75. N.Gh.Eskandari; S.Simovic; C.A.Prestidge, Synergistic Effect of Silica Nanoparticles and Charged Surfactants in the Formation and Stability of Submicron Oil-in-Water Emulsions. *Physical Chemistry Chemical Physics* **2007,** *9*, 6426-6434.

76. H.Hassander; B.Johansson; B.Törnell, The Mechanism of Emulsion Stabilization by Small Silica (Ludox) Particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1989,** *40*, 93-105.

77. J.Wang; F.Yang; J.Tan; G.Liu; J.Xu; D.Sun, Pickering Emulsions Stabilized by a Lipophilic Surfactant and Hydrophilic Platelike Particles. *Langmuir* **2010,** *26*, 5397-5404.

78. A.Bausch; A.Dinsmore; M.Hsu; M.Nikolaides; D.Weitz, Methods and Compositions for Encapsulating Active Agents.2004.US.Patent 2004/0096515 A1.

79. B.R.Midmore, Synergy between Silica and Polyoxyethylene Surfactants in the Formation of O/W Emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1998,** *145*, 133-143.

80. B.P.Binks; A.Desforges, Synergistic Stabilization of Emulsions by a Mixture of Surface-Active Nanoparticles and Surfactant *Langmuir* **2007,** *23*, 1098-1106.

81. R.Pichot; F.Spyropoulos; I.T.Norton, Mixed-Emulsifier Stabilised Emulsions: Investigation of the Effect of Monoolein and Hydrophilic Silica Particle Mixtures on the Stability against Coalescence. *Journal of Colloid and Interface Science* **2009,** *329*, 284-291.

82. S.Tesch; Ch.Gerhardsb; H.Schuberta, Starch Particles for Food Based Pickering Emulsions. *International Congress on Engineering and Food* **2011,** 1, *95-103*

83. S.Tesch; Ch.Gerhardsb; H.Schuberta, Stabilization of Emulsions by OSA Starches. *Journal of Food Science* **2002,** *54*, 167-174.

84. E.Dickinson, Use of Nanoparticles and Microparticles in the Formation and Stabilization of Food Emulsions. *Trends in Food Science and Technology* **2012,** *24*, 4-12.

85. A.Yusoff; B.S.Murray, Modified Starch Granules as Particle-Stabilizers of Oil-in-Water Emulsions. *Food Hydrocolloids* **2011,** *25*, 42-55.

86. J.Bao; J.Xing; D.L.Phillips; H.Corke, Physical Properties of Octenyl Succinic Anhydride Modified Rice, Wheat, and Potato Starches. *Journal of Agricultural and Food Chemistry* **2003,** *51*, 2283-2287.

87. P.O.Kamlesh; G.F.Sylvan, Microcrystalline Cellulose Stabilized Emulsions. *Journal of Dispersion Science and Technology* **1986,** *7*, 543-561.

88. G.O.Philips; D.J.Wedlock; P.A.Williams, Application of Microcrystalline Cellulose. In *Gums and Stabilizers for the Food Industry*, IRL Press: Oxford, 1990; Vol. 5.

89. H.D.Goff Ice Cream. In *Advanced Dairy Chemistry: Lipids*. P.F.Fox; P.L.H.McSweeney, Springer: New York, 2006; Vol. Two.

90. D.Rousseau, Fat Crystals and Emulsion Stability: A Review. *Food Research International* **2000,** *33*, 3-14.

91. M. A. J. S. v. Boekel; P.Walstra, Stability of Oil-in-Water Emulsions with Crystals in the Disperse Phase. *Colloids and Surfaces* **1981,** *3*, 109-118.

92. P.Rousset, Crystallization. In *Physical Properties of Lipids*. A.G.Marangoni and S.Narine, CRC Press: New York, 2002.

93. K.Boode; C.Bisperink; P.Walstra, Destabilization of O/W Emulsions Crystals by Temperature Cycling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1999,** *61*, 55-74.

94. K.W. Smith, Fat Crystal Networks. In *Crystallization Processes in Fats and Lipid Systems*. N.Garti; K. Sato, Marcel Dekker: New York, 2001.

95. J.N.Coupland, Crystallization in Emulsions. *Current Opinion in Colloid and Interface Science* **2002,** *7*, 445-450.

96. R.W.Hartel, Crystal Growth. In *Crystallization in Foods*. Aspen: Gaithersburg, 2001.

97. S.Metin and R.W.Hartel, Crystallization of Fats and Oils. In *Bailey's Industrial Oil and Fat Products*, Sixth ed.; F.Shahidi, Ed. John Wiley & Sons: 2005.

98. W.Kloek; P.Walstra; T. V. Vliet, Nucleation Kinetics of Emulsified Triglyceride Mixtures. *Journal of the American Chemical Society* **2000,** *77*, 643-652.

99. L.W.Phipps, Heterogenous and Homogeneous Nucleation in Supercooled Triglycerides and N -Paraffins. *Transactions of the Faraday Society* **1964,** *60*, 1873-1883.

100. P.Walstra, Secondary Nucleation in Triglyceride Crystallization. *Progress in Colloid and Polymer Science* **1998,** *108*, 4-8.

101. J.W.Mullin, Nucleation. In *Crystallisation*. Third ed.; Butterworth-Heinemann: Oxford, 1993.

102. R.E.Timms, Fractional Crystallisation – The Fat Modification Process for the 21st Century. *European Journal of Lipid Science and Technology* **2005,** *107*, 48-57.

103. A.Huyghebaert, Lipid Crystallization Kinetics. In *Crystallization and Solidification Properties of Lipids*. N.Widlak; R.W.Hartel; S.Narine, AOCS Press: Illinois, 2001.

104. K.Sato; N.Yoshimoto; T.Arishima, Crystallization Phenomena in Fats and Lipids *Journal of Dispersion Science and Technology* **1989,** *10*, 363-392.

105. D.J.Cebula; G.Ziegleder, Studies of Bloom Formation Using X-Ray Diffraction from Chocolates after Long-Term Storage. *European Journal of Lipid Science and Technology* **1993,** *95*, 340-343.

106. D.Johansson; B.Bergenstahl, Sintering of Fat Crystal Network in Oil During Post-Crystallization Processes. *Journal of the American Oil Chemists' Society* **1995,** *72*, 911-920.

107. J.E.Schaap; G.A.M.Rutten, Effect of Technological Factors on the Crystallization of Bulk Milk Fat. *Netherlands Milk and Dairy Journal* **1976,** *30*, 197-206.

108. S.Martini; A.G.Marangoni, Microstructure of Dairy Fat Products. In *Structure of Dairy Products*, A.Tamime, Ed. Blackwell: Oxford, 2007.

109. M.L.Herrera; R.W.Hartel, Effect of Processing Conditions on Crystallization Kinetics of a Milk Fat Model System *Journal of the American Oil Chemists' Society* **2000,** *77*, 1177-1188.

110. A.G.Marangoni; R.W.Hartel, Visualization and Structural Analysis of Fat Crystal Networks. *Journal of Food Technology* **1998,** *52*, 46-51.

111. R.Campos; S.S.Narine; A.G.Marangoni, Effect of Cooling Rate on the Structure and Mechanical Properties of Milk Fat and Lard. *Food Research International* **2002,** *35*, 971-981.

112. S.Martini; M.L.Herrera; R.W.Hartel, Effect of Cooling Rate on Nucleation Behavior of Milk Fat-Sunflower Oil Blends. *Journal of Agricultural and Food Chemistry* **2001,** *49*, 3223-3229.

113. A.J.Wright; M.G.Scanlon; R.W.Hartel; A.G.Marangoni, Rheological Properties of Milkfat and Butter. *Journal of Food Science* **2001,** *66*, 1056-1071.

114. A.J.Dijkstra Modified Processes and Food Uses. In *The Lipid Handbook*. F.D.Gunstone; J.L.Harwood; A.J.Dijkstra, Third ed.; CRC Press: New York, 2007; Vol. Third.

115. N.K.Ojijo; I.Neeman; Sh.Eger; E.Shimoni, Effects of Monoglyceride Content, Cooling Rate and Shear on the Rheological Properties of Olive Oil/Monoglyceride Gel Networks. *Journal of Food Science* **2004,** *84*, 1585-1593.

116. W.Kloek; T.V.Vliet; P.Walstra, Mechanical Properties of Fat Dispersions Prepared in a Mechanical Crystallizer. *Journal of Texture Studies* **2005,** *36*, 544-568.

117. W.Xu; A.Nikolov; D.T.Wasan, Shear-Induced Fat Particle Structure Variation and the Stability of Food Emulsions: II. Effects of Surfactants, Protein, and Fat Substitutes. *Journal of Food Engineering* **2005,** *66*, 107-116.

118. W.Xu; A.Nikolov; D.T.Wasan, Shear-Induced Fat Particle Structure Variation and the Stability of Food Emulsions: I. Effects of Shear History, Shear Rate and Temperature. *Journal of Food Engineering* **2005,** *66*, 97-105.

119. P.Walstra, Emulsion Stability. In *Encyclopedia of Emulsion Technology*, P.Becher, Ed. Marcel Dekker: New York, 1996; Vol. 4.

120. S.Dukhin; J.Sjöblom, Emulsions and Emulsion Stability. In *Kinetics of Brownian and Gravitational Coagulation in Dilute Emulsions*, J.Sjöblom, Ed. Marcel Dekker: New York, 1996.

121. G.Marrucci, A Theory of Coalescence. *Chemical Engineering science* **1969,** *24*, 975-985.

122. K.Boode; P.Walstra, Partial Coalescence in Oil-in-Water Emulsions 1.Nature of the Aggregation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1993,** *81*, 121-137.

123. P.Taylor, Ostwald Ripening in Emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1995,** *99*, 175-185.

124. O.R.Fennema, Lipids. In *Food Chemistry*, W.W.Nawar, Ed. Marcel Dekke: New York, 1996.

125. S.O.Agboola; G.Dalgleish, Enzymatic Hydrolysis of Milk Proteins Used for Emulsion Formation. 1. Kinetics of Protein Breakdown and Storage Stability of the Mulsions. *Journal of Agricultural and Food Chemistry* **1996,** *44*, 3631-3636.

126. E.Dickinsonn, Colloidal Aggregation: Mechanisms and Implications. In *Food Colloids, Biopolymers and Materilas*. E.Dickinson; T. Van Vliet, Royal Society of Chemistry: London, 2003.

127. M.M.Robins, Emulsions - Creaming Phenomena. *Current Opinion in Colloid and Interface Science* **2000,** *5*, 254-273.

128. P.Walstra, Introduction to Aggregation Phenomenon in Food Colloids. In *Food Colloids and Polymers: Stability and Mechanical Properties*, Third ed.; E.Dickinson; P.Walstra, Eds. Royal Society of Chemistry: Cambridge, 1993.

129. M.M.Robins; A.D.Watson; P.J.Wilde, Emulsions Creaming and Rheology. *Current Opinion in Colloid and Interface Science* **2002,** *7*, 419-425.

130. P.Walstra, Priciples of Emulsion Formation. *Chemical Engineering science* **1993,** *48*, 333-350.

131. E.Dickinson, Emulsion Instability. In *Introduction to Food Colloids*. Oxford University Press: Oxford, 1992.

132. S.S.Dukhin; J.Sjöblom; D.T.Wasan; O.Saether, Coalescence Coupled with Either Coagulation or Flocculation in Dilute Emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2001,** *180*, 223-234.

133. D.N.Petsev, Theoretical Analysis of Film Thickness Transition Dynamics and Coalescence of Charged Miniemulsion Droplets. *Langmuir* **2002,** *16*, 2093-2100.

134. R.P.Borwankar; L.A.Lobo; D.T.Wasan, Emulsion Stability - Kinetics of Flocculation and Coalescence. *Colloids and Surfaces* **1992,** *69*, 135-146.

135. U.Sundararaj; C.W.Macosko, Drop Breakup and Coalescence in Polymer Blends: The Effects of Concentration and Compatibilization. *Macromolecules* **1995,** *8*, 2647-2657.

136. D.T.Wasan; J.J.McNamara; S.M.Shah; K.Sampath; N.Aderangi, The Role of Coalescence Phenomena and Interfacial Rheological Properties in Enhanced Oil Recovery: An Overview. *Journal of Rheology* **1979,** *23*, 181-208.

137. K.Boode; P.Walstra; A. E. A. d. Groot-Mostert, Partial Coalescence in Oil-in-Water Emulsions 2. Influence of the Properties of the Fat. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1993,** *81*, 139-151.

138. H.D.Goff, Instability and Partial Coalescence in Whippable Dairy Emulsions. *Journal of Dairy Science* **1997,** *80*, 2620-2630.

139. E.Fredrick; P.Walstra; K.Dewettinck, Factors Governing Partial Coalescence in Oil-in-Water Emulsions. *Advances in Colloid and Interface Science* **2010,** *153*, 30-42.

140. D.F.Darling, Recent Advances in the Destabilization of Dairy Emulsions. *Journal of Dairy Research* **1982,** *49*, 695-712.

141. S.A.Vanapalli; J.N.Coupland, Emulsions under Shear- the Formation and Properties of Partially Coalesced Lipid Structures. *Food Hydrocolloids* **2001,** *16*, 507-512.

142. A.R.Mackie; A.P.Gunning; P. J. Wilde; V.J.Morris, Orogenic Displacement of Protein from the Oil/Water Interface. *Langmuir* **2000,** *16*, 2242-2247.

143. C.Granger; P.Barey; P.Veschambre; M.Cansell, Physicochemical Behavior of Oil-in-Water Emulsions: Influence of Milk Protein Mixtures, Glycerol Ester Mixtures and Fat Characteristics. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2005,** *5*, 235-243.

144. N.I.Hayati; Y.B.C.Man; C.P.Tan; I. N. Aini, Droplet Characterization and Stability of Soybean Oil/Palm Kernel Olein O/W Emulsions with the Presence of Selected Polysaccharides. *Food Hydrocolloids* **2009,** *23*, 233-243.

145. A.S.Kabalnov; E.D.Shchukin, Ostwald Ripening Theory: Applications to Fluorocarbon Emulsion Stability. *Journal of Colloid and Interface Science* **1992,** *38*, 69-97.

146. T.Cosgrove, Emulsions. In *Colloid Science: Principles, Methods and Applications*, Second ed.; B.Vincent, Ed. Wiley-Blackwel: Sussex, 2010.

147. F.D.Gunstone, Fatty acids: Nomenclature, Struture, Isolation and Struture Determination, Biosynthesis and Chemical Synthesis. In *Fatty Acid and Lipid Chemistry*. Aspen Publication: New York, 1996.

148. H.D.Belitz; W.Grosch; P.Schieberle, Lipids. In *Food Chemistry*. Fourth ed.; Springer: Berlin, 2009.

149. A.P.Simopoulos, Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids: Evolutionary Aspects. In *Omega-6/Omega-3 Essential Fatty Acid Ratio: The Scientific Evidence*. A.P.Simopoulos; L.G.Cleland, Karger: Basel, 2003.

150. R.L.Wysong, Essential Fatty Acids. In *Lipid Nutrition: Understanding Fats and Oils in Health and Disease*. Inquiry Press: Michigan, 1990.

151. C.Ruxton, Health Benefits of Omega-3 Fatty Acids. *Nursing Standard* **2004,** *18*, 38-42.

152. J. M. Kremer, Omega-3 Fatty Acid Supplements in Rheumatoid Arthritis. *American Journal of Clinical Nutrition* **2000,** *71*, 349S-3451S.

153. C.R.Harper; T.A.Jacobson, The Role of Omega-3 Fatty Acids in the Prevention of Coronary Heart Disease. *The Archives of Internal Medicine* **2001,** *161*, 2185-2192.

154. I.Toft; K.H.Bonaa; O.C.Ingebretsen; A.Nordoy; T.Jenssen, Effects of Omega-3 Polyunsaturated Fatty Acids on Glucose Homeostasis and Blood Pressure in Essential Hypertension. *Annals of Internal Medicine* **1995,** *123*, 911-918.

155. J.R.Vercellotti; A.J.St.Angelo; A.M.Spanier, Lipid Oxidation in Foods: An Overview. In *Lipid Oxidation in Food*, A.St.Angelo, Ed. American Chemical Society: Washington, 1992.

156. F.Shahidi; Y.Zhong, Lipid Oxidation: Measurement Methods. In *Bailey's Industrial Oil and Fat Products*, Sixth ed.; F.Shahidi, Ed. John Wiley & Sons New Jersy, 2005; pp 357-386.

157. E.N.Frankel, Secondary Products of Lipid Oxidation. *Chemistry and Physics of Lipids* **1987,** *44*, 73-85.

158. J.R.Mancuso; D.J.McClements; E.A.Decker, Iron-Accelerated Cumene Hydroperoxide Decomposition in Hexadecane and Trilaurin Emulsions *Journal of Agricultural and Food Chemistry* **2000,** *48*, 213-219.

159. L.Mei; D.J.McClements; J.Wu; E.A.Decker, Iron-Catalyzed Lipid Oxidation in Emulsions as Affected by Surfactant, pH and Nacl. *Food Chemistry* **1998,** *61*, 307-312.

160. H.Yuji; J.Weiss; P.Villeneuve; L.J.L.Giraldo; M. C. Figueroa-Espinoza; E.A.Decker, Ability of Surface-Active Antioxidants to Inhibit Lipid Oxidation in Oil-in-Water Emulsion *Journal of Agricultural and Food Chemistry* **2007,** *55*, 11052-11056.

161. E.A.Decker; D.J.McClements; W.Chaiyasit; C.Nuchi; M.P.C.Silvestre; L.M.Tong; L.Mei, Factors Influencing Free Radical Formation in Food Emulsions. In *Free Radicals in Food: Chemistry, Nutrition and Health Affects*, C.T.Ho; F.Shahidi, Eds. ACS Press: Washington, 2002.

162. S.S.Kellerby; D.J.McClements; E.A.Decker, Role of Proteins in Oil-in-Water Emulsions on the Stability of Lipid Hydroperoxides *Journal of Agricultural and Food Chemistry* **2006,** *54*, 7879-7884.

163. A.W.Girotti, Lipid Hydroperoxide Generation, Turnover, and Effector Action in Biological Systems. *Journal of Lipid Research* **1998,** *39*, 1529-1553.

164. B.Halliwell; S. Chirico, Lipid Peroxidation: Its Mechanism, Measurement, and Significance. *The American Journal of Clinical Nutrition* 1993, p 715S-724S.

165. M.C.Qiant; G.Devin, Gas Chromatography. In *Food Analysis*. S.S.Nielse, Fourth ed.; Springer: New York, 2010.

166. R.Bhalla; A.Kumarsingh; S.Asachi and K.R.Unnikumar, Lipids:Struture, Funstion and Biotechnology Aspects. In *A Textbook of Molecular Biotechnology*. A.K.Chauhan; A.Varma, I.K.International: New Delhi, 2009.

167. N.C.Shantha; E.A.Decker, Rapid, Sensitive, Iron-Based Spectrophotometric Methods for Determination of Peroxide Values of Food Lipids. *Journal of the Association of Official Agricultural Chemists* **1994,** *77*, 421-424.

168. D.Kritchevsky, Fats and Oils in Human Health. In *Food Lipids*, C.C.Akoh; D.B.Min, Eds. Marcel Dekker: New York, 1998.

169. R.D.O'Brien, Solidification Analysis. In *Fats and Oils: Formulating and Processing for Applications*. Third ed.; CRC Press: New York, 2008.

170. K.G.Berger; R.J.Hamilton, Lipids and Oxygen: Is Rancidity Avoidable in Practice? In *Developments in Oils and Fats*, R.J.Hamilton, Ed. Blackie Academic & Professional: Glasgow, 1995.

171. W.Chaiyasit; R.J.Elias; D. J. McClements; E. A. Decker, Role of Physical Structures in Bulk Oils on Lipid Oxidation *Critical Reviews in Food Science and Nutrition* **2007,** *47*, 299-317.

172. G.Minotti; S.D.Aust, The Role of Iron in the Initiation of Lipid Peroxidation. *Chemistry and Physics of Lipids* **1987,** *44*, 191-208.

173. J.C.Cowan, Key Factors and Recent Advances in the Flavor Stability of Soybean Oil. *Journal of the American Oil Chemists' Society* **1969,** *43*, 300-321A.

174. D.W.Reisch, Antioxidants. In *Food Lipids: Chemistry, Nutrition and Biotechnology*, C.C.Akoh; D.B.Min, Eds. Marcel Dekker: New York, 1998.

175. W.L.Porter, Paradoxical Behavior of Antioxidants in Food and Biological Systems. In *Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspects*, G.M.William, Ed. Princeton:Princeton Scientifc: New Jersy, 1993.

176. F.Shahidi; Y.Zhong, Revisiting the Polar Paradox Theory: A Critical Overview. *Journal of Agricultural and Food Chemistry* **2011,** *59*, 3499-3504.

177. S.H.Huang; A.Hopia; K.Schwarz; E.N.Frankel; J.B.German, Antioxidant Activity of a-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils Vs Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **1996,** *44*, 444-452.

178. E.N.Frankel; S.W.Huang; J.Kanner; J.B.German, Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils Vs Emulsions. *Journal of Agricultural and Food Chemistry* **1994,** *42*, 1054-1059.

179. V.Kristinova; R.Mozuraityte; I.Storr; T.Rustad, Antioxidant Activity of Phenolic Acids in Lipid Oxidation Catalyzed by Different Prooxidants. *Journal of Agricultural and Food Chemistry* **2009,** *57*, 10377-10385.

180. S.Trombino; S.Serini; F.DiNicuolo; L.Celleno; S.Ando; N.Picci; G.Calviello; P.Palozza, Antioxidant Effect of Ferulic Acid in Isolated Membranes and Intact Cells: Synergistic Interactions with a-Tocopherol, B-Carotene, and Ascorbic Acid. *Journal of Agricultural and Food Chemistry* **2004,** *52*, 2411-2420.

181. E.N.Frankel, Food Antioxidants. In *Antioxidants in Food and Biology: Facts and Fiction*. The Oily Press: Bridgewater, 2007.

182. S.Kiokias; Ch.Dimakou; V.Oreopoulou, Effect of Heat Treatment and Droplet Size on the Oxidative Stability of Whey Protein Emulsions. *Food Chemistry* **2007,** *105*, 94-100.

183. M.Kargar; F.Spyropoulos; I.T.Norton, The Effect of Interfacial Microstructure on the Lipid Oxidation Stability of Oil-in-Water Emulsions. *Journal of Colloid and Interface Science* **2011,** *357*, 527-533.

184. L.M.Tong; S.Sasaki; D. J. McClements; E.A.Decker, Mechanisms of the Antioxidant Activity of a High Molecular Weight Fraction of Whey. *Journal of Agricultural and Food Chemistry* **2000,** *48*, 1473-1478.

185. H.Faraji; D.J.McClements; E.A.Decker, Role of Continuous Phase Protein on the Oxidative Stability of Fish Oil-in-Water Emulsions *Journal of Agricultural and Food Chemistry* **2004,** *52*, 4558-4564.

186. R.J.Elias; D.J.McClements; E.A.Decker, Impact of Thermal Processing on the Antioxidant Mechanisms of Continuous Phase β-Lactoglobulin in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2007,** *104*, 1402-1409.

187. M.J.Taylor; T.Richardson, Antioxidant Activity of Skim Milk Effect of Heat and Resultant Sulfhydryl-Groups. *Journal Dairy Science* **1980,** *63*, 1783-1795.

188. R.J.Elias; S.S.Kellerby; E.A.Decker, Antioxidant Activity of Proteins and Peptides. *Critical Reviews in Food Science and Nutrition* **2008,** *48* 430-441.

189. R.J.Elias; D. J. McClements; E. A. Decker, Antioxidant Activity of Cysteine, Tryptophan, and Methionine Residues in Continuous Phase α-Lactoglobulin in Oil-in-Water Emulsions *Journal of Agricultural and Food Chemistry* **2005,** *53*, 10248-10253.

190. A.M.Haahr; Ch.Jacobsen, Emulsifier Type, Metal Chelation and pH Affect Oxidative Stability of N-3-Enriched Emulsions. *European Journal of Lipid Science and Technology* **2008,** *110*, 949-961.

191. J.R.Mancuso; D.J.McClements; E.A.Decker, The Effects of Surfactant Type, pH, and Chelators on the Oxidation of Salmon Oil-in-Water Emulsions *Journal of Agricultural and Food Chemistry* **1999,** *47*, 4112-4116.

192. Y.J.Cho; J.Alamed; D.J.McClements; E.A.Decker, Ability of Chelators to Alter the Physical Location and Prooxidant Activity of Iron in Oil-in-Water Emulsions *Journal of Food Science* **2003,** *68*, 1952-1957.

193. J.R.Mahoney; E.Graf, Role of a-Tocopherol, Ascorbic Acid, Citric Acid and EDTA as Oxidants in a Model System. *Journal Food Science* **1986,** *51*, 1293-1296.

194. M.Diaz; Ch.M.Dunn; D.J.McClements; E.A.Decker, Use of Caseinophosphopeptides as Natural Antioxidants in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2003,** *51*, 2365-2370.

195. S.G.Rival; C.G.Boeriu; H.J.Wichers, Caseins and Casein Hydrolysates. 2. Antioxidative Properties and Relevance to Lipoxygenase Inhibition. *Journal of Agricultural and Food Chemistry* **2001,** *49*, 295-302.

196. B.Chen; D.J.McClements; E.A.Decker, Role of Continuous Phase Anionic Polysaccharides on the Oxidative Stability of Menhaden Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2010,** *58*, 3779-3784.

197. Y.Matsumura; M.Egami; C.Satake; Y.Maeda; T.Takahashi; A.Nakamura; T.Mori, Inhibitory Effects of Peptide-Bound Polysaccharide on Lipid Oxidation in Emulsions. *Food Chemistry* **2003,** *83*, 107-119.

198. K.Shimada; K.Fujikawa; K.Yahara; T.Nakamura, Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. *Journal of Agricultural and Food Chemistry* **1992,** *40*, 945-948.

199. C.S.Boon; Z.Xu; X.Yue; D.J.McClements; J.Weiss; E.A.Decker, Factors Affecting Lycopene Oxidation in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2008,** *56*, 1408-1414.

200. L.Mei; D.J.McClements; E.A.Decker, Lipid Oxidation in Emulsions as Affected by Charge Status of Antioxidants and Emulsion Droplets. *Journal of Agricultural and Food Chemistry* **1999,** *47*, 2267-2273.

201. D.Djordjevic; D.J.McClements; E.A.Decker, Oxidative Stability of Whey Protein-Stabilized Oil-in-Water Emulsions at pH 3: Potential Omega-3 Fatty Acid Delivery Systems (Part B). *Journal of Food Science* **2004,** *62*, C356-C369.

202. L.Mei; E.A.Decker; D.J.McClements, Evidence of Iron Association with Emulsion Droplets and Its Impact on Lipid Oxidation. *Journal of Agricultural and Food Chemistry* **1998,** *46*, 5072-5077.

203. M.Hu; D.J.McClements; E.A.Decker, Impact of Whey Protein Emulsifiers on the Oxidative Stability of Salmon Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2003,** *51*, 1435-1439.

204. M.P.C.Silvestre; W.Chaiyasit; R.G.Brannan; D.J.McClements; E.A.Decker, Ability of Surfactant Headgroup Size to Alter Lipid and Antioxidant Oxidation in Oil-in-Water Emulsions *Journal of Agricultural and Food Chemistry* **2000,** *48*, 2057-2061.

205. W.Chaiyasit; M.P.C.Silvestre; D.J.McClements; E.A.Decker, Ability of Surfactant Hydrophobic Tail Group Size to Alter Lipid Oxidation in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2000,** *48*, 3077-3080.

206. M.Hu; D.J.McClements; E.A.Decker, Lipid Oxidation in Corn Oil-in-Water Emulsions Stabilized by Casein, Whey Protein Isolate, and Soy Protein Isolate. *Journal of Agricultural and Food Chemistry* **2003,** *51*, 1696-1700.

207. D.G.Dalgleish; M.Srinivasan; M.Singht, Surface Properties of Oil-in-Water Emulsion Droplets Containing Casein and Tween 60. *Journal of Agricultural and Food Chemistry* **1995,** *43*, 2351-2355.

208. M.P.Richards; W.Chaiyasit; D.J.McClements; E.A.Decker, Ability of Surfactant Micelles to Alter the Partitioning of Phenolic Antioxidants in Oil-in-Water Emulsions *Journal of Agricultural and Food Chemistry* **2002,** *50*, 1254-1259.

209. Y.Cho; D.J.McClements; E.A.Decker, Ability of Surfactant Micelles to Alter the Physical Location and Reactivity of Iron in Oil-in-Water Emulsion *Journal of Agricultural and Food Chemistry* **2002,** *50*, 5704-5710.

210. C.Nuchi; D.Hernandez; D.J.McClements; E.A.Decker, Ability of Lipid Hydroperoxides to Partition into Surfactant Micelles and Alter Lipid Oxidation Rates in Emulsions. *Journal of Chemical Society* **2002,** *51*, 5522-5527.

211. S.Ogawa; E.A.Decker; D.J.McClements, Production and Characterization of O/W Emulsions Containing Cationic Droplets Stabilized by Lecithin-Chitosan Membranes. *Journal of the American Chemical Society* **2003,** *51*, 2806-2812.

212. U.Klinkesorn; P.Sophanodora; P.Chinachoti; D.J.McClements; E.A.Decker, Production and Characterization of Oil-in-Water Emulsions Containing Droplets Stabilized by Multilayer Membranes Consisting of a-Lactoglobulin, β-Carrageenan and Gelatin *Langmuir* **2005,** *21*, 5752-5760.

213. L.Moreau; H.J.Kim; E.A.DECKER; D.J.McClements, Production and Characterization of Oil-in-Water Emulsions Containing Droplets Stabilized by Beta-Lactoglobulin–Pectin Membranes. *Journal of Agricultural and Food Chemistry* **2003,** *22*, 6612-6617.

214. M.Katsuda; D.J.McClements; L.Miglioranza; E.A.Decker, Physical and Oxidative Stability of Fish Oil-in-Water Emulsions Stabilized with B-Lactoglobulin and Pectin. *Food Chemistry* **2012,** *56*, 5926-5931.

215. D.Djordjevic; L.Cercaci; J.Alamed; D.J.McClements; E.A.Decker, Chemical and Physical Stability of Citral and Limonene in Sodium Dodecyl Sulfate Chitosan and Gum Arabic-Stabilized Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry* **2007,** *55*, 3585-3591.

216. L.A.Shaw; D.J.McClements; E.A.Decker, Spray-Dried Multilayered Emulsions as a Delivery Method for Omega-3 Fatty Acids into Food Systems. *Journal of Agricultural and Food Chemistry* **2007,** *55*, 3112-3119.

217. M.A.Grompone, Sunflower Oil. In *Bailey's Industrial Oil and Fat Products*, F.Shahidi, Ed. John Wiley & Sons: New Jersey, 2005.

218. A.P.Imeson; W.Humphreys, Microcrystalline Cellulose. In *Thickening and Gelling Agents for Food*. A.Imeson, Second ed.; Aspen Publisher: New York, 1997.

219. H.Iijima; K.Takeo, Microcrystalline Cellulose: An Overview In *Handbook of Hydrocolloids*, G.O.Phillips; P.A.Williams, Eds. Woodhead Publishing Limited: Florida, 2000; pp 331-346.

220. S. L. S.-A. Overview, Www.Malven.Com. In 2000.

221. D.Chicea, Revealing Magnetite Nanoparticles Aggregation Dynamics – A SLS and DLS Study. In *Trends in Nanophysics: Theory, Experiment and Technology*. A.Aldea; V.Barsan, Springer: Berlin, 2010.

222. E.Keowmaneechai; D.J.McClements, Influence of EDTA and Citrate on Physicochemical Properties of Whey Protein-Stabilized Oil-in-Water Containing Cacl2. *Journal of Agricultural and Food Chemistry* **2002,** *50*, 7145-7153.

223. N.H.March; M.P.Tosi, Diffusion. In *Introduction to Liquid State Physics*. Allied Publishers Private Limited: Mumbai, 2005.

224. R.Xu, Electrophoretic Light Scatering- Zeta Potential Measurment. In *Particle Characterization: Light Scattering Methods*. Kluwer Academic Publishers: Dordrecht, 2002.

225. G.Klancnik; J.Medved; P.Mrvar, Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC) as a Method of Material Investigation. *Materials and Geoenvironment* **2012,** *57*, 127-142.

226. G.W.H.Höhne; W.F.Hemminger; H.-J.Flammersheim, Types of Differential Scanning Calorimeters and Modes of Operation. In *Differential Scanning Calorimetry*. Second ed.; Springer: Berlin, 2003.

227. AOCS, P-Anisidine Value. In *Official Methods and Recommended Practices of the American Oil Chemists. Method Cd 18-90;*, AOCS Press: : Champaign, 1998.

228. B.Muika; B.Lendl; A.Molina-D´ýaz; M.Ayora-Ca˜nada, Direct Monitoring of Lipid Oxidation in Edible Oils by Fourier Transform Raman Spectroscopy. *Chemistry and Physics of Lipids* **2005,** *134*, 173-182.

229. S.M.Jafari; E.Assadpoor; Y. H. B.Bahandari, Re-Coalescence of Emulsion Droplets During High-Energy Emulsification. *Food Hydrocolloids* **2008,** *22*, 1191-1202.

230. Ch.Qian; D.J.McClements, Formation of Nanoemulsions Stabilized by Model Food-Grade Emulsifiers Using High-Pressure Homogenization: Factors Affecting Particle Size. *Food Hydrocolloids* **2011,** *25*, 1000-1008.

231. E.Dickinson; M.Golding, Rheology of Sodium Caseinate Stabilized Oil-in-Water Emulsions. *Journal of Colloid and Interface Science* **1997,** *191*, 166-176.

232. Q.Guo; T.H.Mu, Emulsifying Properties of Sweet Potato Protein: Effect of Protein Concentration and Oil Volume Fraction. *International Dairy Journal* **2011,** *25*,98-106

233. H.T.Osborn; C.C.Akoh, Effect of Emulsifier Type, Droplet Size, and Oil Concentration on Lipid Oxidation in Structured Lipid-Based Oil-in-Water Emulsions. *Food Chemistry* **2004,** *84*, 451-456.

234. Ch.Su; S.Gunasekaran, Effects of Protein Concentration and Oil-Phase Volume Fraction on the Stability and Rheology of Menhaden Oil-in-Water Emulsions Stabilized by Whey Protein Isolate with Xanthan Gum. *Food Hydrocolloids* **2009**,23, 165-174

235. R.J.Sims; J.Fioriti; J.Trumbetas, Effects of Sugars and Sugar Alcohols on Autoxidation of Safflower Oil in Emulsions. *Journalof the American Oil Chemists' Society* **1979,** *56*, 742-745.

236. D. J.McClements; E.Dickinson; S. R.Dungan; J. E.Kinsella; J.G.Ma; M. J. W.Povey, Effect of Emulsifier Type on the Crystallization Kinetics of Oil-in-Water Emulsions Containing a Mixture of Solid and Liquid Droplets. *Journal of Colloid and Interface Science* **1993,** *160*, 293-297.

237. P.F.Fox; A.L.Kelly, The Caseins. In *Proteins in Food Processing*. R. Y. Yada, Woodhead Publishing Limited: Cambridge, 2004.

238. Y. E. Sun; W.D.Wang; H.W.Chen; Ch.Li, Autoxidation of Unsaturated Lipids in Food Emulsion. *Critical Reviews in Food Science and Nutrition* **2011,** *51*, 453–466.

239. A.K.Anal; A.Tobiassen; J.Flanagan; H.Singh, Preparation and Characterization of Nanoparticles Formed by Chitosan–Caseinate Interactions. *Colloids and Surfaces : Biointerfaces* **2008,** *65*, 104-110.

240. H.Ma; P.Forssell; R.Partanen; R.Seppänen; J. Buchert; H. Boer, Sodium Caseinates with an Altered Isoelectric Point as Emulsifiers in Oil/Water Systems. *Journal of Agricultural and Food Chemistry* **2009,** *57*.3800-3807

241. S. M. V. Ruth; J.P.Roozen; M.A.Posthumus; F.H.M.Jansen, Volatile Composition of Sunflower Oil-in-Water Emulsions During Initial Lipid Oxidation: Influence of pH. *Journal of Agricultural and Food Chemistry* **1999,** *47*, 4365-4369.

242. E.A.Decker; B.Chen; S.Panya, Understanding Antioxidant Mechanisms in Preventing Oxidation in Foods. In *Oxidation in Foods and Beverages and Antioxidant Applications: Understanding Mechanisms of Oxidation and Antioxidant Activity*, E.A.Decker; R.J.Elias; D.J.McClements, Woodhead Publishing: London, 2010.

243. M.P.Sidorova; L.E.Ermakova; N.E.Kotel'nikova; N.P.Kudina, Electrosurface Properties of Microcrystalline Cellulose of Different Origin in 1 : 1 Electrolyte Solutions. *Colloid Journal* **2001,** *63*, 100-107.

244. R.Chanamai; D.J.McClements, Comparison of Gum Arabic, Modified Starch, and Whey Protein Isolate as Emulsifiers: Influence of pH, Cacl2 and Temperature. *Journal of Food Science* **2002,** *67*, 120-125.

245. R.Chanamai; D.J.McClements, Impact of Weighting Agents and Sucrose on Gravitational Separation of Beverage Emulsions. *Journal of Agricultural and Food Chemistry* **2000,** *48*, 5561-5565.

246. Y.Yaginuma; T.Kijima, Effect of pH on Rheological Properties of Microcrystalline Cellulose Dispersions. *Journal of Dispersion Science and Technology* **2006,** *27*, 365-370.

247. G.Azuma; N.Kimura; M.Hosokawa; K.Miyashita, Effect of Droplet Size on the Oxidative Stability of Soybean Oil Tag and Fish Oil Tag in Oil-in-Water Emulsion. *The Journal of Oleo Science* **2009,** *59*.329-338

248. K.Shimada; H.Muta; Y.Nakamura; H.Okada; K.Matsuo; S.Yoshioka; T.Matsudaira; T.Nakamura, Iron-Binding Property and Antioxidative Activity of Xanthan on the Autoxidation of Soybean Oil in Emulsion. *Food Chemistry* **1994,** *42*.1607-1611

249. D.Paraskevopoulou; D.Boskou; A.Paraskevopoulou, Oxidative Stability of Olive Oil-Lemon Juice Salad Dressings Stabilized with Polysaccharides. *Food Chemistry* **2007,** *101*, 1197-1204.

250. F.Leal-Caldero; V.Schmitt; J.Bibette, Emulsification. In *Emulsion Science: Basic Principles*. Second ed.; Springe: New York, 2007.

251. K. L.Mittal; R.D.Vold, Effect of the Initial Concentration of Emulsifying Agents on the Ultracentrifugal Stability of Oil-in-Water Emulsions. *Journal of the American Oil Chemists' Society* **2011,** *49*, 527-532.

252. A.Patist; S.SBhagwat; K.W.Penfield; P.Aikens; D.O.Shah, On the Measurment of Critical Micelle Concentrations of Pure and Technical-Grade Nonionic Surfactants. *Journal of Surfactants and Detergents* **2000**,3, 53-58.

253. R.Pichot; F.Spyropoulos; I.T.Norton, O/W Emulsions Stabilised by Both Low Molecular Weight Surfactants and Colloidal Particles:. *Journal of Colloid and Interface Science* **2010,** *352*.128-135

254. L.Lethuaut; F.Métro; C.Genot, Effect of Droplet Size on Lipid Oxidation Rates of Oil-in-Water Emulsions Stabilized by Protein. *Journal of the American Oil Chemists' Society* **2002,** *79*, 425-430.

255. J.M.Valderrama; A.M.Rodriguez, M.A.Cabrerizo; M.J.Ruiz, Interfacial Phenomena Underlying the Behavior of Foam and Emulsion. In *Structure and Functional Properties of Colloidal Systems*. R. Hidalgo-Alvarez, CRC Press Taylor and Francis Group: New York, 2011; Vol. 146.

256. I.Campbell; I.Norton; W.Morley, Factors Controlling the Phase Inversion of Oil-in-Water Emulsions. *Netherlands Milk and Dairy Journal* **1996,** *50*, 167-182.

257. J.Vereeckena; W.Meeussenb; I.Fouberta; A.Lesafferb; J.Woutersc; K.Dewettincka, Comparing the Crystallization and Polymorphic Behaviour of Saturated and Unsaturated Monoglycerides. *Food Research International* **2009,** *42*, 1415-1425

258. N.Krog, Crystallization Properties and Lyotropic Phase Behavior of Food Emulsifiers: Relation to Technical Applications. In *Crystallization Processes in Fats and Lipid Systems*, N.Garti; K.Sato, Eds. Marcel Dekker: New York, 2001.

259. Y.H.Hui, Food Science: Properties and Products Food Components and their Properties. In *Handbook of Food Science, Technology, and Engineering*. Taylor & Francis: Boca Raton, 2006.

260. V.D.Graef; B.Goderis; P.V.Puyvelde; I.Fouberta; K.Dewettinck, Development of a Rheological Method to Characterize Palm Oil Crystallizing under Shear. *European Journal of Lipid Science and Technology* **2008,** *110*, 521-529.

261. F.G.F.Qin; Sh.Premathilaka; X. D. Chen; K.W.Free, The Shaft Torque Change in a Laboratory Scraped Surface Heat Exchanger Used for Making Ice Slurries. *Asia-Pacific Journal of Chemical Engineering* **2007,** *2*, 618-630.

262. J. E. Norton; P. J. Fryer, Investigation of Changes in Formulation and Processing Parameters on the Physical Properties of Cocoa Butter Emulsions. *Journal of Food Engineering* **2012,** *113*, 329-336.

263. D.W.Bruijine; A.Bot, Fabricated Fat-Based Foods. In *Food Texture: Measurement and Perception*. A.J.Rosenthal, Aspen Publishers Maryland, 1999.

264. B.J.D.LeReverend; M.S.Taylor; I.T.Norton, Design and Application of Water-in-Oil Emulsions for Use in Lipstick Formulations. *International Journal of Cosmetic Science* **2010,** *33*, 263-268.

265. S.A.Vanapalli; J.Palanuwech; J.N.Coupland, Influence of Fat Crystallization on the Stability of Flocculated Emulsions. *Journal of Agricultural and Food Chemistry* **2000,** *50*, 5224-5228.

266. I.T.Norton; P.FryeR; S.Moore, Product/Process Integration in Food Manufacture: Engineering Sustained Health. *American Institute of Chemical Engineers* **2006,** *52*, 1632-1640.

267. J.E.Norton; P.Fryer; J.Parkinson; P.W.Cox, Development and Characterisation of Tempered Cocoa Butter Emulsions Containing up to 60% Water. *Journal of Food Engineering* **2009,** *95*, 172-178.

268. D.Rousseau; S.M.Hodge, Stabilization of Water-in-Oil Emulsions with Continuous Phase Crystals. *Colloids and Surfaces A. Physicochemical and Engineering Aspects* **2005,** *260*, 229-237.

269. E.R.Morris; T.J.Foster, Role of Conformation Inssynergistic Interactions of Xanthan. *Carbohydrate Polymers* **1993**, 133-137.

270. Ch.Su; S.Gunasekaran; M.P.Richards, Effect of Xanthan Gum on Physicochemical Properties of Whey Protein Isolate Stabilized Oil-in-Water Emulsions. *Food Hydrocolloids* **2007,** *21*, 555-564.

271. V.Gudipati; S.Sandra; D.J.McClement; E.A.Decker, Oxidative Stability and in Vitro Digestibility of Fish Oil-in-Water Emulsions Containing Multilayered Membranes. *Journal of Agricultural and Food Chemistry* **2010**, 58,8093-8099.