In Vitro Gastrointestinal Digestion of Oil-in-Water Emulsions

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Technology in Food Technology
at Massey University, Auckland
New Zealand

Limin (Jessie) Li

2012

Abstract

Oil-in-water (O/W) emulsions are widely used as a dispersion system for oil or fat or as a delivery system for lipophilic bioactive compounds in aqueous food products. There is a growing interest among food scientists in understanding the digestion behaviour of O/W emulsions when they are ingested and pass through the gastrointestinal (GI) tract. In recent years, a number of researches have been carried out to investigate the lipid digestion of emulsions using *in-vitro* models such as simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) that mimic the biological conditions of human bodies because of the complexity of *in vivo* study. However, most studies have been conducted to study the effect of gastric or intestinal digestion using SGF or SIF, and the studies on the effect of sequential digestion of emulsions first in SGF and then in SIF have been very limited. The objective of this study was therefore to investigate the effect of *in vitro* digestion of emulsions sequentially in SGF and SIF on the physicochemical properties and lipolysis of emulsions. In this study, sodium caseinate, whey protein isolate (WPI) and Tween 20 were used as emulsifiers to prepare O/W emulsions (20% soy oil and 1% emulsifier). The mean particle size and particle size distribution, zeta potential and microstructure of freshly prepared emulsions were initially measured, and the changes in the physicochemical properties of emulsions occurring during digestion in SGF or SIF and sequentially in SGF and SIF were analysed. The hydrolysis of fatty acids from emulsified lipid core was also determined during digestion in SIF after gastric digestion. In acidic simulated gastric conditions (pH 1.6 and 3.2 mg/mL pepsin), sodium caseinate-stabilized emulsions showed extensive flocculation with some coalescence, resulting in change in the size and microstructure of the emulsions. In contrast, the emulsions stabilized with WPI or Tween 20 showed no pronounced changes over time during 2 hrs of gastric digestion. In simulated intestinal conditions (pH 7.5, bile salts and pancreatin), a massive coalescence by pancreatic lipase took place in both sodium caseinate and WPI-stabilized emulsions, leading to a pronounced increase and change in the droplet size and microstructures, whereas Tween 20-stabilized emulsions were relatively stable with much less droplet coalescence and size increase. After sequential digestion in SGF and SIF, protein-stabilized O/W emulsions showed more extensive aggregation and coalescence of droplets in comparison with their digestion in SIF only without gastric digestion, whereas Tween 20-stabilized emulsions were relatively stable with only some extent of coalescence after 2 hrs of its

sequential digestion in SIF after SGF. The amounts of free fatty acids released in SIF after gastric digestion were similar between three types of emulsions and were not affected significantly by the gastric digestion prior to the intestinal digestion. The overall results indicated that the digestion behaviour of emulsions was affected by types of emulsifiers, and that the sequential digestion of emulsions in SGF and SIF resulted in more pronounced changes in the emulsion particle size and microstructure compared to the digestion in SGF or SIF. However, the rate of lipid digestion was not affected by the sequential digestion. The results of this study provide a significant insight into the effect of sequential gastric and intestinal digestion on the size and properties of emulsion systems and its effect being different depending on type of emulsifiers used to stabilise oil droplets.

Acknowledgements

I am sincerely thankful to my chief supervisor Dr. Sung Je Lee for giving me the opportunity to study for my masters in Food Technology at Massey University. The research present in this thesis would not have been possible without his patient guidance, consistent encouragement, and great help. I am very grateful for his scientific comments and academic advice on my research and writing up this thesis. It was a great experience to work under his supervision.

I am sincerely thankful to my co-supervisor Dr. Ai Qian Ye (Riddet Institute) for his support and guidance during my research at Riddet Institute. His continued encouragement and help and his intelligent ideas and scientific knowledge in emulsions have been a great help in completing my research project.

I am truly thankful to Professor Harjinder Singh for giving me the chance to conduct my research project at Riddet Institute with a financial support.

I would like to thank Riddet Institute laboratory managers Ms. Janiene Gilliland and Mr Chris Hall for their support (trainings, laboratory induction) and help in laboratory work. I would like to offer my special thanks to Jack Cui for his guidance on my overall practical work and his consistent encouragement during my study. I would also like to thank Mr Garry Radford (IFNHH) for his guidance on using homogenizer, Dr Jianyu Chen for her patient guidance on using Laser Scanning Confocal Microscopy (LSCM) and my officemates, Mr Mallesh Peram and Mr Anant Dave, for their great help in running SDS-PAGE gels.

My special thanks also go to my flatmates, Weifong Pok, Karren Khaw and Xianhui Geng, for their encouragement, accompany and support during my study. I would also like to offer my great thanks to all my officemates, Marllesh Peram, Anant Dave, Sylvia Chung, and Davastotra Poddar, for their academic advices and support.

Last but not the least, I would like to thank my beloved parents and sister for their unconditional support and continuous encouragement whenever in need.

Table of Contents

ABSTRACT ..... **I**

ACKNOWLEDGEMENTS……**III**

TABLE OFCONTENTS……...**V**

LIST OF FIGURES……..……**VIII**

CHAPTER 1. INTRODUCTION **1**

CHAPTER 2. LITERATURE REVIEW **3**

2.1 Introduction 3
2.2 Emulsion formation 5
2.3 Emulsion stability 5
2.3.1 Depletion flocculation *8*
2.3.2 Bridging flocculation *9*
2.3.3 Coalescence *11*
2.4 Protein emulsifiers and small molecule surfactants 12
2.4.1 Protein emulsifiers *12*
2.4.1.1 Sodium caseinate 13
2.4.1.2 Whey Proteins 14
2.4.2 Small molecule surfactants *16*
2.4.2.1 Tween 20 17
2.5 Factors affecting emulsion stability 18
2.5.1 pH *18*
2.5.2 Ionic strength *19*
2.5.2.1 Monovalent ions 19
2.5.2.2 Divalent ions 20
2.6 In vitro emulsion digestion studies 21
2.7 Gastric digestion 23
2.7.1 Acidic pH and ionic strength *23*
2.7.2 Pepsin *24*
2.8 Intestinal digestion 25
2.8.1 pH and ionic strength *25*
2.8.2 Bile salts *25*
2.8.3 Pancreatic lipase *26*
2.9 Conclusions 27

CHAPTER 3. MATERIALS AND METHODS **28**

3.1 Materials 28
3.2 Emulsion Preparation 29
3.3 Simulated Gastric and Intestinal Fluids 30
3.4 In Vitro Digestion of Emulsions with SGF, SIF and SGF/SIF 30
3. 5 Analyses of Emulsions 31
3.5.1 Particle size and size distribution *31*

3.5.2 ζ-potential *32*
3.5.3 Confocal laser scanning microscopy *33*
3.5.4 pH-stat titration *34*
3.5.5 Electrophoresis *34*
3.5.5.1 Sample preparation 34
3.5.5.2 SDS-PAGE 35
3.5.5.3 Statistical analysis 35

CHAPTER 4. IN VITRO GASTROINTESTINAL DIGESTION 36

OF OIL-IN-WATER EMULSIONS STABILIZED BY SODIUM CASEINATE .. 36

4.1 ABSTRACT 36
4.2 INTRODUCTION 36
4.3 RESULTS AND DISCUSSION 38
4.3.1 Digestion of emulsions in SGF *38*
4.3.1.1 Droplet size and microstructure 38
4.3.1.2 Zeta potential 41
4.3.1.3 SDS-PAGE 42
4.3.2 Digestion of emulsions in SIF *44*
4.3.2.1 Droplet size and microstructure 44
4.3.2.2 Zeta potential 46
4.3.3 Sequential digestion of emulsions in SGF and SIF *47*
4.3.3.1 Droplet size and zeta potential 47
4.3.3.2 Microstructures of emulsions 49
4.3.3.3 Free fatty acid release 51
4.4 CONCLUSIONS 53

CHAPTER 5. IN VITRO GASTROINTESTINAL DIGESTION 54

OF OIL-IN-WATER EMULSIONS STABILIZED BY TWEEN 20 54

5.1 ABSTRACT 54
5.2 INTRODUCTION 54
5.3 RESULTS AND DISCUSSION 56
5.3.1 In vitro SGF digestion of Tween 20-stabilzied emulsions *56*
5.3.1.1 Particle size and size distribution 56
5.3.1.2 Zeta (ζ) potential 58
5.3.1.3 Microstructures of emulsions treated with SGF 59
5.3.2 In-vitro SIF digestion of Tween 20-stabilzied emulsions *60*
5.3.2.1 Particle size and size distribution 60
5.3.2.2 Zeta potential 62
5.3.2.3 Microstructures of emulsions treated with SIF 63
5.3.3 In vitro sequential digestion of Tween 20 emulsions in SGF and SIF *64*
5.3.3.1 Particle size and size distribution 64
5.3.3.2 Microstructures of Tween 20 emulsions 65

5.3.3.3. Zeta potential 68
5.3.3.4. Hydrolysis of fatty acids 70
5.4 CONCLUSIONS 72
CHAPTER 6. IN VITRO GASTROINTESTINAL DIGESTION OF 73
OIL-IN-WATER EMULSIONS STABILIZED BY WPI 73
6.1 ABSTRACT 73
6.2 INTRODUCTION 73
6.3 RESULTS AND DISCUSSION 76
6.3.1 WPI-stabilized emulsion behaviour in SGF 76
6.3.1.1 Droplet size, zeta potential and microstructure 76
6.3.1.2 SDS-PAGE analysis 79
6.3.2.1 Droplet size distribution and microstructure images 82
6.3.3.1 Average droplet size 85
6.3.3.2 Size distribution and microstructure 87
6.3.3.3 Zeta potential 90
6.3.3.4 Free fatty acid release 92
6.4 CONCLUSIONS 94
CHAPTER 7. CONCLUSIONS 95
REFERENCES 96
APPENDICES 103

List of Figures

Figure 2.1 Various types of physical destabilization of O/W emulsions. 6

Figure 2.2 Depletion flocculation in an O/W emulsion. Particles approach due to an osmotic pressure gradient pushing out the unadsorbed biopolymer. 9

Figure 2.3 Bridging flocculation in an oil-in-water emulsion. 10

Figure 2.4 Coalescence in an oil-in-water emulsion. 11

Figure 2. 5 Chemical structure of Tween 20 (Di Marzio et al., 2011). 17

Figure 3.1 Two-stage high pressure homogenizer. 29

Figure 3.2 Schematic representation of sequential digestion of emulsions in SGF and SIF. 31

Figure 3.3 Malvern Mastersizer 2000. 32

Figure 3.4 Laser Scanning Confocal Microscopy. 33

Figure 4.1 The volume ($d_{4,3}$) and surface ($d_{3,2}$) mean diameters of original sodium caseinate-stabilized emulsion (20 wt% soybean oil and 1.0 wt% protein) and of emulsions after digestion in SGF (pH 1.6) containing pepsin for different times. 39

Figure 4.2 Particle size distributions of sodium caseinate-stabilized emulsions (20 wt% soy bean oil, 1.0 wt% protein) in SGF containing no pepsin and after digestion in SGF containing pepsin for 1, 10, 30, 60 min and 2 hrs. 39

Figure 4.3 Confocal micrographs of sodium caseinate-stabilized emulsions: (A) original emulsion; (B) emulsion mixed with SGF (no pepsin); emulsions digested in SGF containing pepsin for (C) 10 min, (D) 30 min, (E) 60 min and (F) 120 min. All samples were stained with Nile Red (for oil) and Fast Green (for protein). Scale bar = 10 μm. . 40

Figure 4.4 Changes in zeta potentials of sodium caseinate-stabilized emulsions during digestion in SGF (pH 1.6, pepsin) and SIF (pH 7.5, pancreatin and bile salt) as a function of digestion time. 0 min represents the emulsions mixed with SGF or SIF without containing enzymes (pepsin or pancreatin) 41

Figure 4.5 SDS-PAGE patterns of (A) the interfacial proteins obtained from the cream phase and (B) the proteins from the serum phase of sodium-caseinate-stabilized emulsions after digestion in SGF containing pepsin for different times: lane 1, molecule weight size markers; lane 2, sodium caseinate solution; lane 3, original emulsion; lane 4, digestion for 1 min; lane 5, digestion for 10 min; lane 6, digestion for 30 min; lane 7, digestion for 60 min; lane 8, digestion for 120 min. 43

Figure 4.6 Changes in the particle diameters $d_{3,2}$ and $d_{4,3}$ of sodium caseinate-stabilized emulsions (20 wt% soy oil and 1.0 wt% NaCas) after digestion in SIF (pH 7.5, pancreatin and bile salts) for different times. 44

Figure 4.7 Changes in the particle size distributions of sodium caseinate-stabilize emulsions (20 wt% soybean oil and 1.0 wt% protein) after digestion in SIF (pH 7.5, pancreatin and bile salts) for different times. 45

Figure 4.8 Confocal laser scanning microscope (CLSM) images of sodium caseinate-stabilized emulsions after digestion in SIF for different times. Original emulsion (a), and emulsions digested in SIF containing pancreatin and bile salts for 1 min (b), 10 min (c), 30 min (d), 60 min (e) and 120 min (f). Scale bar = 10 μm. 45

Figure 4.9 Change in average droplet size ($d_{4,3}$) of sodium caseinate-stabilized emulsions (20 wt% soya oil, 1.0 wt% protein) after digestion in SGF containing pepsin for 1, 30 and 120 min and then further digestion in SIF containing pancreatic lipase for different times. 47

Figure 4.10 Particle size distributions of sodium caseinate-stabilized emulsions digested in SGF containing pepsin for (A) 30 min and (B) 2 hrs and then digested in SIF containing pancreatic lipase. 48

Figure 4.11 Zeta potentials of sodium caseinate-stabilized emulsions measured during digestion in SIF after digestion in SGF for 30 min and 2 hrs. 49

Figure 4.12 Confocal micrographs of sodium-caseinate-stabilized emulsions digested in SGF containing pepsin for (A) 30 min and (B) 2 hs and then in SIF containing pancreatic lipase for different times. All samples were stained with Nile Red (for oil) and Fast Green (for protein). Scale bar = 10 μm. 50

Figure 4.13 The release of free fatty acids (μmol/mL emulsion) hydrolyzed from NaCas-emulsions during digestion in SIF for 2 hrs after gastric treatment for different times. (A) SIF containing 5.0 mg/mL bile salts and 1.6 mg/mL pancreatin and (B) SIF containing 1.6 mg/mL pancreatin and no bile salts. 52

Figure 5.1 The volume ($d_{4,3}$) and surface ($d_{3,2}$) mean particle diameters of Tween 20-stabilized emulsions (20 wt% soy oil and 1.0 wt% Tween 20) during digestion in SGF (pH 1.6) containing pepsin. 57

Figure 5.2 Particle size distributions of Tween 20-stabilized emulsions (20 wt% soy oil, 1.0 wt% Tween 20) before and duing digestion in SGF for 2 hrs. 57

Figure 5.3 Electrical charges (ζ-potentials) of Tween 20-stabilized emulsions measured during digestion of emulsions in SGF (pH 1.6, containing pepsin) for 2 hrs. 59

Figure 5.4 Confocal microscopic images of Tween 20-stabilized emulsions during digestion in SGF for 2 hrs. Original emulsion (pH 6.71) (a); emulsions in SGF digested for 1 min (b), 10 min (c), 30 min (d), 60 min (e) and 2 hrs (f). Scale bar represents 10 μm. 60

Figure 5.5 Changes in particle diameters $d_{3,2}$ and $d_{4,3}$ (μm) of Tween 20 emulsion droplets (20 wt% soy oil and 1.0 wt% Tween 20) during digestion in SIF (pH7.5, pancreatin and bile salts) as a function of time. 61

Figure 5. 6 Changes in the particle diameters $d_{3,2}$ and $d_{4,3}$ (μm) of Tween 20 emulsions (20 wt% soy oil and 1.0 wt% Tween 20) during digestion in SIF (pH 7.5, pancreatin and bile salts) as a function of time. 62

Figure 5.7 Zeta potentials of Tween 20-stabilized emulsions during digestion in SIF (pH 7.5, pancreatin and bile salt) as a function of incubation time..... 63

Figure 5.8 Confocal laser scanning microscope (CLSM) image of Tween 20-stabilized emulsions during digestion in SIF for 2 hrs. Original Tween 20-stabilized emulsion (a), emulsions digested in SIF containing pancreatin and bile salts for 1 min (b), 10 min (c), 30 min (d), 60 min (e) and 2 hrs (f). The scale bar represents 10 μm. 64

Figure 5.9 Mean particle diameters $d_{4,3}$ (μm) of Tween 20-stabilized emulsions during digestion in SIF (containing pancreatin) over 2 hrs after gastric treatment as a function of incubation time. 66

Figure 5.10 Particle size distributions of Tween 20-stabilized emulsions measured during sequential digestion with SGF and SIF. (A) after mixing with SGF (without pepsin stirring for 5 minutes); (B) and (C) after mixing with SGF (containing pepsin) for 30 and 120 min, respectively. 67

Figure 5.11 Representative confocal laser scanning microscopic (CLSM) images of microstructures of Tween 20-stabilized emulsions taken during sequential digestion in SIF for different times after gastric treatment. (A): emulsions with pre-treatment with SGF containing no pepsin; (B) and (C): emulsions with pre-treatment with SGF containing pepsin for 30 min and 2 hrs, respectively. 68

Figure 5.12 Zeta potentials of Tween 20-stabilized emulsions during digestion with SGF, SIF and SGF/SIF..... 69

Figure 5.13 The release of free fatty acids (μmol/mL emulsion) hydrolyzed from Tween-20 emulsions during digestion in SIF for 2 hrs after gastric treatment for different times. (A) SIF containing 5.0 mg/mL bile salts and 1.6 mg/mL pancreatin and (B) SIF containing 1.6 mg/mL pancreatin and no bile salts. 71

Figure 6.1 The volume ($d_{4,3}$) and surface ($d_{3,2}$) mean particle diameters of WPI-stabilized emulsions (20 wt% soy oil and 1.0 wt% WPI) during digestion in SGF (pH 1.6) containing pepsin. 77

Figure 6.2 Particle size distributions of WPI-stabilized emulsions (20 wt% soy oil, 1.0 wt% WPI) before and duing digestion in SGF for 2 hrs. 78

Figure 6.3 Confocal laser scanning microscope (CLSM) image of WPI-stabilized emulsions during digestion of emulsions in SGF (pH 1.6, containing pepsin) for 2 hrs.

Original emulsions (a), original emulsions digested in SGF containing pepsin for 1 min (b), 10 min (c), 30 min (d), 60 min (e) and 2 hrs (f). The scale bar represents 10 μm. ..78

Figure 6.4 The ζ-potentials of WPI-stabilized emulsions measured during digestion in SGF (pH 1.6) and SIF (pH 7.5, pancreatin and bile salt), respectively, as a function of incubation time.....79

Figure 6.5 SDS-PAGE of WPI-stabilized emulsions during digestion in SGF for 2 hrs. Cream phase of emulsions (A); Serum phase of emulsions (B). Lanes from left to right: Molecular weight markers (Lane 1); 1% WPI protein (Lane 2); WPI emulsion mixed with RO water in 1:1 ratio (0.5% WPI protein) (Lane 3); WPI emulsion (1 % WPI protein) mixed with SGF in 1:1 ratio containing no enzyme (Lane 4), containing pepsin after digestion for 1 min (Lane 5), 10 min (Lane 6), 30 min (Lane 7) and 2 hrs (Lane 8). 81

Figure 6.6 Mean particle diameters $d_{4,3}$ (μm) and $d_{3,2}$ (μm) of WPI-stabilized emulsions during digestion in SIF (containing pancreatin) over 2 hrs as a function of incubation time.....83

Figure 6.7 Size distributions of WPI emulsions (20 wt% soy oil and 1.0 wt% WPI) during digestion in SIF (pH 7.5, pancreatin and bile salts) as a function of time.....83

Figure 6.8 Confocal laser scanning microscope (CLSM) images of WPI-stabilized emulsions during digestion in SIF for 2 hrs. Original WPI-stabilized emulsion (a), WP-stabilized emulsions digested in SIF containing pancreatin and bile salts for 1 min (b), 10 min (c), 30 min (d), 60 min (e) and 2 hrs (f). The scale bar represents 10 μm. 84

Figure 6.9 Mean particle diameters $d_{4,3}$ (μm) (A) and $d_{3,2}$ (μm) (B) of WPI-stabilized emulsions during digestion in SIF (containing pancreatin) over 2 hrs after gastric treatment as a function of incubation time.....86

Figure 6.10 Particle size distributions of WPI-stabilized emulsions after sequential digestion with both SGF and SIF. (A) after mixing with SGF (without pepsin stirring for 5 minutes); (B), (C) and (D) after mixing with SGF (containing pepsin) for 1, 30 and 120 min, respectively. 88

Figure 6.11 Images of emulsions taken over time during digestion. (A) SGF for 2 hrs , (B) SIF for 2 hrs, (C) SGF 30 min/SIF 1 min -2 hrs and (D) SGF 2 hrs/SIF 1 min – 2 hrs. Numbers from 1 to 5 represent 1 min, 10 min, 30 min, 1 hr and 2 hr incubation times, respectively. 89

Figure 6.12 Confocal laser scanning microscope (CLSM) images of WPI-stabilized emulsions during the subsequent digestion in SIF for 1 min - 2 hrs (A) after digestion with SGF (without pepsin stirring for 5 minutes); (B), (C) and (D) after digestion with SGF (containing pepsin) for 1, 30 and 120 min, respectively. 90

Figure 6.13 The ζ-potentials of WPI-stabilized emulsions measured during digestion with SIF for 2 hrs after gastric treatment for different incubation times (1 min, 30 min and 2 hrs).....91

Figure 6. 14 The release of free fatty acids (μmol/mL emulsion) hydrolyzed from WPI-emulsions during digestion in SIF for 2 hrs after gastric treatment for different times. (A) SIF containing 5.0 mg/mL bile salts and 1.6 mg/mL pancreatin and (B) SIF containing 1.6 mg/mL pancreatin and no bile salts. 93