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Development of novel nanoemulsions as delivery systems

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2016

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**A thesis presented in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy in Food Technology
at Massey University, New Zealand**

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2016**

ABSTRACT

In the past decades, emulsions have been widely used as delivery systems for incorporating bioactive compounds into foods. With the advancing of nanotechnology, smaller particles in the nanometric range (i.e. nanoemulsions) can be created with better properties that are more advantageous than conventional emulsions in terms of their stability to gravitational separation, optical clarity and better absorption of nutrients in drug delivery (with increased bioavailability). In particular, emulsification and solvent evaporation method has been used to produce nanoemulsions with optimum results. However, like conventional emulsions, protein-stabilised nanoemulsions become unstable when exposed to certain environmental stresses such as high temperatures, salt addition and extreme pH changes. Additionally, liquid emulsions are difficult to transport and use in some food systems while being susceptible to microbial spoilage. To remedy, a dry, stable emulsion system has to be obtained for their prospective future in food applications.

The objective of this research was to develop nanoemulsions with useful attributes. The thesis consists of three main parts in which the first part studied the formation and properties of nanoemulsions using emulsification and solvent evaporation method; the second part delved into the making of dried nanoemulsion powders and the third part focused on the structural modifications of nanoemulsions and encapsulation of a bioactive compound lutein.

To begin, an experimental study to optimise the conditions for producing nanoemulsions using emulsification and solvent evaporation methodology was performed under different processing conditions (microfluidisation pressures and number of passes), organic phase ratios and materials (oil types and emulsifiers). It was found that smaller oil droplets (around 80 nm in diameter) were achieved when increasing the microfluidisation pressure up to 12000 psi (80 MPa) for 4 passes at an organic phase ratio of 10:90. There was a progressive decrease in particle size with increasing emulsifier concentration up to a 1% (w/w) level for whey protein isolate (WPI) and lactoferrin but it did not decrease further at higher concentration. On the other hand, much larger oil droplets were formed in Tween 20 emulsions (120 – 450 nm). The environmental study showed that lactoferrin and Tween 20 emulsions have

a better stability to pH changes (pH 2 – 12) and salt addition (0 – 500 mM NaCl or 0 – 90 mM CaCl₂) than WPI stabilised nanoemulsions.

After successful preparation of nanoemulsions, liquid nanoemulsions were converted to dried powders by spray drying or freeze drying. The nanoemulsions were mixed with different wall materials consisting of maltodextrin alone, trehalose alone or a 1:1 ratio of maltodextrin and trehalose at 10, 20 or 30% (w/w) solid concentration. Results showed that the powders containing 20% trehalose have better powder properties with lower moisture content and water activity, higher bulk density and good reconstitution in water. The freeze-dried powders showed excellent wettability and dispersibility in water but lower encapsulation efficiency than spray dried powders.

In another part of study, nanoemulsions with modified interfacial structure were used to improve their stability to environmental stresses. The interactions between WPI and lactoferrin in aqueous solutions were first studied to explore the feasibility of using these two proteins to form complex interfacial structures at the droplet surface in the emulsions. Based on ζ -potential and turbidity measurements, both proteins were shown to interact with each other via electrostatic interactions at pH values between 6 and 8. The adsorption of protein layers on a gold surface that mimics the hydrophobic oil surface was also confirmed by a quartz crystal microbalance with dissipation (QCM-D) study.

Next, a series of bi-layer nanoemulsions at different pH values and lactoferrin concentrations were prepared so as to determine the best conditions on the overall emulsion stability. It was shown that the stability of emulsions was dependent on both pH and lactoferrin concentration. At pH values close to pI of WPI (around pH 5), the nanoemulsions remained unstable regardless of the lactoferrin concentration used (0.25 – 5% w/w). The nanoemulsions at pH 6 were also unstable at low concentrations (0.5 – 1% w/w) presumably due to “bridging flocculation” and exhibited phase separation. Consequently, a lactoferrin concentration of 3% (w/w) was used to produce bi-layer nanoemulsions at pH 6. At pH 7 – 10, the bi-layer nanoemulsions were stable at all lactoferrin concentrations and formed a bi-layer structure at the interface of droplet.

The formulated nanoemulsions (single layer and bi-layer emulsions) were subjected to a variety of environmental stresses and *in vitro* digestion under simulated gastrointestinal conditions. The emulsion stability to pH changes and salt addition was improved in the bi-layer emulsions containing WPI and lactoferrin when compared to the single layer nanoemulsions stabilised by WPI alone. However, the bi-layer emulsions were more susceptible to destabilisation on heating at temperatures above 60°C. The *in vitro* digestion of bi-layer nanoemulsions was similar to single layer nanoemulsions in which the protein hydrolysis of the interfacial layers results in extensive droplet flocculation.

In subsequent formulations, lutein was incorporated in the emulsions as a model of bioactive compound for the application of nanoemulsions as a novel delivery system. The nanoemulsions well encapsulated lutein in their matrices with an encapsulation efficiency of 80% and contained small oil droplets (70 – 80 nm). All the emulsions were physically stable under the tested conditions up to 28 days at different storage temperatures (5, 20 and 40°C). However, there was a significant decrease in lutein content during storage especially at higher temperatures due to oxidative degradation. Nevertheless, the bi-layer nanoemulsions showed a better stability to lutein degradation. Based on *in vitro* cell toxicity studies on Caco-2 cells using MTT assay, both nanoemulsions did not show toxicity as the cell viability was more than 80% at 10 times or more dilution after 24 hours of incubation. The cellular uptake of lutein was higher in bi-layer nanoemulsions when compared to single layer emulsions.

The present work demonstrated that nanoemulsions can be formed using emulsification and a solvent evaporation method. Dried microcapsules of nanoemulsions were formed with similar properties as their original nanoemulsions after reconstitution in water. The nanoemulsions with bi-layer interfacial structure have better stability to environmental changes than single layer emulsions. Nanoemulsions did not show more toxicity than their corresponding conventional emulsions with large oil droplets produced without the use of organic solvent. These have important implications in the use of nanoemulsions for encapsulation lutein or other bioactive compounds for applications in foods and beverages.

ACKNOWLEDGEMENTS

It was indeed my pleasure to study at Massey University (School of Food & Nutrition). First of all, I would like to thank all my supervisors, Dr. Sung Je Lee, Associate Professor Kelvin Goh and Professor Matt Golding for their supervision, guidance and support throughout my study. I am honoured to have Dr. Lee as my chief supervisor. It has been a great learning experience to work under his supervision. His thorough thinking, scientific knowledge, suggestions, valuable inputs and advices have helped me to complete this research. This thesis would not have been possible without the great input from him. I am also thankful to my co-supervisor, Associate Professor Goh for his guidance and continuous support in the experimental work by providing his constructive feedbacks and suggestions as well as in writing of the thesis.

I am very grateful to the Ministry of Business, Innovation and Employment (MBIE) for funding this research project through the Royal Society of New Zealand as well as providing the travel grants to attend conferences both locally and overseas.

I would also like to thank Professor Conan Fee, Dr. Simone Dimartino and Ms. Rayleen Fredericks (Chemical and Process Engineering Department, University of Canterbury) for giving me the opportunity to work in their laboratory. It was indeed a pleasure to work with Dr. Simone on the QCM-D to study the interactions between protein molecules. His advice and feedbacks were useful to the research work.

I am also thankful to Dr. Fran Wolber for her teaching and guidance on the cell culture studies. It has been a wonderful experience to work with her and the little laboratory tricks that she shared with me.

I would like to thank all the laboratory managers, Ms. Michelle Tamehana, Mr. Steve Glasgow and Ms. Gabrielle Plimmer from School of Food & Nutrition and Ms. Janiene Gilliland and Mr. Chris Hall from Riddet Institute. They have been very helpful to provide the trainings on the use of laboratory equipment and ordering of chemicals. I am also grateful to Ms. Michelle McGrath for her assistance on the HPLC. I would also like to acknowledge the help from Ms. Jordon Taylor and Ms.

Niki Murray (Manawatu Microscopy and Imaging Centre) for preparing the samples for TEM and SEM imaging. I am thankful to Ms. Yvonne Parkes and Ms. Christine Ramsay for their help on the administrative works.

I would also like to thank the students from Singapore, Ms. Lam Yi Shi and Mr. Chiang Jie Hong for helping me with some of the experimental works and the data they provided.

This journey would not have been more than enjoyable without the fun and support of my friends, Yap Sia Yen, Karen Khaw, Noor Soffalina, Daisy Wen, Anynda Yuris, Li Mo, Deng Le and fellow Singaporean friends. It is a pleasure to know them here especially Soffalina who had helped me got my driver license. There was so much wonderful and memorable time we spent in the laboratory doing our research.

Finally, I would like to thank my grandma, my mom and my brother for their unconditional love and continuous support.

TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	vi
LIST OF FIGURES	x
LIST OF TABLES	xvi
LIST OF SYMBOLS	xviii
LIST OF ABBREVIATIONS	xix
LIST OF PUBLICATIONS AND CONFERENCE PROCEEDINGS	xxii
Chapter One: Introduction	1
1.1 Background Information.....	1
1.2 Overview of Thesis	4
Chapter Two: Literature Review	5
2.1 Development of Nanoemulsions	5
2.1.1 Defining conventional emulsions and nanoemulsions.....	5
2.1.2 Materials used to make nanoemulsions	6
2.1.3 Methods used to produce nanoemulsions	13
2.1.4 Characteristics and physicochemical properties of nanoemulsions...	22
2.1.5 Potential applications of nanoemulsions in food industry	31
2.2 Drying Methods on Dehydration of Nanoemulsions	32
2.2.1 Spray drying versus freeze drying methods.....	32
2.2.2 Wall material components	35
2.3 Nanoemulsions as Delivery System for Bioactive Compounds	39
2.3.1 Lutein as a bioactive compound	39
2.3.2 Current studies on the use of nanoemulsions as delivery systems....	42
2.4 Design of Novel Structured Emulsions.....	43
2.5 Physiological Behaviour and Toxicological Study.....	46
2.5.1 Gastrointestinal digestion and absorption behaviour of emulsions ..	46
2.5.2 <i>In vitro</i> cytotoxicity and cellular uptake studies	49
2.6 Concluding Remarks.....	51
Chapter Three: Materials & Methods	52
3.1 Materials	52
3.1.1 Whey protein isolate (WPI)	52
3.1.2 Lactoferrin.....	52

3.1.3	Corn oil	52
3.1.4	Chemicals.....	52
3.2	Preparation of Methods of Nanoemulsions.....	53
3.2.1	Preparation of solutions	54
3.2.2	Preparation of nanoemulsions.....	54
3.3	Characterisation Methods	55
3.3.1	Particle size and size distribution.....	55
3.3.2	Zeta potential (ζ -potential) measurements.....	56
3.3.3	Transmission electron microscopy (TEM)	57
3.4	Data Analysis	58
Chapter Four: Development of Nanoemulsions Using Emulsification and Solvent Evaporation		59
4.1	Abstract.....	59
4.2	Introduction.....	60
4.3	Materials and Methods.....	61
4.3.1	Materials	61
4.3.2	Formulation of nanoemulsions	61
4.3.3	Effects of environmental conditions on nanoemulsions	63
4.3.4	Characterisation of nanoemulsions	63
4.4	Results and Discussion	64
4.4.1	Preparation of nanoemulsions using emulsification and solvent evaporation technique.....	64
4.4.2	Comparison of nanoemulsions and conventional emulsions	66
4.4.3	Effect of organic phase ratios and WPI concentrations on nanoemulsions	69
4.4.4	Effect of organic phase ratios and homogenisation parameters on nanoemulsions	73
4.4.5	Influence of oil types on nanoemulsions	77
4.4.6	Influence of emulsifier types and concentrations on nanoemulsions	79
4.4.7	Environmental stability of nanoemulsions.....	84
4.5	Conclusions.....	94
Chapter Five: Influence of Wall Materials and Drying Methods on Physicochemical Properties of Dehydrated Nanoemulsions		95
5.1	Abstract.....	95
5.2	Introduction.....	96
5.3	Materials and Methods.....	97
5.3.1	Materials	97

5.3.2	Preparation of nanoemulsions with wall solutions for spray drying..	98
5.3.3	Characterisation of nanoemulsions with wall materials before spray drying.....	99
5.3.4	Drying of nanoemulsions	99
5.3.5	Analysis of dried nanoemulsions powders	100
5.3.6	Reconstitution of nanoemulsions	103
5.3.7	Data analysis	103
5.4	Results and Discussion	103
5.4.1	Properties of nanoemulsions added with different wall materials ..	103
5.4.2	Properties of spray dried nanoemulsions with different wall materials	105
5.4.3	Reconstitution of spray dried nanoemulsions	119
5.4.4	Comparison of nanoemulsion powders produced by spray drying and freeze drying.....	122
5.5	Conclusions.....	127
Chapter Six: Interactions between WPI and Lactoferrin in Aqueous Solution and Interfacial Structures monitored by QCM-D		128
6.1	Abstract.....	128
6.2	Introduction.....	128
6.3	Materials and Methods.....	130
6.3.1	Materials	130
6.3.2	Preparation of protein solutions	131
6.3.3	Analysis of protein solutions	131
6.3.4	Quartz crystal microbalance with dissipation (QCM-D) measurements	131
6.3.5	Data analysis	133
6.4	Results and Discussion	134
6.4.1	Characteristics of individual protein solutions and their mixture	134
6.4.2	Adsorption of protein bi-layers on hydrophobic surface	137
6.4.3	Effect of pH on the adsorption of WPI and lactoferrin bi-layer	142
6.4.4	Adsorption of protein complex on hydrophobic surface	146
6.5	Conclusions.....	149
Chapter Seven: Physicochemical Properties and <i>In vitro</i> Gastrointestinal Digestion of Nanoemulsions stabilised by WPI and/or Lactoferrin.....		150
7.1	Abstract.....	150
7.2	Introduction.....	151
7.3	Materials and Methods.....	152

7.3.1	Materials	152
7.3.2	Preparation of bi-layer nanoemulsions	153
7.3.3	Effect of environmental conditions on nanoemulsions	155
7.3.4	Characterisation of nanoemulsions	155
7.3.5	<i>In vitro</i> gastrointestinal digestion.....	155
7.4	Results and Discussion	157
7.4.1	Effect of pH and lactoferrin concentration on the adsorption at the droplet surface	157
7.4.2	Environmental stability of nanoemulsions.....	169
7.4.3	Protein hydrolysis of nanoemulsions using <i>in vitro</i> gastrointestinal model	178
7.5	Conclusions.....	184
Chapter Eight: Encapsulation and Stability of Lutein in Protein-stabilised Nanoemulsions and Cytotoxicity using Caco-2 cell line		185
8.1	Abstract.....	185
8.2	Introduction.....	186
8.3	Materials and Methods.....	188
8.3.1	Materials	188
8.3.2	Preparation of lutein conventional emulsion and nanoemulsion	189
8.3.3	Characterisation of lutein nanoemulsions	190
8.3.4	Analysis of lutein content in nanoemulsions using HPLC	190
8.3.5	Cell cultures	191
8.3.6	<i>In vitro</i> cytotoxicity of lutein nanoemulsions	192
8.3.7	Cellular uptake of lutein from nanoemulsions.....	193
8.4	Results and Discussion	194
8.4.1	Physicochemical properties of lutein loaded conventional emulsions and nanoemulsions	194
8.4.2	Stability of lutein nanoemulsions during storage.....	197
8.4.3	<i>In vitro</i> cytotoxicity of lutein nanoemulsions	205
8.4.4	Cellular uptake of lutein from nanoemulsions	208
8.5	Conclusions.....	210
Chapter Nine: Overall Conclusions & Recommendations		211
	Future work	213
REFERENCES.....		xxiii
APPENDICES		xl

LIST OF FIGURES

Figure 2.1 Schematic representations of mechanical devices used to produce emulsions: (a) high pressure valve homogeniser, (b) microfluidiser and (c) ultrasonic probe homogeniser.	15
Figure 2.2 Schematic illustration of movement of organic solvent in oil droplets during preparation of nanoemulsions using emulsification and solvent displacement-evaporation. The aqueous phase contains water and emulsifiers.	20
Figure 2.3 Examples of TEM images of β -carotene nanodispersions using (a) resin embedding and (b) freeze-fracture replica methods.	25
Figure 2.4 Schematic representations of the thickness of interfacial layer (δ) on the droplet radii (r) in (a) conventional emulsion and (b) nanoemulsion.	28
Figure 2.5 Schematic illustrations of spray dryer and freeze dryer (showing the major components).	33
Figure 2.6 Schematic illustrations of air flow movements in spray dryers: (a) co-current, (b) counter current and (c) mixed flow patterns.	34
Figure 2.7 Chemical structures of (a) lutein and (b) lutein esters.	40
Figure 2.8 Schematic representations of emulsion structures that may be formed in emulsions containing two different biopolymers denoted as “A” and “B”: (a) single layer, (b) bilayer and (c) mixed layer.	44
Figure 2.9 Schematic illustrations of cell absorption by (a) paracellular and (b) transcellular mechanisms.	48
Figure 3.1 Schematic illustration of a combined method of high pressure homogenisation and solvent evaporation used to produce nanoemulsions.	53
Figure 3.2 Pictures of (a) laboratory scale microfluidiser (M-110P) and (b) rotary evaporator.	55
Figure 3.3 Pictures of (a) Zetasizer Nano ZS and (b) Mastersizer 2000 equipped with the Hydro 2000MU.	56
Figure 3.4 Picture of a transmission electron microscope.	58
Figure 4.1 Particle size distributions of WPI-stabilised nanoemulsion during preparation. A coarse emulsion was formed by mixing the aqueous phase and organic phase using high shear mixer. The coarse emulsion was homogenised using a microfluidiser at 80 MPa for 4 cycles and evaporated using a rotary evaporator (50°C; 153 mBar) to remove ethyl acetate.	65
Figure 4.2 Particle size distributions of WPI stabilised conventional emulsion and nanoemulsion (denoted as CE and NE, respectively) adjusted to 0.5% (w/w) oil with photographs inserted.	67
Figure 4.3 TEM images of WPI stabilised (a) nanoemulsions and (b) conventional emulsions adjusted to 0.5% (w/w) oil.	68

Figure 4.4 Photographs of nanoemulsions prepared with different concentrations of WPI at different organic to aqueous phase ratios of (a) 10:90 and (b) 20:80 (after evaporation) adjusted to 0.5% (w/w) oil.	69
Figure 4.5 Mean particle diameter (Z-Average) of nanoemulsions prepared with different concentrations of WPI at different organic to aqueous phase ratios of 10:90 and 20:80 before and after evaporation and adjusted to 0.5% (w/w) oil.	72
Figure 4.6 Mean ζ -potential of nanoemulsions prepared with different concentrations of WPI at different organic to aqueous phase ratios of 10:90 and 20:80 after solvent evaporation and adjusted to 0.5% (w/w) oil.	72
Figure 4.7 Photographs of WPI-stabilised nanoemulsions prepared at different homogenisation pressures for 4 cycles at different organic phase ratios of (a) 10:90 and (b) 20:80 and adjusted to 0.5% (w/w) oil.	74
Figure 4.8 Particle size distributions and mean particle diameter (Z-Average) of WPI-stabilised nanoemulsions prepared at different homogenisation pressures for 4 cycles at different organic phase ratios of (a) 10:90 and (b) 20:80 and adjusted to 0.5% (w/w) oil.	74
Figure 4.9 Photographs of WPI-stabilised nanoemulsions prepared at 80 MPa with different number of homogenisation cycles for different organic phase ratios of (a) 10:90 and (b) 20:80 and adjusted to 0.5% (w/w) oil.	76
Figure 4.10 Particle size distributions and mean particle diameter (Z-Average) of WPI-stabilised nanoemulsions prepared at 80 MPa with different number of homogenisation cycles for different organic phase ratios of (a) 10:90 and (b) 20:80 and adjusted to 0.5% (w/w) oil.	76
Figure 4.11 Particle size distributions of WPI-stabilised nanoemulsions prepared with different types of oils, corn oil (CR), coconut oil (CC) and lemon oil (LO), at organic phase ratio of 10:90 and adjusted to 0.5% (w/w) oil.	78
Figure 4.12 Photographs of nanoemulsions prepared with different types and concentrations of emulsifiers at organic phase ratio of 10:90 and adjusted to 0.5% (w/w) oil.	79
Figure 4.13 Mean particle diameter (Z-Average) of nanoemulsions prepared with different types and concentrations of emulsifiers at organic phase ratio of 10:90 and adjusted to 0.5% (w/w) oil.	80
Figure 4.14 Mean ζ -potential of nanoemulsions prepared with different types and concentrations of emulsifiers at organic phase ratio of 10:90 and adjusted to 0.5% (w/w) oil.	82
Figure 4.15 TEM images of nanoemulsions prepared with different types of emulsifiers, (a) WPI, (b) lactoferrin and (c) Tween 20 at the same emulsifier concentration (1% w/w). Nanoemulsions were prepared at organic phase ratio of 10:90 and adjusted to 0.5% (w/w) oil.	83
Figure 4.16 Influence of heating temperatures on the mean particle diameter (Z-Average) of nanoemulsions stabilised by different types of emulsifiers.	86
Figure 4.17 Influence of heating temperatures on the mean ζ -potential of nanoemulsions stabilised by different types of emulsifiers.	86

Figure 4.18 Photographs of nanoemulsions prepared with different types of emulsifiers at different pH levels.	88
Figure 4.19 Influence of pH changes on the mean particle diameter of nanoemulsions stabilised by different types of emulsifiers.	88
Figure 4.20 Influence of pH changes on the mean ζ -potential of nanoemulsions stabilised by different types of emulsifiers.	89
Figure 4.21 Influence of NaCl concentrations on the mean particle diameter (Z-Average) of nanoemulsions stabilised by different types of emulsifiers.	91
Figure 4.22 Photographs of nanoemulsions prepared with different types of emulsifiers adjusted to different CaCl_2 concentrations.	91
Figure 4.23 Influence of CaCl_2 concentrations on the mean particle diameter of nanoemulsions stabilised by different types of emulsifiers.	93
Figure 4.24 Influence of CaCl_2 concentrations on the mean ζ -potential of nanoemulsions stabilised by different types of emulsifiers.	93
Figure 5.1 Particle size distributions (by intensity and volume) of WPI-stabilised nanoemulsions (0.5% w/w oil) mixed with (a) maltodextrin, (b) trehalose and (c) a 1:1 ratio of maltodextrin and trehalose at 10, 20 and 30% (w/w) before spray drying.	104
Figure 5.2 Viscosity of WPI-stabilised nanoemulsions (0.5% w/w oil) mixed with different wall materials (10, 20 and 30% w/w) before spray drying measured at shear rate 10s^{-1}	105
Figure 5.3 Particle size distribution (by volume) of spray dried powders prepared with (a) maltodextrin, (b) trehalose and (c) a 1:1 ratio of maltodextrin and trehalose at 10, 20 and 30% (w/w).	109
Figure 5.4 SEM images (outer and inner structures) of spray dried powders prepared with different concentrations of maltodextrin: (a) 10%, (b) 20% and (c) 30% (w/w).	111
Figure 5.5 SEM images (outer and inner structures) of spray dried powders prepared with different concentration of trehalose: (a) 10%, (b) 20% and (c) 30% (w/w). ..	113
Figure 5.6 SEM images (outer and inner structures) of spray dried powders prepared with different concentration of a mixture of 1:1 ratio of maltodextrin and trehalose: (a) 10%, (b) 20% and (c) 30% (w/w).	115
Figure 5.7 Particle size distributions (by intensity and volume) of reconstituted nanoemulsions prepared with (a) maltodextrin, (b) trehalose and (c) a 1:1 ratio of maltodextrin and trehalose at 10, 20 and 30% (w/w) dry matter.	121
Figure 5.8 Particle size distributions of spray dried and freeze dried nanoemulsion powders containing 20% (w/w) trehalose.	123
Figure 5.9 SEM images (outer and inner structures) of dried nanoemulsion powders containing 20% (w/w) trehalose produced by different methods: (a) spray drying and (b) freeze drying.	124
Figure 5.10 Particle size distributions by (a) intensity and (b) volume of initial nanoemulsions and reconstituted nanoemulsions containing 20% (w/w) trehalose produced by spray drying or freeze drying.	126

Figure 6.1 Mean ζ -potential of 1% (w/w) protein solutions of WPI and lactoferrin and 1% (w/w) of protein mixtures of WPI and lactoferrin (1:1 ratio) at different pH values.	136
Figure 6.2 Mean optical density (at 600 nm) of 1% (w/w) protein solutions of WPI and lactoferrin and 1% (w/w) protein mixtures of WPI and lactoferrin (1:1 ratio) at different pH values, including a photograph of the protein mixtures.	136
Figure 6.3 Frequency and dissipation shift versus time at 7 th overtone for the sequential adsorption of WPI first and then lactoferrin on the quartz crystal surface with alternate rinse intervals with water at pH 6.	140
Figure 6.4 Frequency and dissipation shift versus time at 7 th overtone for the sequential adsorption of WPI first and then lactoferrin on the quartz crystal surface with alternate rinse intervals with water at pH 6.	141
Figure 6.5 Frequency and dissipation shift versus time at 7 th overtone for (a) the adsorption of WPI on the quartz crystal surface (first layer) and (b) the adsorption of lactoferrin on the WPI-coated quartz crystal surface (second layer) at various pHs.	145
Figure 6.6 Thickness of secondary layer after adsorption of lactoferrin on the WPI-coated surface at different pH from 2 to 10 using the Sauerbrey model.	146
Figure 6.7 Frequency and dissipation shift versus time at 7 th overtone for the adsorption of protein complex of WPI and lactoferrin on the quartz crystal surface with water rinse after 5 h at pH 6.	148
Figure 6.8 Dissipation shift versus frequency shift plot at 7 th overtone during the adsorption of protein complex of WPI and lactoferrin at pH 6.	149
Figure 7.1 Schematic illustration of interfacial deposition of lactoferrin molecules on WPI-coated oil droplets to produce bi-layer nanoemulsions.	154
Figure 7.2 Mean ζ -potential of droplets in single layer emulsions (0.5% w/w oil and 1.0% WPI) prepared at different pH values (pH 2 to 10).	158
Figure 7.3 Mean particle diameter of droplets in single layer emulsions (0.5% w/w oil and 1.0% WPI) prepared at different pH values (pH 2 to 10).	158
Figure 7.4 Photographs of bi-layer emulsions (0.5% w/w oil, 1.0% w/w WPI and 0.0 to 5.0% w/w LF) at different pH values: (a) pH 2, (b) pH 4 (c) pH 5, (d) pH 6, (e) pH 7, (f) pH 8, (g) pH 9 and (h) pH 10.	160
Figure 7.5 Mean ζ -potential of droplets in bi-layer emulsions with increasing lactoferrin concentrations (0 – 5% w/w) prepared at different solution pH from 2 to 10.	161
Figure 7.6 Mean particle diameter of droplets in bi-layer emulsions with increasing lactoferrin concentrations (0 – 5% w/w) prepared at different solution pH from 2 to 10.	161
Figure 7.7 TEM images of (a) nanoemulsions containing 1% (w/w) WPI (single layer) at pH 6 and (b) those nanoemulsions containing 1% (w/w) WPI and 3% (w/w) lactoferrin (bi-layer) adjusted to 0.5% (w/w) oil at different pH of 2, 4, 5, 6, 7 and 10.	163

Figure 7.8 Mean ζ -potential versus lactoferrin concentration of bi-layer nanoemulsions at different pH values: (a) pH 6, (b) pH 7, (c) pH 8, (d) pH 9 and (e) pH 10.	165
Figure 7.9 Schematic illustrations of interactions between WPI-coated droplets and lactoferrin molecules in bi-layer emulsions at different lactoferrin concentrations and solution pH (not drawn to scale).	168
Figure 7.10 Influence of heat treatment at different temperatures for 15 minutes on the mean ζ -potential of single layer and bi-layer emulsions.	171
Figure 7.11 Influence of heating temperatures at different temperatures for 15 minutes on the mean particle diameter (Z-Average) of single layer and bi-layer emulsions.	171
Figure 7.12 Influence of pH changes on the mean particle diameter of single layer and bi-layer emulsions.	174
Figure 7.13 Influence of pH changes on the mean ζ -potential of single layer and bi-layer emulsions.	174
Figure 7.14 Influence of NaCl concentrations on the mean particle diameter (Z-Average) of single layer and bi-layer emulsions.	175
Figure 7.15 Influence of NaCl concentrations on the mean ζ -potential of single layer and bi-layer emulsions.	176
Figure 7.16 Influence of CaCl_2 concentrations on the mean ζ -potential of single layer and bi-layer emulsions.	177
Figure 7.17 Influence of CaCl_2 concentrations on the mean particle diameter of single layer and bi-layer emulsions.	177
Figure 7.18 SDS-PAGE analysis of nanoemulsions containing (a) 1% (w/w) WPI (single layer emulsion) and (b) those containing 1% (w/w) WPI and 3% (w/w) lactoferrin (bi-layer emulsion) diluted to the same protein concentration as the WPI emulsions during <i>in vitro</i> digestion when mixed with SGF and SIF in sequence at different time intervals (0, 5, 15, 30, 60, 65, 75, 90 and 120 minutes). Mw: molecular weight standards; E: original emulsion; A: mixing of emulsion after gastric phase with SIF. SDS-PAGE analysis of 1% protein solution of (i) WPI or (ii) lactoferrin subjected to the same digestion conditions.	181
Figure 7.19 Influence of simulated gastrointestinal tract conditions on the mean particle diameter of single layer and bi-layer emulsions.	183
Figure 7.20 Influence of simulated gastrointestinal tract conditions on the mean ζ -potential of single layer and bi-layer emulsions.	183
Figure 8.1 Particle size distributions and photographs of lutein conventional emulsions and nanoemulsions: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	194
Figure 8.2 TEM images of lutein loaded conventional emulsions and nanoemulsions: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	195
Figure 8.3 Mean particle size (Z-Average) of lutein loaded conventional emulsions and nanoemulsions during storage at different temperatures (5, 20 & 40°C) for 28	

days: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	198
Figure 8.4 Photographs of lutein conventional emulsions and nanoemulsions during storage at different temperatures (5, 20 & 40°C) for 28 days: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	199
Figure 8.5 Total colour changes of lutein conventional emulsions and nanoemulsions during storage at different temperatures (5, 20 & 40°C) for 28 days: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	201
Figure 8.6 Relative content of lutein conventional emulsions and nanoemulsions during storage at different temperatures (5, 20 & 40°C) for 28 days: (a) WPI-stabilised conventional emulsion, (b) WPI-stabilised nanoemulsions and (c) WPI-lactoferrin stabilised nanoemulsions.	202
Figure 8.7 Arrhenius plot of lutein conventional emulsion and nanoemulsions stored at 5, 20 and 40°C.	205
Figure 8.8 Viability of Caco-2 cells as determined by MTT assay after incubation for 24 hours with (a) individual components and (b) blank emulsions stabilised by WPI and/or lactoferrin at different dilution time from 10 to 1000.	206
Figure 8.9 Viability of Caco-2 cells as determined by MTT assay after incubation with lutein loaded emulsions for (a) 24 hours and (b) 72 hours at different dilution time from 10 to 1000.	208
Figure 8.10 Cellular uptake of lutein by Caco-2 cell monolayers incubated with lutein loaded conventional emulsions and nanoemulsions with single or bi-layer interfacial layer.	210

LIST OF TABLES

Table 2.1 Physicochemical properties of caseins and whey proteins in bovine milk.	11
Table 2.2 Properties and uses of selected organic solvents used in preparation of nanoemulsions.	21
Table 2.3 Summary of some wall materials used in microencapsulation by drying methods in foods.	38
Table 2.4 Physicochemical properties of lutein.	41
Table 2.5 Summary of physiological conditions and some possible physicochemical processes when emulsions pass through the GI tract during digestion.	48
Table 4.1 Composition of conventional emulsion and nanoemulsion prepared at organic phase ratio of 10:90.	61
Table 4.2 Variations of the emulsion composition and conditions used in the preparation of nanoemulsions.	62
Table 4.3 Mean particle diameter (Z-Average) and PDI of coarse emulsion and nanoemulsion before and after evaporation.	65
Table 4.4 Mean particle diameter (Z-Average) and mean ζ -potential of conventional emulsion and nanoemulsion.	67
Table 4.5 Physicochemical properties of different types of oil used to prepare nanoemulsions and the characteristics of emulsions formed.	78
Table 5.1 Formulations with different wall materials and their theoretical fat content in powders assuming removal of all moisture via evaporation during spray drying.	98
Table 5.2 Mean moisture content and water activity of spray dried powders prepared with different wall materials containing 10, 20 or 30% (w/w) in feed solutions. ...	107
Table 5.3 Particle size and bulk density of spray dried powders prepared with different wall materials containing 10, 20 or 30% (w/w).	108
Table 5.4 Total, surface oil content and encapsulation efficiency of powders prepared with different wall materials containing 10, 20 and 30% (w/w) dry matter.	117
Table 5.5 Wettability and dispersibility of spray dried powders prepared with different wall materials containing 10, 20 and 30% (w/w) dry matter.	118
Table 5.6 Brix readings of nanoemulsions with wall solutions before and after spray drying process and the amount of powders added for reconstitution.	119
Table 5.7 Mean particle size (Z-Average) and ζ -potential of initial nanoemulsions and nanoemulsions after reconstitution prepared with different wall materials at pH 7.	120
Table 5.8 Properties of spray dried and freeze dried nanoemulsion powders containing 20% (w/w) trehalose.	123

Table 5.9 Wettability and dispersibility of spray dried and freeze dried nanoemulsion powders containing 20% (w/w) trehalose.	125
Table 5.10 Mean particle size (Z-Average) and ζ -potential of initial nanoemulsions and nanoemulsions after reconstitution at pH 7.	125
Table 6.1 Thickness of different interfacial structures of WPI and lactoferrin adsorbed on SAM modified hydrophobic gold surface at pH 6.	142
Table 7.1 Composition of bi-layer nanoemulsions formed by mixing WPI-stabilised nanoemulsions (2% WPI, 0.5% w/w oil) with an equal amount of lactoferrin solution (1:1 ratio) at different concentration.	154
Table 7.2 Values of ζ_0 , ζ_{Sat} , C_{Sat} and R_2 obtained by fitting the equation (7.1) to the experimental values and lactoferrin concentration.	165
Table 8.1 Composition of lutein loaded conventional emulsion and nanoemulsion.	189
Table 8.2 Particle characteristics of lutein loaded conventional emulsions and nanoemulsions at pH 6.	196
Table 8.3 Change in colour parameters of lutein loaded conventional emulsions and nanoemulsions during storage at different temperatures.	200
Table 8.4 Rate constant, coefficient and activation energy of lutein conventional emulsions and nanoemulsions at different temperatures of 5, 20 and 40°C.	204

LIST OF SYMBOLS

C	Mass sensitivity constant
D	Translational diffusion coefficient
$D_{3,2}$	Surface weighted mean
$D_{4,3}$	Volume weighted mean
$f(\kappa a)$	Henry's function
g	Gravitational acceleration
h	Thickness
K	Boltzmann's constant
k	Rate constant
n	Overtone number
r	Radius
T	Temperature
U_E	Electrophoretic mobility
v	Creaming velocity
$t_{1/2}$	Half-life
ΔD	Dissipation shift
Δf	Frequency shift
Δm	Adsorbed mass
ΔP	Laplace pressure
γ	Interfacial tension
δ	Interfacial layer thickness
η	Viscosity
λ	Wavelength
ε	Dielectric constant
μ	Shear elastic modulus
ρ	Density
ρ_{eff}	Effective surface density
ϕ	Volume fraction
ω	Angular frequency of oscillation

LIST OF ABBREVIATIONS

AMD	Age-related macular degeneration
AMY	Amylase
ANOVA	Analysis of Variance
APS	Ammonium persulphate
BHT	butylated hydroxyl toluene
CaCl ₂	Calcium chloride
CC	Coconut oil
CCP	Colloidal calcium phosphate
CIE	Commission Internationale de L'Eclairage
CR	Corn oil
DE	Dextrose Equivalence
DLS	Dynamic Light Scattering
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
EE	Encapsulation efficiency
GI	Gastrointestinal
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
pI	Isoelectric point
kBar	Kilobar
kDa	Kilodalton
L	Lipase
LBL	Layer-by-layer
LCT	Long chain triglycerides
LF	Lactoferrin
LO	Lemon oil
LSCM	Laser Scanning Confocal Microscopy
MCT	Medium chain triglycerides
MD	Maltodextrin
MPa	Mega pascal
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Na ₃ N	Sodium azide
NaCas	Sodium caseinate
NaCl	Sodium chloride
nm	nanometre
O.D.	Optical Density
O/W	Oil-in-water
PDI	Polydispersity Index
PIT	Phase inversion temperature
pmol	picomol
psi	Pound force per square inch
QCM-D	Quartz Crystal Microbalance with Dissipation
RNase	Ribonuclease
ROS	Reactive oxygen species
SAM	Self-assembled monolayer
SCT	Short chain triglycerides
S.D.	Standard Deviation
SDS	Sodium dodecyl sulphate
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SLS	Static Light Scattering
SNEDDS	Self-Nanoemulsifying Drug Delivery System
SOR	Surfactant to oil ratio
SPR	Surface plasmon resonance
TEM	Transmission Electron Microscopy
TEMED	N,N,N',N'-tetramethylethylene diamine
TR	Trypsin
TRE	Trehalose
WI	Whiteness Index
WPC	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate

W/O	Water-in-oil
α -lac	Alpha-lactalbumin
β -lg	Beta-lactoglobulin
ζ -potential	Zeta-potential

LIST OF PUBLICATIONS & CONFERENCE PROCEEDINGS

1. Teo, A., S. Dimartino, S. J. Lee, K. K. T. Goh, J. Wen, Indrawati, O., S. Ko & H. S. Kwak (2016). Interfacial structures of whey protein isolate (WPI) and lactoferrin on hydrophobic surfaces in a model system monitored by quartz crystal microbalance with dissipation (QCM-D) and their formation on nanoemulsions. *Food Hydrocolloids*, 56, 150-160.
2. Teo, A., Goh, K. K. T., Wen, J., Oey, I., Ko, S., Kwak, H. S. & Lee, S. J. (2016). Physicochemical properties of whey protein, lactoferrin and Tween 20 stabilised nanoemulsions: effect of temperature, pH and salt. *Food Chemistry*, 197(Part A), 297-306.
3. Anges Teo, Sung Je Lee & Kelvin K. T. Goh (2015). Stability of lutein in protein-stabilized nanoemulsions prepared by emulsification and solvent evaporation method, 19th International Conference of Functional Food Center, 17-18 November, Kobe, Japan.
4. Teo, A., S. Dimartino, K. K. T. Goh, J. Wen, Indrawati, O., S. Ko, H. S. Kwak, M. Golding & S. J. Lee. (2015). Characterisation of interfacial bi-layer or complex structures of nanoemulsions coated with WPI and lactoferrin studied by QCM-D, NZIFST Annual Conference, 29 June-2 July, Palmerston North, New Zealand.
5. Teo, A., Goh, K. K. T. & Lee, S. J. (2014). Nanoparticles and nanoemulsions. In: Noomhorm, A., Ahmad, I. & Anal, A. K., *Functional Foods and Dietary Supplements: Processing Effects and Health Benefits* (pp. 405-435). United Kingdom: Wiley Blackwell.
6. Teo, A., Lee, S. J. & Goh, K. K. T. (2014). Modulation of interfacial composition on the physico-chemical stability and lipid digestibility of nanoemulsions stabilised by whey protein isolates (WPI) and lactoferrin, Food Structure and Functionality Forum Symposium from Molecules to Functionality, 30 March-2 April, Amsterdam, The Netherlands.
7. Teo, A., Lee, S. J. & Goh, K. K. T. (2012). Nanotechnology in emulsions: preparation and characterisation of protein-stabilised nanoemulsions and their stability against heat, ionic strength and pH changes, NZIFST Annual Conference, 26-28 June, Hamilton, New Zealand.