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Behaviour of Emulsion Gels in the Human Mouth and Simulated Gastrointestinal Tract

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Abstract

Food structure greatly impacts the digestion process of food in the human body. Food structure design is a potential strategy to modulate food digestion and develop foods with controlled nutrient digestion and release. This research aimed to understand the dynamic processes of digestion of whey protein emulsion gels from the mouth to the intestine. Therefore, a series of heat-set whey protein emulsion gels, the structure of which was designed by varying NaCl concentration (10, 25, 100 and 200 mM) and oil droplet size (1, 6 and 12 μm), were formed. Mechanical properties in linear viscoelastic region, large deformation and fracture region and microstructure of gels were evaluated. *In vivo* oral processing and *in vitro* oral-to-gastrointestinal digestion of whey protein emulsion gels with different structures were investigated with a focus on the effect of gel structure on the gel disintegration and lipid digestion.

Results showed that the gel strength increased with increasing NaCl concentration. At the micro-scale, the gel structure became from homogenous to porous with increasing NaCl concentration from 10 to 200 mM. The fragmentation degree of whey protein emulsion gels in the mouth showed a positive linear relationship with the gel strength (i.e. a higher gel strength, the greater gel fragmentation degree). During oral processing, the small oil droplets ($\sim 0.45 \mu\text{m}$) incorporated in the protein matrix were stable without oil droplet release. During gastric digestion, the bolus of the gel containing 10 mM NaCl (soft gel) disintegrated much faster than that of the gel containing 200 mM NaCl (hard gel) in the human gastric simulator (HGS). The disintegration of the soft gel in the HGS was caused by both the abrasion and fragmentation while the abrasion was the predominant mechanism of the disintegration of the hard gel. The larger particle size of the soft gel bolus slowed down the emptying of gels from the HGS. With continued digestion, the emptying of both gels from the

HGS was accelerated by gel disintegration. The gel structure greatly influenced the gel disintegration at the micro-scale. The soft gel particles were gradually disrupted into individual oil droplets, with the protein matrix dissolving after gastric digestion for 4 hours while the hard gel particles still retained the oil droplets inside the protein matrix. The colloidal structure of emptied gastric digesta, which generated from original gel structure, still significantly impacted the digestion of whey protein emulsion gels in an *in vitro* intestinal model. In general, the colloidal structure of the emptied gastric digesta of the hard gel hindered the breakdown of gel particles and hydrolysis of oil droplets more effectively than that of the soft gel. The remaining structure within the hard gel particles limited the free motion of oil droplets, which led to a lower degree of coalescence and breakup of oil droplets. Interestingly, coalescence appeared to occur between neighboring oil droplets inside the emptied gastric digesta of the hard gel during intestinal digestion.

The structure of the gels containing 100 mM NaCl became from aggregated particle to emulsion-filled with increasing oil droplet size from 1 to 12 μm . The gel strength also decreased with the increase of droplet size. For the gels containing large oil droplets (6 and 12 μm), oil droplets were released from the protein matrix along with some coalescence during oral processing. During gastric digestion, a higher degree of coalescence of oil droplets occurred and coalesced oil droplets creamed to form a top oil layer. This slowed down the emptying of gels from the HGS. For the gels containing 1 μm oil droplets, most oil droplets still retained in the protein matrix during oral and gastric digestion with minimal instability of oil droplets. In addition, increasing interactions between oil droplets and protein matrix by decreasing oil droplet size hindered the protein hydrolysis.

Overall, this research provides an understanding of the way in which food

disintegrates in the human body and highlights the role of food structure on the digestion of food in the human body. These findings could assist in designing the functional new foods that deliver health benefits (e.g. lipid regulation, encapsulation and release of nutrients) and improving human health related to food digestion (e.g. dysphagia, dyspepsia).

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Table of Contents

Abstract	I
Acknowledgement	V
Table of Contents	IX
List of Figures	XV
List of Tables	XXI
List of Symbols	XXII
List of Abbreviations	XXII
List of Peer-reviewed Publications	XXIII
Chapter 1 Introduction	1
Chapter 2 Literature Review	5
2.1 Emulsions	5
2.1.1 Emulsion formation.....	6
2.1.2 Emulsion stability	6
2.1.2.1 Gravitational separation.....	7
2.1.2.2 Flocculation	7
2.1.2.3 Coalescence	8
2.2 Whey proteins	9
2.2.1 β -lactoglobulin	9
2.2.2 α -lactalbumin	10
2.2.3 BSA.....	10
2.3 Thermal denaturation of whey proteins.....	11
2.3.1 Molecular basis of protein interactions.....	11
2.3.2 Heat-induced aggregation of β -Lg.....	12
2.3.3 Heat-induced aggregation of other whey proteins	14
2.3.4 Heat-induced aggregates of β -Lg, α -La and BSA in whey protein mixtures..	15
2.4 Gelation of whey proteins.....	16
2.4.1 Heat-set whey protein gels.....	16
2.4.2 Cold-set whey protein gels.....	18
2.4.2.1 Salt-induced gels.....	18
2.4.2.2. Acid-induced gels	18
2.5 Emulsion gels	19
2.5.1 Formation of protein emulsion gels	20

2.5.2 Active or inactive fillers	22
2.6 Upper gastrointestinal tract.....	24
2.6.1 Mouth.....	24
2.6.1.1 Oral cavity	24
2.6.1.2 Teeth	25
2.6.1.3 Oral surfaces.....	25
2.6.1.4 Saliva.....	26
2.6.2 Stomach.....	27
2.6.2.1 Anatomy of stomach.....	27
2.6.2.2 Gastric motility and emptying	29
2.6.2.3 Gastric secretion	29
2.6.3 Small intestine.....	32
2.7 Disintegration of solid foods in the human body	37
2.7.1 Oral processing	37
2.7.2 Food breakdown in the stomach	40
2.7.2.1 Acid effect.....	41
2.7.2.2 Ion effect.....	42
2.7.2.3 Swelling effect.....	44
2.7.2.4 Enzyme effect	46
2.7.2.5 Gastric motility effect	46
2.7.2.6 Food structural effect.....	47
2.7.3 Gastric emptying	50
2.7.3.1 Pyloric sieving	50
2.7.3.2 Pyloric trituration.....	51
2.7.3.3 Meal particle size.....	51
2.7.3.4 Food composition	52
2.7.3.5 Food calorie content	52
2.7.3.6 Food density	53
2.7.3.7 Food rheology.....	53
2.7.4 Food breakdown in the intestine	54
2.8 Modulation of lipid digestion	55
2.9 Conclusions	60
Chapter 3 Materials and Methods.....	63
3.1 Materials	63
3.1.1 WPI	63
3.1.2 Soybean oil.....	63
3.1.3 Chemicals.....	63

3.1.4 Enzymes	64
3.1.5 Artificial saliva	64
3.1.6 Simulated gastric fluid (SGF)	64
3.1.7 Simulated intestinal fluid (SIF)	64
3.2 Methods	65
3.2.1 Protein solution preparation	65
3.2.2 Emulsion preparation	65
3.2.3 Emulsion gel preparation	65
3.2.4 Small strain oscillatory rheology	66
3.2.5 Large deformation properties of gels	68
3.2.5.1 Compression test.....	68
3.2.5.2 Cutting test.....	69
3.2.6 <i>In vivo</i> oral processing	69
3.2.6.1 Selection of panellists.....	69
3.2.6.2 Mastication experimental procedure	70
3.2.7 <i>In vitro</i> oral processing.....	71
3.2.8 Measurement of masticated gel or <i>in vitro</i> gel bolus	71
3.2.9 Quantification of released oil droplets	73
3.2.10 <i>In vitro</i> gastric digestion.....	73
3.2.10.1 Human gastric simulator (HGS).....	73
3.2.10.2 Gel swelling.....	77
3.2.10.3 Gastric emptying.....	77
3.2.10.4 pH measurement.....	78
3.2.10.5 Photos of emptied gastric digesta	79
3.2.10.6 Measurement of gel disintegration	79
3.2.11 <i>In vitro</i> intestinal digestion.....	80
3.2.12 Measurement of free fatty acid release	81
3.2.13 Particle size characterization by laser diffraction	81
3.2.14 Zeta-potential	83
3.2.15 Tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	85
3.2.15.1 Preparation of stock solutions.....	86
3.2.15.2 Gel preparation	87
3.2.15.3 Sample preparation.....	88
3.2.15.4 Running of electrophoresis.....	88
3.2.15.5 Staining and destaining.....	89
3.2.16 Confocal laser scanning microscopy (CLSM)	89

3.3.17 Transmission light microscopy	91
3.3.18 Statistical analysis	91
Chapter 4 Effect of Gel Characteristics on Breakdown Properties of Whey Protein Emulsion Gels in the Human Mouth¹	93
4.1 Abstract.....	93
4.2 Introduction	94
4.3 Results	95
4.3.1 Droplet size and zeta-potential of oil droplets	95
4.3.2 Emulsion gel formation.....	96
4.3.3 Mechanical properties of the gels	100
4.3.3.1 Large deformation properties before fracture.....	100
4.3.3.2 Fracture properties.....	101
4.3.4 In-mouth behaviour.....	104
4.3.4.1 Mastication parameters.....	104
4.3.4.2 Analysis of masticated gel.....	104
4.3.4.3 Oil droplet release.....	111
4.3.5 Correlation between physical/mechanical properties and degree of gel fragmentation	114
4.4 Discussion.....	116
4.5 Conclusions	121
Chapter 5 Effect of Structure of Protein Matrix on Gastric Digestion of Whey Protein Emulsion Gels²	123
5.1 Abstract.....	123
5.2 Introduction	124
5.3 Results	125
5.3.1 Gel swelling in the gastric environment	125
5.3.2 pH profiles in the HGS.....	126
5.3.3 Physicochemical characteristics of emptied gastric digesta	127
5.3.3.1 Image of emptied gastric digesta.....	127
5.3.3.2 Solid content of emptied digesta	129
5.3.3.3 Particle size distributions of emptied digesta	130
5.3.3.4 Stability of oil droplets	134
5.3.3.5 Microstructure of emptied digesta.....	137
5.3.3.6 SDS-PAGE of protein patterns in emptied digesta.....	139
5.3.4 Gastric digestion of gels at the low level of pepsin	142
5.3.4.1 Particle size distributions of emptied digesta	142
5.3.4.2 Stability of oil droplets	143
5.3.4.3 Microstructure	145

5.3.4.4 Protein hydrolysis	147
5.4 Discussion.....	148
5.5 Conclusions	152
Chapter 6 Disintegration Kinetics of Food Gels During Gastric Digestion and its Role on Gastric Emptying³	155
6.1 Abstract.....	155
6.2 Introduction	156
6.3 Results and Discussion	158
6.3.1 Physical characteristics of initial gel bolus	158
6.3.2 Disintegration kinetics	160
6.3.3 Assessment of emptying from HGS	168
6.3.4 Relationship between disintegration kinetics and emptying from the HGS ..	172
6.4 Disintegration mechanisms of gels during gastric digestion.....	174
6.5 Conclusions	176
Chapter 7 Behaviour of Whey Protein Emulsion Gels during Oral and Gastric Digestion: Effect of Droplet Size⁴	177
7.1 Abstract.....	177
7.2 Introduction	178
7.3 Results and discussion.....	179
7.3.1 Gel structure and mechanical properties	179
7.3.2 Breakdown properties of gels in the human mouth	184
7.3.3 Characterization of the simulated gel bolus	187
7.3.4 <i>In vitro</i> gastric digestion.....	191
7.3.4.1 pH	191
7.3.4.2 Gastric emptying.....	191
7.3.4.3 Protein hydrolysis	194
7.3.4.4 Breakdown of gel particles and behaviour of oil droplets.....	196
7.4 Mechanisms of disintegration of emulsion gels containing ~ 1 and 12 µm oil droplets	202
7.5 Conclusions	204
Chapter 8 Impact of Colloidal Structure of Gastric Digesta on <i>in vitro</i> Intestinal Digestion of Whey Protein Emulsion Gels	205
8.1 Abstract.....	205
8.2 Introduction	206
8.3 Results and discussion.....	208
8.3.1 Physicochemical properties of emptied gastric digesta	208
8.3.2 Breakdown of gel particles during intestinal digestion.....	214
8.3.3 Stability of oil droplets.....	219

8.3.4 Microstructure	224
8.3.5 Lipolysis	229
8.4 Possible mechanisms of lipolysis of oil droplets incorporated in the hard gel ...	233
8.5 Conclusions	235
Chapter 9 Overall Conclusions, Discussion and Recommendations for Future Work.....	237
9.1 Overall conclusions and discussion	237
9.2 Recommendations	244
Bibliography	247
DRC 16 Forms	285

List of Figures

Figure 2-1 Schematic presentation of idealized emulsion-filled gel (A), protein-stabilized emulsion gel (B), and a mixture of both emulsion gels (C). The grey cycles filled with blue color represent the emulsified oil droplets. The grey color outside these filled cycles represents the protein matrix.	20
Figure 2-2 Contrasting effects of active and inactive fillers on storage of heat-set whey protein emulsion gels. The logarithm of G'/G_m' , where G_m' is the modulus of the gel matrix, is plotted against the oil volume fraction ϕ . ● represents active filler and ■ represents inactive filler. Different effects of the applied shear stress on the active filler (upper diagram) and the inactive filler (lower diagram) are shown schematically (Dickinson et al. 2012).	23
Figure 2-3 An anatomic diagram of oral organ (Chen, 2009).	24
Figure 2-4 The teeth in human mouth (Xu & Bronlund, 2010).	25
Figure 2-5 Anatomy of the stomach of man (Tortora et al., 2008).	28
Figure 2-6 3-D model of the average human stomach (Ferrua et al., 2010).	28
Figure 2-7 Glands in the mucosa of the body the human stomach (Ganong et al., 2005).	31
Figure 2-8 Anatomy of the small intestine (Wikipedian-contributors, 2014).	33
Figure 2-9 Cholate (A) and schematic representation of the amphiphilic structure of bile salts (B).	35
Figure 2-10 One disk-shaped bile salt-lipid mixed micelle (A) and lipid digestion and passage to intestine mucosa; the circular structures are bile salts micelles with lipolytic products in cores (B) (Ganong et al., 2005).	36
Figure 2-11 A generalized texture profile analysis curve obtained from the instron universal testing machine (Bourne, 2002).	39
Figure 2-12 Diagram of ions equilibrium of a food particle in the stomach. □, ⊕, +, and − represent fixed charges, counterions, mobile positive charges and mobile negative charges, respectively.	43
Figure 2-13 General structure of TAG (Small, 1991). The three R groups stand for different acyl groups. With the carbon in the 2-position in the plane of the page and the 1- and 3-carbons behind the plane of the page, if the −OH is drawn to the left then the top carbon becomes sn-1, the mid carbon becomes sn-2, and the bottom carbon becomes sn-3.	56
Figure 2-14 Mechanism of lipolysis in the intestine (Wilde, & Chu, 2011).	58
Figure 3-1 Oscillatory shear strain (γ), shear rate ($\dot{\gamma}$) and shear stress (τ). The shear strain, rate and stress oscillate with frequency ω . [Adapted from (Bird et al., 1987)]....	67
Figure 3-2 Photographs of whey protein emulsion gels (A: 10 mM and B: 200 mM NaCl) during compression by the texture analyzer.	68
Figure 3-3 Image depicting the fracture wedge set geometry.	69
Figure 3-4 Image of an HGS and illustration of latex stomach chamber (A), (1) SGF; (2) plastic tubes for secretion; (3) pump; (4) latex chamber; (5) mesh bag; (6) roller; (7) belt; (8) pulley; (9) shaft; (10) angle gear; (11) Love-Joy joint; (12) fan heater for temperature control. Illustration of the human gastric simulator (B). The schematic diagram of grinding of solids in one sequence of contraction of the HGS (C).	76
Figure 3-5 Schematic diagram of the zeta-potential of a particle. (Wikipedia contributors, 2014).	85
Figure 3-6 Principle of confocal laser scanning microscopy (Wikipedia contributors, 2015).	90

Figure 4-1 Particle size and zeta-potential of whey protein stabilized oil-in-water emulsions containing different NaCl concentrations.	96
Figure 4-2 Typical photographs of original whey protein emulsion gels containing 10 (A), 25 (B), 100 (C) and 200 mM (D) NaCl.	98
Figure 4-3 Development of viscoelastic properties of whey protein emulsion gels containing 10 (A), 25 (B), 100 (C) and 200 (D) mM NaCl.	99
Figure 4-4 Confocal micrographs of whey protein emulsion gels containing 10 (A), 25 (B), 100 (C) and 200 (D) mM NaCl and particle size distributions of oil droplets within gels. Red represents oil and green represents protein.	100
Figure 4-5 Normalized force versus normalized time curves after fracture point of whey protein emulsion gels. A, B, C and D represent the gels containing 10, 25, 100 and 200 mM NaCl, respectively.	103
Figure 4-6 Typical image of whey protein emulsion gel boluses (A: 10, B: 25, C: 100 and D: 200 mM NaCl)	106
Figure 4-7 Typical image of particles within each masticated whey protein emulsion gel (A: 10, B: 25, C: 100 and D: 200 mM NaCl) retained in each sieve (0.038, 0.425, 0.85, 1.4, 2 and 3.15 mm, respectively) after washing.	110
Figure 4-8 Average particle size distributions of fragments of whey protein emulsion gels upon chewing obtained from 8 human subjects. The symbols (×, ♦, ▲ and ■) represent whey protein emulsion gels containing 10, 25, 100 and 200 mM NaCl, respectively.	111
Figure 4-9 CLSM of boluses of whey protein emulsion gels containing 10 (A), 25 (B), 100 (C) and 200 (D) mM NaCl and particle size distributions of oil droplets within masticated gels. Colour in red represents the oil and green the protein.	113
Figure 4-10 Oil droplet release measured by centrifugation.	114
Figure 4-11 Correlation between physical/mechanical properties and fragmentation degree of whey protein emulsion gels. (a), represents the hardness measured by the fracture wedge set; (b), represents the mechanical property index $(R/E)^{0.5}$; (c), represents the mechanical property index $(ER)^{0.5}$; (d), represents toughness (R).	115
Figure 5-1 Swelling kinetics of whey protein emulsion gels in the SGF.	126
Figure 5-2 pH change during <i>in vitro</i> digestion of whey protein emulsion gels in the HGS.	127
Figure 5-3 Typical images of emptied gastric digesta of whey protein emulsion gels during gastric digestion in the presence of pepsin.	128
Figure 5-4 Solid content of emptied digesta as a function of time.	129
Figure 5-5 Volume-weighted average diameter ($D_{4,3}$) of emptied digesta as a function of time.	130
Figure 5-6 Particle size distributions of emptied digesta: A1, soft gel without pepsin; A2, soft gel with pepsin; B1, hard gel without pepsin; B2, hard gel with pepsin.	133
Figure 5-7 Volume-weighted average diameter ($D_{4,3}$) of oil droplets in emptied digesta as a function of time.	134
Figure 5-8 Particle size distributions of oil droplets in emptied digesta: A1, soft gel without pepsin; A2, soft gel with pepsin; B1, hard gel without pepsin; B2, hard gel with pepsin.	136
Figure 5-9 Microstructure of emptied digesta as a function of time: A (soft gel) and B (hard gel). x400 and x3000 represent the low and high magnification respectively. Green colour represents protein, red colour represents the oil phase, and black colour represents air or water.	138
Figure 5-10 Tricine SDS-PAGE patterns under reducing conditions of proteins in emptied digesta in the absence of pepsin: A, soft gel; B, hard gel.	140

Figure 5-11 Tricine SDS-PAGE patterns under reducing conditions of proteins in emptied digesta in the presence of pepsin: A, soft gel; B, hard gel.	141
Figure 5-12 Evolution of particle size distributions of emptied digesta during gastric digestion at the low level of pepsin.	142
Figure 5-13 $D_{4,3}$ of emptied gastric digesta of the soft gel as a function of time at the low level of pepsin.	143
Figure 5-14 Evolution of size distribution of oil droplets in the emptied gastric digesta of the soft gel as a function of time at the low level of pepsin.	144
Figure 5-15 $D_{4,3}$ of oil droplets in the emptied gastric digesta of the soft gel as a function of time at the low level of pepsin.	145
Figure 5-16 Microstructure of emptied gastric digesta of the soft gel as a function of time at the low level of pepsin. Green colour represents protein, red colour represents the oil phase, and black colour represents air or water.	146
Figure 5-17 Tricine SDS-PAGE patterns under reducing conditions of proteins in emptied digesta of the soft gel at the low level of pepsin.	147
Figure 5-18 Schematic diagram of the breakdown of soft and hard gel particles during gastric digestion. Green colour represents the protein matrix; red colour represents oil droplets.	152
Figure 6-1 Photographs of gel bolus. A and B represent the soft and hard gels, respectively.	159
Figure 6-2 Comparison of the amount of gel particles of different sizes in the combined digesta at different digestion times. A1 and A2 represent the soft gels with and without pepsin, respectively. B1 and B2 represent the hard gels with and without pepsin.	162
Figure 6-3 Mean particles size distributions of the combined digesta at different digestion times. A1 and A2 represent the soft gels with and without pepsin respectively and B1 and B2 represent the hard gels with and without pepsin.	165
Figure 6-4 Disintegration profiles of whey protein emulsion gels in the HGS. Data dots are experimentally measured values and the lines represent the fitting of the data according to the Weibull model (see text and Table 6-2). A and B represent the soft and hard gels, respectively.	167
Figure 6-5 Gel retention in the HGS over the digestion time of 300 min. A and B represent the soft and hard gels, respectively. Lines represent the fitting of the data to the Siegel's model using the parameters in Table 6-3.	169
Figure 6-6 Linear relationship between the emptying from the HGS and disintegration kinetics of whey protein emulsion gels in the presence of pepsin (30 – 300 min).	173
Figure 6-7 Relationship between emptying of gels from the HGS and gel disintegration (0-300 min).	174
Figure 6-8 Disintegration mechanisms of the soft and hard gels during gastric digestion.	175
Figure 7-1 CLSM images of whey protein emulsion gels (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). Green colour represents protein, red colour represents the oil phase, and black colour represents air or water. LM and HM represent the low and high magnification respectively. All gels contain 100 mM NaCl.	180
Figure 7-2 Schematic diagram of the emulsion-filled gel (A) and the particle aggregated gel (B). Green colour and red colour represent protein and oil, respectively. All gels contain 100 mM NaCl.	181
Figure 7-3 Normalized force versus normalized time curves after fracture point of whey protein emulsion gels. A, B and C represent the gels containing 1, 6 and 12 μm oil droplets, respectively. All gels contain 100 mM NaCl.	183
Figure 7-4 CLSM images of boluses of whey protein emulsion gels after human	

mastication and oil droplet distributions in the gel boluses (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). Green colour represents protein, red colour represents the oil phase, and black colour represents air or water. All gels contain 100 mM NaCl.....	186
Figure 7-5 Mean particle size distribution of fragments of whey protein emulsion gels containing oil droplets of different sizes after chewing (obtained from 8 human subjects). All gels contain 100 mM NaCl.	187
Figure 7-6 Mean particle size distributions of simulated gel bolus produced from whey protein emulsion gels containing oil droplets of different sizes. All gels contain 100 mM NaCl.	189
Figure 7-7 Particle size distributions of oil droplets in the simulated gel bolus produced from whey protein emulsion gels containing oil droplets of different sizes (A) and their oil droplet release during the oral processing (B). All gels contain 100 mM NaCl.....	190
Figure 7-8 Profiles of pH of whey protein emulsion gels during <i>in vitro</i> gastric digestion (A, B and C: gels containing 1, 6 and 12 μm oil droplets, respectively). All gels contain 100 mM NaCl.....	191
Figure 7-9 Gastric emptying profiles of whey protein emulsion gels with varied droplet size distributions in the HGS. All gels contain 100 mM NaCl.	192
Figure 7-10 Images of top oil layer after 300 min of <i>in vitro</i> gastric digestion (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). All gels contain 100 mM NaCl.	193
Figure 7-11 Tricine SDS-PAGE patterns under reducing conditions of proteins in emptied digesta (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). All gels contain 100 mM NaCl.	195
Figure 7-12 Evolution of the volume-weighted diameter ($D_{4,3}$) (-1) and size distributions (-2) of emptied digesta (i.e. gel fragments) of gels with varied droplet size (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). All gels contain 100 mM NaCl.	197
Figure 7-13 Evolution of the volume-weighted diameter ($D_{4,3}$) (-1) and size distributions (-2) of oil droplets in emptied digesta of gels with varied droplet size during gastric digestion (A, B and C: gels containing 1, 6 and 12 μm oil droplets respectively). All gels contain 100 mM NaCl.	198
Figure 7-14 Microstructure of emptied gastric digesta at low magnification (LM). A, B and C represent gels containing 1, 6 and 12 μm oil droplets, respectively. Green colour represents protein, red colour represents the oil phase, and black colour represents air or water. All gels contain 100 mM NaCl.....	200
Figure 7-15 Microstructure of emptied gastric digesta at high magnification (HM). A, B and C represent gels containing 1, 6 and 12 μm oil droplets, respectively. Green colour represents protein, red colour represents the oil phase, and black colour represents air or water. All gels contain 100 mM NaCl.....	201
Figure 7-16 Schematic diagram of disintegration of gels containing ~ 1 and 12 μm oil droplets in the human mouth and human gastric simulator. The aggregated gel is the gel containing ~ 1 μm oil droplets; the particle-filled gel is the gel containing ~ 12 μm oil droplets.....	203
Figure 8-1 Particle size distributions of gastric digesta of the soft and hard gels emptied at 60 and 240 min.....	210
Figure 8-2 Size distributions of oil droplets in gastric digesta of the soft and hard gels emptied at 60 and 240 min.....	212
Figure 8-3 CLSM images of gastric digesta of the soft (A) and hard (B) gels emptied at 60 and 240 min. Red colour represents the oil and green colour represents the protein.	

Scale bar is 5 μm	214
Figure 8-4 Evolution of particle size distributions of emptied gastric digesta after intestinal digestion (0, 15, 30, 60, 90, 120 and 150 min). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.....	217
Figure 8-5 Change in $D_{4,3}$ and $D_{3,2}$ of emptied gastric digesta after intestinal digestion as a function of time (min). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.....	218
Figure 8-6 Evolution of oil droplet size distributions of emptied gastric digesta after intestinal digestion at different times (0, 15, 30, 60, 90, 120 and 150 min). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.....	222
Figure 8-7 Change in $D_{4,3}$ and $D_{3,2}$ of oil droplets in emptied gastric digesta after intestinal digestion as a function of time (min). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.	224
Figure 8-8 CLSM images of emptied gastric digesta after intestinal digestion at different times (0, 30, 90 and 150 min) at low magnification (scale bar is 50 μm).). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.....	226
Figure 8-9 CLSM images of emptied gastric digesta after intestinal digestion at different times (0, 30, 90 and 150 min) at high magnification (scale bar is 5 μm).). A: gastric digesta of the soft gel emptied at 60 min, B: gastric digesta of the soft gel emptied at 240 min, C: gastric digesta of the hard gel emptied at 60 min and D: gastric digesta of the hard gel emptied at 240 min.....	227
Figure 8-10 Microphotographs (transmission light) of intestinal digesta of whey protein emulsion gels. A, B, C and D represent the 60 min gastric digesta of the soft gel, 240 min gastric digesta of the soft gel, 60 min gastric digesta of the hard gel and 240 min gastric digesta of the hard gel, respectively.	229
Figure 8-11 Free fatty acid release of gastric digesta emptied at 60 and 240 min during intestinal digestion. The unit of fatty acid release of A is μmol per mL digesta; the unit of fatty acid release of B is μmol per gram gel.	232
Figure 8-12 Mechanisms of lipolysis of oil droplets of the 60 min gastric digesta of whey protein emulsion gel (hard gel) during intestinal digestion.....	234

List of Tables

Table 2-1 General characteristics of molecular interactions between protein molecules in solution [Adapted from (Bryant and McClements, 1998)].	12
Table 2-2 Principles enzymes in pancreatic juice (Ganong et al., 2005).	34
Table 2-3 Composition and melting temperature of some natural lipids (Small, 1991).	57
Table 4-1 Mechanical properties (large deformation) of whey protein emulsion gels.	101
Table 4-2 Fracture properties of whey protein emulsion gels.	102
Table 4-3 Mastication parameters of whey protein emulsion gels.	104
Table 6-1 Cohesive force of simulated gel bolus	158
Table 6-2 Parameters of fitted curves of gel disintegration in the HGS.	166
Table 6-3 Parameters of fitted model of gastric emptying	171
Table 7-1 Mechanical properties of whey protein emulsion gels	182
Table 7-2 Mastication parameters of whey protein emulsion gels with different oil droplet sizes.	184
Table 8-1 Solid content and pH of the emptied gastric digesta.	208
Table 8-2 Particle size of the emptied gastric digesta.	211
Table 8-3 Particle size of oil droplets in the emptied gastric digesta.	212
Table 8-4 Initial lipolysis rate of emptied gastric digesta during intestinal digestion ..	231

List of Symbols

G'	Shear storage modulus
D _{4,3}	Average volume-weighted diameter
D _{3,2}	Average surface-weighted diameter
pI	Isoelectric point

List of Abbreviations

α -La	α -lactalbumin
β -Lg	β -lactoglobulin
WPI	Whey protein isolate
Ig	Immunoglobulin
IgG	Immunoglobulin G
IF	Lactoferrin
SA	Serum albumin
BSA	Bovine serum albumin
TAG	Triglyceride
SDS	Sodium dodecyl sulfate
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
HGS	Human gastric simulator

List of Peer-reviewed Publications

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Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2014). Behaviour of whey protein emulsion gel during oral and gastric digestion: effect of droplet size. *Soft matter*, 10(23), 4173-4183.

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