Structured emulsion gel systems for delivery of bioactive compounds

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Abstract

The structure of solid/semi-solid foods greatly impacts on how the food is broken down and digested in the human body, which affects its sensory perception, and the bioaccessibility of nutrients. In this project, heat-set whey protein emulsion gel was used as a model system for solid/semi-solid foods for the delivery of capsaicinoids (CAP); the capsaicinoids were dissolved in the emulsion droplets. The aim was to investigate the effect of emulsion gel structure on the breakdown properties and sensory perception of the gel in human mouth and to understand how gel structure affects its digestion behaviour as well as the release of capsaicinoids during *in vitro* gastrointestinal digestion. Small and large deformation properties as well as the microstructure of the emulsion gel were evaluated. Eighteen human subjects were used to investigate *in vivo* oral processing behaviour and sensory perception. The Human Gastric Simulator (HGS) was used for *in vitro* dynamic gastric digestion and pH-stat for simulated intestinal digestion. Human intestinal epithelial cells Caco-2 were used to evaluate the irritation effect of gastric digesta by the quantification of human interleukin-8 (IL-8) production using enzyme-linked immunosorbent assay (Elisa).

Based on the rheological properties, the gels were classified into three groups: semi-solid gel (whey proteins as emulsifier, 10 mM NaCl with $d_{4,3}$ of ~ 0.2 μm); soft and elastic gels (whey proteins as emulsifier, 10 mM NaCl with $d_{4,3}$ of ~ 4, 1 and 0.5 μm); hard and brittle gels (whey proteins as emulsifier, 100 mM NaCl with $d_{4,3}$ of ~ 4, 1, 0.5 and 0.2 μm). Results from *in vivo* study indicated that the degree of gel fragmentation during mastication was positively correlated with gel hardness (represented by Young's modulus). A higher degree of fragmentation led to a greater surface exposure during mastication and, therefore, a greater release of capsaicinoid molecules, resulting in

greater mouth burn perception. Results from *in vitro* gastrointestinal digestion of CAP-loaded soft gel and CAP-loaded hard gel showed that the hard gel was disintegrated and hydrolysed slower than the soft gel during gastric digestion. The rate and extent of lipid digestion during intestinal digestion were affected by several factors, such as fat content, gel structure, gel particle size and initial oil droplet size of the gastric digesta. Generally, the soft gel had higher degree of lipid digestion, mainly because of its soft gel structure and lower fat content. The bioaccessibility of CAP was found to be positively correlated with the extent of lipid digestion.

The effect of active (whey proteins as emulsifier) versus inactive (Tween 80 as emulsifier) filler particles of CAP-loaded emulsion gels was also investigated. CAP-loaded Tween-80-coated oil droplets were not bound within the whey protein matrix (i.e. emulsion gels containing inactive filler particles) and appeared to be flocculated and heterogeneously distributed in the gel matrix; this led to drastically decreased gel strength. On the other hand, the whey-protein-coated oil droplets had strong interactions with surrounding whey protein matrix contributing to gel strength, and the oil droplets were relatively evenly distributed in gel matrix in CAP-loaded whey protein emulsion gels (i.e. emulsion gels containing active filler particles). During *in vivo* oral processing, CAP-loaded Tween 80 emulsion gels were readily broken down into small fragments in the mouth at small deformations with less chewing and released large amounts of oil droplets from the gel matrix. In general, the mouth burn perception was positively correlated with degree of gel fragmentation. The large amounts of oil droplets released from the gel matrix during mastication and the inhomogeneous distribution of the oil droplets of the CAP-loaded Tween 80 emulsion gels also contributed to their greater mouth burn perception. During *in vitro* gastric digestion, the gel with inactive filler particles was disintegrated and emptied out faster than gel with active filler particles, due to its

significantly smaller masticated particle size entering the stomach. Large amounts of oil droplets were released during gastric digestion from the gel with inactive filler particles while gel with active filler particles had minor release of oil droplets at the end of digestion. During intestinal digestion, the presence of Tween 80 in gel with inactive filler particles has slowed down the rate and extent of lipolysis, because Tween 80 had certain resistance against replacement by bile salts from the interface. Moreover, the Tween 80 molecules, once displaced by bile salts from the interface, would also participate in the formation of mixed micelles and help solubilize the released CAP molecules, therefore, leading to improved bioaccessibility of CAP.

An *in vitro* method was developed to quantify the gastric irritation of CAP-loaded food formulations during gastric digestion. Results suggest that Caco-2 cells had immune responses to CAP-loaded samples by secreting significant amounts of IL-8, confirming that CAP molecules are inflammatory to Caco-2 cells. The emulsion gel structure was modified using different emulsifiers: whey proteins versus Tween 80. The gastric digesta from CAP-loaded Tween 80 emulsion gel was able to stimulate more IL-8 production than CAP-loaded whey protein emulsion gel. Tween 80 was found to be a proinflammatory factor to Caco-2 cells and could stimulate IL-8 secretion.

Overall, this research provided new information on the use of solid/semi-solid systems for delivery of capsaicinoids and how food structure affects disintegration and digestion behaviour and eventually the release of capsacinoids. The outcomes have potential for designing functional foods containing capsaicinoids, with increased incorporation of capsaicinoids in the foods / pharmaceuticals, reduced irritation in the mouth and stomach and increased bioaccessibility in the intestine.

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List of Abbreviations

CAP	Capsaicinoids
WPI	Whey protein isolate
β-Lg	β-lactoglobulin
α-La	α-lactalbumin
BSA	Bovine serum albumin
Ig	Immunoglobulin
HGS	Human gastric simulator
SSF	Simulated salivary fluid
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
TAG	Triacyl glyceride
FFA	Free fatty acid
SDS	Sodium dodecyl sulfate
CLSM	Confocal laser scanning microscope
HPLC	High performance liquid chromatography
IL-8	Interleukin-8
IL-1β	Interleukin-1β
ELISA	Enzyme-linked immunosorbent assay
MTT	Thiazolyl blue tetrazolium bromide
EDTA	Ethylenediaminetetraacetic acid

List of Peer-reviewed Publications

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Chapter 1: Introduction

The emulsion-based systems for delivery of lipid-soluble bioactive compounds, such as capsaicinoids, β-carotene and curcumin have been widely studied in recent decades. The advantages of using such delivery systems include increased solubility, reduced irritation, increased stability and increased bioaccessibility, etc. (Ahmed, Li, McClements, & Xiao, 2012; Lin, Liang, Zhong, Ye, & Singh, 2018; Lu, Cao, Ho, & Huang, 2016; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013). These studies mainly focus on liquid emulsion systems, which have been extensively studied for the delivery of bioactive compounds in relation to their digestion behaviour in the gastrointestinal tract. However, limited information is available on the use of solid/semi-solid emulsion-based delivery systems.

When a food product is ingested, it goes through a wide range of physical and biochemical processes as it moves along the mouth, stomach and intestines. Different from the liquids, the solid/semi-solid foods undergo more complicated processes during digestion, as they usually take longer time to digest and empty from the stomach; their digestion behaviour would be different, which will impact on the release behaviour of the nutrients and their bioaccessibility (Dias, Zhu, Thompson, Singh, & Garg, 2019). The solid/semi-solid food requires several chewing cycles during oral processing, which lead to particle size reduction and formation of a bolus that is safe for swallowing; mastication also contributes to texture perception and the release of flavours from the food (Foegeding et al., 2011). The degree of particle size reduction depends critically on the structural and rheological properties of the food (Agrawal, Lucas, Prinz, & Bruce, 1997; Chen, Khandelwal, Liu, & Funami, 2013; Lucas, Prinz, Agrawal, & Bruce, 2002). In the stomach, the food particles are further disintegrated and digested under the effects of

peristaltic movements of the stomach wall and the secretion of digestive enzymes and gastric acid. The small intestine is the major site for food digestion and nutrient uptake. Although understanding food breakdown and digestion is crucial for food structural design for different purposes such as regulating lipid digestion, improving bioaccessibility of targeted nutrients, etc. little information is available for solid/semi-solid systems.

Soft solid systems have drawn increasing interest because of their potential applications in a number of industries from pharmaceuticals and cosmetics to food formulations (Dickinson, 2012; Guo, Ye, Bellissimo, Singh, & Rousseau, 2017; Ubbink, Burbidge, & Mezzenga, 2008). Many foods, such as puddings, dairy desserts, set yoghurt and sausages, are categorized as solid/semi-solid foods. All these foods have a structure that resembles an emulsion gel (Rosa, Sala, van Vliet, & van de Velde, 2006). An emulsion gel denotes a complex colloidal system that exists as both an emulsion and a gel (Dickinson, 2012). It is widely used as a model system for solid/semi-solid foods, to investigate the structure, rheology, disintegration and digestion behaviour in the human mouth and subsequent gastrointestinal tract (Chen & Dickinson, 1998b, 1999; Guo, Ye, Lad, Dalgleish, & Singh, 2014a, 2014b, 2016; Gwartney, Larick, & Foegeding, 2004; Ye & Taylor, 2009). The structure and rheological properties of the emulsion gel can be tailored so that the disintegration and digestion behaviour in the GI tract would be altered (Guo et al., 2014a, 2014b, 2016). Emulsion gel has great potential to be used as delivery system for bioactive compounds. However, how gel structure and digestion behaviour affect the release behaviour of the bioactive compounds and their bioaccessibility must be established.

Capsaicinoids, the lipophilic bioactive compounds presented in many peppers from the genus *Capsicum*, are responsible for causing the sensory responses of burning, pain and irritation when peppers are consumed. The beneficial activities of capsaicinoids include analgesic, anti-carcinogenic, anti-inflammatory, as well as beneficial influences on the gastrointestinal system (Srinivasan, 2016). However, their low water solubility and their acute burning sensation have limited their use in many food, nutraceutical and pharmaceutical applications. Some studies have investigated the use of liquid emulsions as delivery systems for capsaicinoids (Lu et al., 2016; Popescu, Chiutu, Mircioiu, & Dima, 2013) and found that emulsion-based systems can reduce irritation and increase bioaccessibility. Yet, there is no information on solid/semi-solid delivery systems for capsaicinoids and their release behaviour during oral processing and subsequent gastrointestinal digestion.

The overall hypothesis of this project is that incorporating capsaicinoids in solid/semi-solid foods leads to a reduction in the irritation / burning effect in mouth and stomach. Also, by designing food with different structures, it is possible to tailor the breakdown and digestion behaviour of the CAP-loaded food formulations and control the release and bioaccessibility of capsaicinoids during digestion.

In this project, heat-set whey protein emulsion gel was used as the model system for solid/semi-solid foods and the delivery system for capsaicinoids; the capsaicinoids were dissolved in the emulsion droplets, to achieve increased solubility and reduced irritation (Lawless, Hartono, & Hernandez, 2000). The aim was to study the effect of emulsion gel structure on the oral processing and sensory perception of the gel *in vivo* and understand how gel structure affects its breakdown and digestion behaviour as well as the release behaviour and bioaccessibility of capsaicinoids during *in vitro* gastrointestinal digestion.

The emulsion gel structure was modified by varying oil droplet size ($d_{4,3}$ ~ 4, 1, 0.5 and 0.2 μm), NaCl concentration (10 mM, 100 mM and 200 mM) and type of emulsifier (whey proteins versus Tween 80). The *in vivo* oral processing and sensory analysis were performed with 18 trained human participants; the *in vitro* dynamic gastric digestion was performed in a Human Gastric Simulator developed by Kong and Singh (2010) and the *in vitro* intestinal digestion was performed in a pH-stat; Caco-2 cells were used for quantification of gastric irritation. The overall aim was to provide a deeper understanding on the processes of food digestion and the bioaccessibility of targeted nutrients in relation to food structure and food digestion behaviour.

The main objectives of this project were:

1) To investigate the breakdown properties of emulsion gels containing capsaicinoids in human mouth as well as the sensory perception (i.e. mouth burn perception and mouth-feel firmness) of the gels, in relation to emulsion gel structure. It was hypothesised that by varying oil droplet size, ionic strength and type of emulsifier of the emulsion gel, the structure as well as the rheological properties of the emulsion gel would be altered, which would have an influence on oral processing behaviour and sensory perception. An increase in gel strength would require more chewing cycles and ends up with higher degree of fragmentation. The higher degree of fragmentation during oral processing could directly cause higher mouth burn perception because of greater surface exposure.
2) To understand the disintegration and digestion behaviour of emulsion gels containing capsaicinoids with varied gel structure during *in vitro* dynamic gastric digestion and subsequent *in vitro* intestinal digestion. The hypothesis was that gel with higher mechanical strength would be disintegrated and digested slower than gel with low

mechanical strength, leading to lower bioaccessibility of capsaicinoids after *in vitro* gastrointestinal digestion; also, gel formed with Tween 80 as emulsifier would be broken down faster because of its loose gel structure compared to gel formed with whey proteins as emulsifier, and that the presence of Tween 80 could improve the bioaccessibility of capsaicinoids.

3) To develop an *in vitro* method using human intestinal epithelial cells (Caco-2) to quantify gastric irritation of digested emulsion gels containing capsaicinoids, by measuring the stimulated secretion of inflammatory mediators (i.e. human interleukin-8, or IL-8). The hypothesis was that Caco-2 cells could respond normally to stimulations presented in the gastric digesta and produce IL-8 as an indication of irritation /inflammation; also, gel formed with Tween 80 as emulsifier would cause higher gastric irritation effect compared to gel formed with whey proteins as emulsifier, because Tween 80 emulsion gel would be broken down faster with large amounts of oil droplet release during gastric digestion. This would lead to elevated release of CAP molecules and therefore, would induce higher IL-8 production.

These objectives were accomplished through 5 research chapters. The outcomes from *in vivo* oral processing and sensory analysis of emulsion gels containing capsaicinoids with varying oil droplet size, NaCl concentration and type of emulsifier are presented in Chapter 4 and Chapter 5. The *in vitro* gastrointestinal digestion behaviour of emulsion gels containing capsaicinoids and bioaccessibility of capsaicinoids are reported in Chapter 6 and Chapter 7. The *in vitro* method using Caco-2 cells to quantify gastric irritation of digested emulsion gels containing capsaicinoids was developed and is reported in Chapter 8.

Chapter 2: Literature review

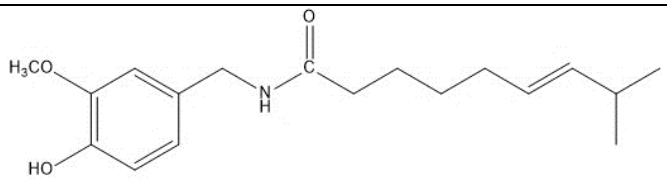
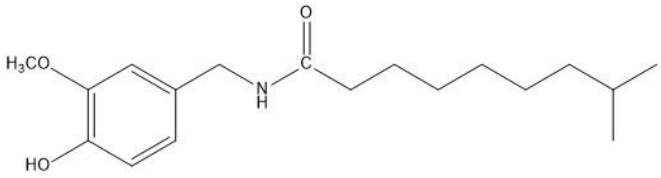
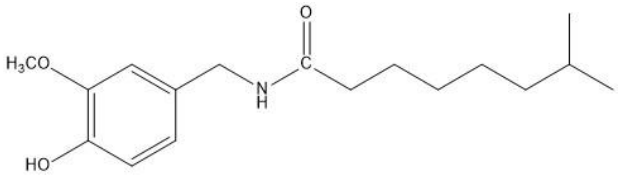
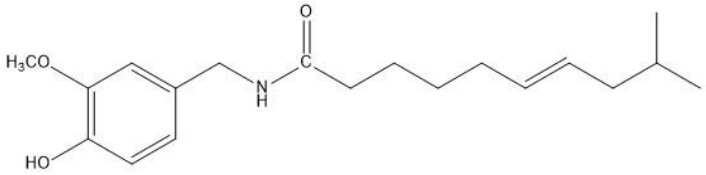
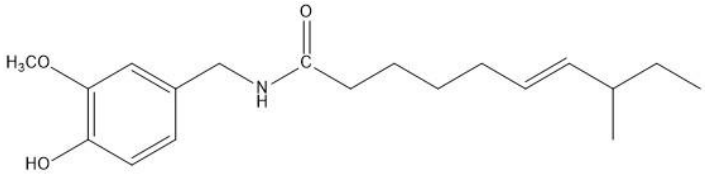
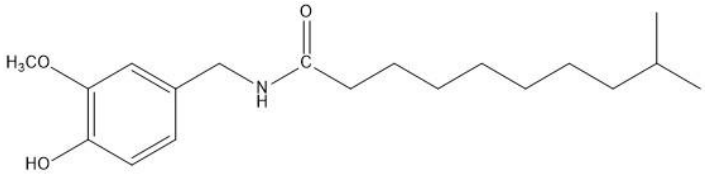
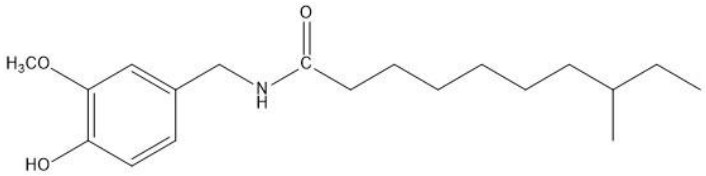
This chapter provides the review on capsaicinoids, whey proteins, Tween 80, emulsions and emulsion gels, Caco-2 cell line, sensory evaluation techniques and the upper gastrointestinal tract. Current liquid-based delivery systems for capsaicinoids were reviewed. Factors affecting the disintegration and digestion of solid/semi-solid foods in the human body were also discussed and reviewed. The research gaps were identified, i.e. the lack of information on the use of solid/semi-solid emulsion-based systems for the delivery of capsaicinoids and the effect of food structure on its digestion behaviour as well as the release of capsaicinoids in the gastrointestinal tract.

2.1 Capsaicinoids

Capsaicinoids, the pungent crystalline bioactive compounds presented in many peppers from the genus *Capsicum*, are a mixture of at least seven closely related capsaicinoids varying in acid side chains (Table 2-1): capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin I and II (1%) and homodihydrocapsaicin I and II (1%) (Bennett & Kirby, 1968; Govindarajan & Salzer, 1986; Iwai, Suzuki, Fujiwake, & Oka, 1979; Kawada, Watanabe, Katsura, Takami, & Iwai, 1985; Kosuge & Furuta, 1970). They are biosynthesized in the placenta through cinnamic acid pathway by condensation reaction of fatty acids and vanillyl amine, and could be degraded by the action of peroxidases (Contreras-Padilla & Yahia, 1998; Nelson, 1919; Thiele, Mueller-Seitz, & Petz, 2008). Capsaicinoids are unevenly distributed in the fresh pepper pods, where the cross wall contains 67.5%, the placenta contains 30.4%, the seed contains 1.7% and the outer wall contains 0.4% (Huffman, Schadle, Villalon, & Burns, 1978). Capsaicinoids are lipophilic; the water solubility reported for a mixture of

65% capsaicin and 35% dihydrocapsaicin was 0.06 g/L, as measured by conventional saturation method (exceeded capsaicinoids powder were distributed in distilled water and magnetically stirred for a week; then the suspension was stored undisturbed for two days allowing the undissolved capsaicin to sediment; the supernatant was then taken and analysed by HPLC for quantification). The water solubility increased to 6.4 g/L by firstly dissolving capsaicinoids in organic solvents such as ethanol, methanol or acetone, then mixing with water, and gradually evaporating the organic solvent (Turgut, Newby, & Cutright, 2004).

Table 2-1 Chemical structures of different capsaicinoids

Capsaicinoids and analogues	Chemical structure
Capsaicin	
Dihydrocapsaicin	
Nordihydrocapsaicin	
Homocapsaicin I	
Homocapsaicin II	
Homodihydrocapsaicin I	
Homodihydrocapsaicin II	

2.1.1 Biological activities of capsaicinoids

Dietary or topical capsaicin (the pungent principle) has been reported to have various biological activities such as anti-carcinogenic, analgesic, anti-inflammation, anti-microbial, weight reduction, beneficial influences on cardiovascular and gastrointestinal systems, etc. (Srinivasan, 2016). The mechanisms of these activities are attributed to the chemical interactions between capsaicin and a series of receptor proteins called transient receptor potential vanilloid (TRPV) or vanilloid receptor (VR). TRPV is a subfamily of non-selective cation channels and it consists of 6 subtypes. TRPVs are located predominantly on the plasma membrane of sensory neurons (Szallasi & Blumberg, 1999); however, they are also found, in a much less degree, in some non-neuronal tissues such as mast cells (Bíró et al., 1998b), glioma cells (Bíró et al., 1998a) and gastric epithelial cells (Faussone-Pellegrini et al., 2005; Lo et al., 2005). TRPVs can be activated through the stimulations of various chemical substances (e.g. capsaicin, resiniferatoxin) and physical factors (e.g. noxious heat, abrasion) (Szallasi & Blumberg, 1999).

The analgesic activity of topical capsaicin is closely related to its actions on transient receptor potential vanilloid subtype 1 (TRPV-1) or vanilloid receptor subtype 1 (VR-1). TRPV-1, famously known as the capsaicin receptor, is excited when the temperature is between 37 and 45 °C or below 37 °C when capsaicin is present (Srinivasan, 2016). Upon the activation of TRPV-1, mitochondrial permeability is increased thus permits the influx of Ca^{2+} into the neuron cell. As a result, the neuron is depolarized. Meanwhile, the activated neuron will release endogenous neurotransmitters called substance P. Both actions allow the sensory signal to move forward to the next neuron and eventually reach central nerve system and express burning sensation. However, the prolonged activation of TRPV-1 by a single high dose or repeated low doses of capsaicin will cause the exhaust

of substance P and the deactivation of the voltage-gated ion channels (a protective mechanism in order to prevent exceeded Ca^{2+} influx). As a consequence, the neuron cell is desensitized and the transmission of the sensory signal is blocked. The pain is then mitigated (Maggi & Meli, 1988; Simone, Nolano, Johnson, Wendelschafer-Crabb, & Kennedy, 1998). The typical capsaicin concentration used in topical analgesia cream is 0.025% to 0.075% for temporary pain-relief (Derry, Lloyd, Moore, & McQuay, 2009).

In addition to its analgesic activity, capsaicin is known to have anti-carcinogenic activity against various cancer cells in both *in vitro* and *in vivo* studies (Choi, Jung, & Oh, 2010; Dou, Ahmad, Yang, & Sarkar, 2011; Ito et al., 2004; Mori et al., 2006; Oh et al., 2008; Thoennissen et al., 2010; Yang et al., 2010). The mechanism is complicated and concentration dependent; it could vary in different cancer cell lines or act through multiple pathways. In several *in vitro* gastric cancer cell studies, the effects of capsaicin on apoptosis were by enhancing the transcription of two proto-oncogenes and tumour suppressor gene p53 at > 1 mM (Kim et al., 1997), or by reducing the expression of the anti-apoptotic protein Bcl-2 or tNOX protein at ≥ 100 μM (Lo et al., 2005; Wang, Chuang, Su, Li, & Chueh, 2011; Wang, Chueh, Chang, Yang, & Shao, 2009), or by increasing the expression of TRPV-6 (instead of TRPV-1) and increasing mitochondrial transmembrane permeability at ≥ 10 μM (Chow, Norng, Zhang, & Chai, 2007). In various *in vitro* colon cancer cell studies, capsaicin was found to promote the activation of peroxisome proliferator-activated receptor γ (PPARγ) at ≥ 100 μM (Kim et al., 2004), or activate adenosine monophosphate-activated protein kinase and increase the expression of the inactive form of acetyl-CoA carboxylase at ≥ 25 μM (Kim, Hwang, Kwak, Lee, & Park, 2007), which will then result in cancer cell apoptosis. In an *in vivo* study using male F344 rats as model, dietary capsaicin was reported to reduce 60% of the incidence of azoxymethane-induced colonic adenocarcinoma by elevating the

expression of phase II enzymes, glutathione S-transferase and quinone reductase (Yoshitani, Tanaka, Kohno, & Takashima, 2001).

People have always thought that consuming spicy foods would promote the chances of getting peptic ulcer, but this may not be entirely true. As a matter of fact, whether dietary capsaicin has beneficial or harmful effects on gastrointestinal systems is dose and/or duration dependent (Luo, Peng, & Li, 2011). A high dose or long duration of capsaicin often leads to the exhaust of capsaicin sensitive afferent nerves, which may have negative impacts on the gastrointestinal systems (Wang et al., 2005). However, a low dose or short duration of capsaicin application could promote gastrointestinal benefits. The beneficial effects on gastrointestinal systems were illustrated in various animal studies where their gastric mucosa was injured by the application of hydrochloric acid, ethanol, ammonia, aspirin or indomethacin (Szolcsányi & Barthó, 2001) and human subjects with gastric diseases including ulcer, gastritis, polyps and erosions (Mózsik, Szolcsányi, & Dömötör, 2007). Capsaicin could prevent the ethanol- and indomethacin-induced gastric mucosal injury and enhance the gastric transmucosal potential difference (GTPD) (Mózsik et al., 2007). Meanwhile, capsaicin was reported to complement with Lafutidine (an anti-ulcer drug) by enhancing the release of nitric oxide (NO) and calcitonin gene-related peptide (CGRP) (Nishihara, Nozawa, Nakano, Ajioka, & Matsuura, 2002), thus modulate the microcirculation of the gastric mucosa, maintain its integrity (Chen & Guth, 1995; Holzer et al., 1993), stimulate the secretion of mucus bicarbonate (Brown, Hanson, & Whittle, 1992; Brown, Keates, Hanson, & Whittle, 1993) and increase the recovery process of chronic gastric ulcer (Konturek, Brzozowski, Majka, Pytko-Polonczyk, & Stachura, 1993).

Moreover, dietary capsaicin was also proved to exert beneficial modulations on small intestine ultra-structure (Prakash & Srinivasan, 2010). Capsaicin was found to increase the activities of brush-border membrane enzymes such as leucine amino peptidase, glycyl-glycine dipeptidase and ϒ-glutamyl transpeptidase in the jejunal mucosa, which may reduce the steric repulsion of membrane lipids on enzyme proteins and, therefore, change the conformation of the enzymes. Moreover, dietary capsaicin was able to increase the absorptive surface of small intestine by altering its ultra-structure, especially through the increase in the perimeter and length of the microvilli, thus increasing the absorption and bioavailability of micronutrients.

2.1.2 Sensory properties of capsaicinoids

Scoville heat units (SHU) and American Spice Trade Association (ASTA) heat units are commonly used to describe the pungency level of heat-producing components, where 1 ppm capsaicin corresponds to 16 Scoville units or 1 ASTA units (Collins, Wasmund, & Bosland, 1995). In SHU method, capsaicinoids are extracted by ethanol, the score is given as the ethanol volume used to extract capsaicinoids from one grain of ground capsicum to give a detectable but weak pungency (Scoville, 1912). Several disadvantages are identified for this method: the effect of extraction time, lack of reproducibility, lack of reference standards, poor statistical validity, the error of central tendency, build up effect of pungency, increased taste threshold and rapid taste fatigue (Gillette, Appel, & Lego, 1984; Govindarajan, Narasimhan, & Dhanaraj, 1977). Accordingly, ASTA has adopted a standardized method to identify and quantify capsaicinoids by high performance liquid chromatography (HPLC) (American Spice Trade, 1985).

Five pungency levels are defined according to SHU: very highly pungent (> 80000 SHU), highly pungent (25000 – 70000 SHU), moderately pungent (3000 – 25000 SHU),

mildly pungent (700 – 3000 SHU) and non-pungent (0 – 700 SHU) (Weiss, 2002). Different capsaicinoids have different Scoville scores in their purified forms: the main component, capsaicin is scored 16000000 SHU; dihydrocapsaicin 15000000 SHU; nordihydrocapsaicin 9100000 SHU; homocapsaicin and homodihydrocapsaicin 8600000 SHU (Robbins, 2000).

Nevertheless, capsaicinoids are not consumed directly as purified powders; the pungency level of a certain food containing capsaicinoids is influenced by many factors: the concentrations of each capsaicinoid, the carrier type (water- or oil-based), fat content, serving temperature, the presence of other compounds (e.g. alcohol, milk proteins), etc. (Baron & Penfield, 1996; Govindarajan et al., 1977; Korel, Bağdatlioğlu, Balaban, & Hişil, 2002; Lawless et al., 2000; Todd, Bensinger, & Biftu, 1977).

Korel et al. (2002) discovered that the pungency level is linearly correlated with capsaicin, dihydrocapsaicin and total capsaicinoids content. Apart from this, Lawless et al. (2000) compared capsaicin thresholds and suprathresholds in oil- and water-based systems, the results showed that capsaicin is much easier to be detected in water-based systems than in oil-based systems. Capsaicin was rated from weak to very strong ranging from 0.3 to 10 mg/L in water-based systems, while the same pungency was achieved in oil-based systems over a range of 10 to 316 mg/L.

Desensitization is another effect that needs to be considered when conducting sensory test on capsaicin. Desensitization is caused by the exhaust of substance P from the sensory neurons and the conformational changes of receptor proteins which eventually closes the channel pore and stopped signal transmission (Szallasi & Blumberg, 1999). Therefore, it is essential to avoid the build-up effect of pungency by the use of neutral foods such as puffed rice and conducting time intervals in between samples, also clean

the palate thoroughly and stop the test when the panellists feel like the next sample could be very pungent (i.e. the carry over effect becomes significant) (Govindarajan et al., 1977).

The difference in pungent sensations for frequent and infrequent chili consumers is also attributed to desensitization. Lawless, Rozin, and Shenker (1985), Prescott and Stevenson (1995) and Ludy and Mattes (2012) compared between regular and irregular chili consumers and found that regular chili consumers are less sensitive and always gave lower ratings to the same samples as compared to irregular chili consumers. This also means that it is able to increase capsaicin tolerance by simply consuming more frequently.

2.1.3 Recent studies on capsaicinoids delivery systems

Capsaicinoids have shown great potential for their biological activities but their irritation effect on skin and mucosa through the digestion pathways and their low water solubility have limited their applications in functional foods. Capsaicinoids are more soluble in oil than in water; capsaicinoids are perceived as less pungent in oil-based systems than in water-based systems at the same concentration (Lawless et al., 2000). Due to these reasons, oil-in-water emulsion-based systems have been investigated for delivery of capsaicinoids, where capsaicinoids are dissolved in the emulsion droplets, for the purposes of reduced irritation, controlled release and increased bioaccessibility / bioavailability (Lu et al., 2016; Popescu et al., 2013; Zhu et al., 2015a; Zhu et al., 2015b).

The definition of the bioaccessibility of a nutrient is the quantity or fraction released from the food matrix during digestion in the gastrointestinal tract that can be absorbed (Heaney, 2001). It is usually assessed by *in vitro* digestion procedures, i.e. by simulated gastric and intestinal digestion, sometimes with Caco-2 cell uptake studies as well (Courraud, Berger, Cristol, & Avallone, 2013). Lu et al. (2016) investigated the impact

of different delivery systems on the bioaccessibility of capsaicin after *in vitro* lipid digestion and found that nanoemulsion had bioaccessibility of about 80%, which was much higher than that of the free unformulated capsaicin (capsaicin suspended in water; bioaccessibility of about 10%). The authors attributed this to the rapid digestion ability and high dissolution rate of medium chain triglycerides (used as the oil phase and carrier for capsaicin), which assisted in the formation of micelles during lipolysis and therefore, increased bioaccessibility. However, their delivery system was liquid-based with a relatively simple composition, which did not represent a complex food matrix. Also, they performed an animal study feeding rats with different CAP-loaded formulations to determine gastric mucosa irritation. The intact stomachs of rats were removed two hours after oral gavage, fixed by 10% formaldehyde solution, dehydrated and then stained. They found that CAP-loaded nanoemulsion was able to decrease gastric mucosa irritation, as compared to free formulated capsaicin. The possible reasons were that capsaicin were encapsulated in the nanoemulsion system, and therefore reduced direct contact between capsaicin and the surface of the gastric mucosa. Also, the CAP-loaded lipid droplets were at nanoscale, which may biologically adhere to the gastric tract, and thus prolong the retention time and avoid rapid release of capsaicin into the stomach.

The bioavailability is defined as the fraction of the ingested or applied nutrient or compound that ends up in the systemic circulation and utilized (Wood, 2005). It is usually evaluated by *in vivo* animal or human studies. The bioavailability, rates of metabolism and metabolites vary as capsaicin can be administered either topically, orally or injected intradermal/intraplantar for different purposes. The peak concentration time after administration is different in different organs / locations (Suresh & Srinivasan, 2010). Pershing, Reilly, Corlett, and Crouch (2004) suggested that the absorption of capsaicin from topical creams into stratum corneum was very rapid, however the metabolism was

very slow (about 20 h) with the main metabolites to be vanillic acid and vanillylamine (Chanda, Bashir, Babbar, Koganti, & Bley, 2008). Moreover, the maximal concentration of capsaicin in the skin is dependent on capsaicin relative solubility in the carriers, where alcohol-based solvents (such as isopropyl alcohol) are able to deliver greater amounts than non-polar solvents (such as mineral oil) (Pershing et al., 2004). The oral bioavailability of capsaicin has also been widely studied (Feng et al., 2018; Peng et al., 2015; Zhang et al., 2016; Zhu et al., 2014; Zhu et al., 2015a; Zhu et al., 2015b). For instance, Feng et al. (2018) performed an *in vivo* study using mice and found that by encapsulating capsaicin in nanoparticles, increased oral bioavailability was achieved. There was decreased irritation and increased hypolipidemic activity, because of their nano-scale characeristics, relatively stable structures and increased solubility, as compared to free capsaicin suspension.

The main absorption sites for dietary capsaicin are the stomach and whole intestine by passive processes which do not require transporter proteins, and the total absorption capacity ranged from 50 to 90% in various animal models (Donnerer, Amann, Schuligoi, & Lembeck, 1990; Kawada, Suzuki, Takahashi, & Iwai, 1984; Leelahuta, Glinsukon, & Wangpanish, 1983; Suresh & Srinivasan, 2010). Capsaicin can reversibly open the tight junctions (Beggs, Liu, Kwan, & Salter, 2010; Hu, Easton, & Fraser, 2005; Kaiser et al., 2015), which could further improve its bioaccessibility.

2.2 Whey proteins

Whey proteins are a mixture of mainly β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA), immunoglobulin (Ig) and proteose-peptone fractions (Fox & McSweeney, 2009). Whey proteins have great nutritional value as well as functional properties including gelation, emulsifying and foaming abilities. Emulsifying and

foaming abilities are attributed to the amphiphilic nature of the proteins, which exhibit great interface activity through the formation of an interfacial layer and reduces interfacial tension (Kinsella & Whitehead, 1989). Gelation occurs by aggregating and cross-linking of whey protein molecules through either hydrophobic interactions, hydrogen bonding or disulphide bonding, and eventually forms a three-dimensional solid-like network (gel). By manipulating the protein gel network structure, it is able to develop soft solids with desirable properties (Morr & Ha, 1993).

2.2.1 β-lactoglobulin

β-lactoglobulin (β-Lg) is the most abundant protein in whey. It composes 10% of the total milk proteins and around 58% of the whey proteins (Kilara & Vaghela, 2004). B-Lg consists of 162 amino acids; it has a molecular weight of around 18.4 kDa and a hydrodynamic radius of around 2 nm. Two genetic variants are found in β-Lg: A and B, which are different in the substitution of an aspartic acid in variant A and a glycine in variant B (Swaisgood, 1982). The molecule contains no phosphorus and five cysteine residues: Cys-66, Cys-106, Cys-119, Cys-121 and Cys-160. Two disulphide bonds were formed: one near the surface of the molecule formed between Cys-66 and Cys-160 and one inside the molecule formed between Cys-106 and Cys-119; Cys-121 remains as a free sulfhydryl group (Brownlow et al., 1997; Qin et al., 1998; Sawyer, 2013; Sawyer & Kontopidis, 2000). The molecular structure of β-Lg is shown in Figure 2-1. It is composed of nine β strands, eight of which are arranged to configure a β barrel with the ninth β-strand on the side of the first strand. The lone α helix is positioned on the surface of the molecule. The barrel has a hydrophobic centre which can bind hydrophobic molecules inside (Kilara & Vaghela, 2004).

Depending on the pH, temperature, etc., the quaternary structure of β-Lg can vary among monomers, dimers or oligomers. β-Lg remains in monomer below pH 3.0 and above pH 8.0; it associates into an octamer between pH 3.1 and pH 5.1 at low temperatures and high protein concentrations. At other pH values (including the pH of milk), β-Lg is likely to exist as dimers. These dimers have a spherical shape with a diameter of around 18 Å (Whitney, 1977). When the pH drops from neutral to acidic pH, β-Lg dissociates from dimers into monomers, while still maintaining the native structure (Kuwata, Hoshino, Era, Batt, & Goto, 1998; Sakurai & Goto, 2002; Uhrínová et al., 2000). Therefore, β-Lg is resistant under gastric digestion because of its stability under acidic pH conditions and is considered to be one of the causes for allergy in human infant milk (Peram, Loveday, Ye, & Singh, 2013).

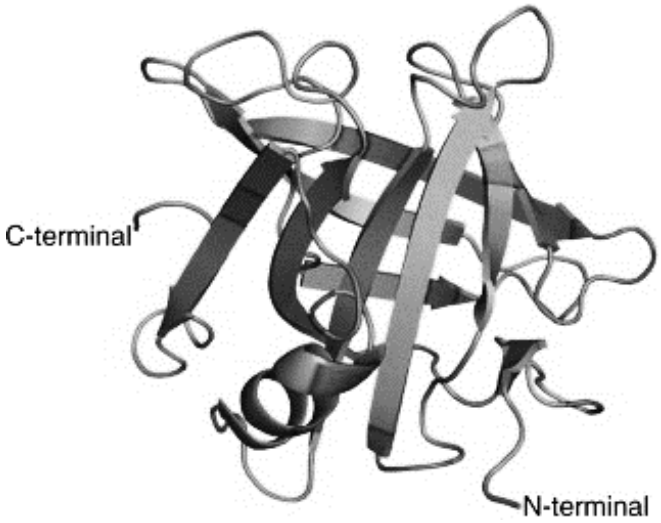


Figure 2-1 Molecular structure of β-lactoglobulin (Cheison, Leeb, Letzel, & Kulozik, 2011)

2.2.2 α-lactalbumin

α-lactalbumin (α-La) is the second most abundant protein in whey, which composes around 2% of the total milk proteins and 13% of the whey proteins. It is a globular protein that consists of 123 amino acids and is about 14.2 kDa in molecular weight. The molecule has four disulphide linkages and no phosphate groups (Kilara & Vaghela, 2004). α-La contains two domains: the α-domain (i.e. largely contains α-helix) and the β-domain (i.e. largely contains β-sheets). There is a deep crack dividing these two domains, while they are also connected by disulphide bridge between residues Cys-73 and Cys-91, forming a loop that could bind calcium. Overall, the structure of α-La is stabilized by four disulphide linkages: Cys-28 and Cys-111, Vys-6 and Cys-120, Cys-61 and Cys-77 and Cys-73 and Cys-91 (Brew, 2013; Permyakov & Berliner, 2000). Figure 2-2 presents the X-ray structure of α-La derived from native buffalo and recombinant bovine protein.

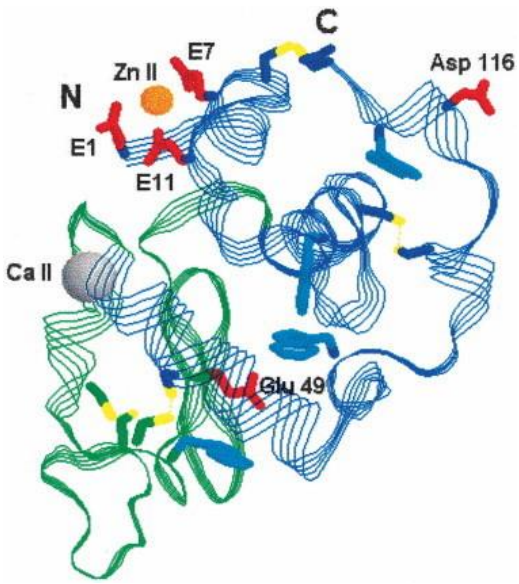


Figure 2-2 X-ray structure of α-La derived from native buffalo and recombinant bovine protein. Trp residues are shown in blue and disulphide linkages are shown in yellow. The residues shown in red colour take part in binding Zn^{2+} ions (Permyakov & Berliner, 2000)

2.2.3 Bovine serum albumin

Bovine serum albumin (BSA) composes of about 5% of the whey proteins, it is one of the most prevalent proteins in plasma (Schreiber & Urban, 1978). BSA is the major carrier for hormones, drugs and metabolites in the blood (Bujacz, 2012). Its molecular weight is about 69 kDa. It has three structurally similar domains with 17 disulphide linkages, one free sulfhydryl group and no phosphorus. Each domain comprises ten helix and can be separated into two sub-domains: A and B, consisting six and four helixes respectively. The two sub-domains are linked by a long loop. The loop flexibility ensures the change of orientations of the sub-domains, while the helix bending accounts for the conformational flexibility between three domains. The gaps among domains and sub-domains can be used as binding sites for various ligands (Bujacz, 2012).

2.2.4 Immunoglobulins

The immunoglobulins (Ig) composes at least 2% of the total milk proteins. Four categories of Ig are identified in milk: IgG1, IgG2, IgA and IgM. They share a similar basic structure, composing of two heavy chains with molecular weights of 50 kDa – 70 kDa, and two light chains with molecular weights of 20 kDa – 25 kDa (Swaisgood, 1982).

2.2.5 Molecular basis of protein functionality

The protein functionality inevitably relies on the structures of the protein molecules and the interactions between them (Bryant & McClements, 1998). Therefore, a thorough understanding on the molecular basis of protein functionality is necessary for the development and application of protein ingredients. There are a series of important molecular interactions that modulate the conformation and aggregation of proteins in foods, summarized in Table 2-2.

Table 2-2 Features of molecular interactions between protein molecules in aqueous solution (Bryant & McClements, 1998)

Type of interaction	Sign	Strength	Range	pH	Ionic strength	Temperature
Hydrophobic	Attractive	Strong	Long	No	No	Increases
Hydrogen bonding	Attractive	Weak	Short	No	No	Decreases
Van de Waals	Attractive	Weak	Short	No	No	–
Disulphide bonds	Attractive	Very strong	Short	Yes	No	–
Hydration	Repulsive	Strong	Short	No[a]	No[a]	Decreases
Steric repulsion	Repulsive	Strong	Short	No	No	–
Electrostatic	Attractive or Repulsive	Weak → Strong[b]	Short → Long[b]	Yes	Decreases	Increases

[a]Indirectly relies on pH and ionic strength since these parameters affect the degree of protein hydration.

[b]Depends on pH and ionic strength.

2.2.6 Interfacial properties of whey proteins

Whey proteins are interface active, that is, they adsorb to the air-water or oil-water interfaces at relatively low concentrations and reduce interfacial tension. The interface properties of whey proteins are vital in food applications that require emulsification at water-oil or water-air interfaces, in various food products such as ice cream, soup, dressing, etc (Leman & Kinsella, 1989). The protein molecules would go through a series of complex reactions to form an interfacial layer that covers and stabilizes the emulsion droplets. The amphiphilic nature of whey proteins drives them to quickly diffuse and adsorb at the newly formed interfaces generated during homogenization or whipping. The movement of protein migration from solution to the interface is thermodynamically favourable, because of the partial loss of the conformational and hydration energy of the protein at the interfaces (Leman & Kinsella, 1989). The adsorbed protein molecules would unfold partially to expose a high proportion of their hydrophobic side to the non-aqueous phase and their hydrophilic side to the aqueous phase. The tendency of the protein molecules to stay between the aqueous and non-aqueous phases allows them to stay adsorbed at the interface, and therefore reducing the interfacial tension. Subsequently, the adsorbed and partially unfolded protein molecules have to go through a series of intramolecular and intermolecular rearrangements to form a strong viscoelastic surface layer that can protect the oil droplets or air bubbles. There is no doubt that the flexibility of the molecular chains is critical for ensuring the proteins to function well as an emulsifier, however, other physicochemical properties are also necessary in forming the protective interfacial layer structure. These properties include the distribution and number of hydrophilic and hydrophobic residues, the distribution and number of charged residues and reactive sulfhydryl residues, etc (Morr & Ha, 1993).

At neutral pH, the globular β-Lg is present as dimers in solutions, associated by non-covalent interactions. The β-Lg monomer (18.4 kDa) has two intramolecular disulphide bonds and a hidden free sulfhydryl group (Dickinson, 1998). Upon adsorption of β-Lg onto the oil-water or air-water interfaces, much of the globular structure remains; however, there are still some partial denaturation and macromolecular rearrangements (Dickinson, Goddard, & Ananthapadmanabhan, 1993). The adsorbed, partially unfolded globular protein is in a state somewhere in between fully denatured and native. The structure resembles the alleged molten globule state, which has a native-like secondary structure and a disordered tertiary structure (Dickinson & Matsumura, 1994; Hirose, 1993). The adsorbed β-Lg monolayer can be seen as a pseudo-two-dimensional system of deformable particles that are densely packed and have strong intramolecular and intermolecular interactions combining hydrogen, hydrophobic and ionic bonds (de Feijter & Benjamins, 1982). The β-Lg monolayer was reported to have a thickness of 2-3 nm at air/water interface over the pH range of 5.5 – 7.0, measured by neutron reflectivity (Atkinson, Dickinson, Horne, & Richardson, 1995; Horne, Atkinson, Dickinson, Pinfield, & Richardson, 1998). α-La is able to transit to a molten globule state during emulsification (Matsumura, Mitsui, Dickinson, & Mori, 1994). However, α-La has a different native secondary structure in solution and contains no free sulfhydryl group. At room temperature and neutral pH, β-Lg was found to be slightly more favourable than α-La to adsorb at water-oil interface in emulsions formed with whey protein isolate and whey protein concentrate (Demetriades, Coupland, & McClements, 1997; Kulmyrzaev, Bryant, & McClements, 2000; Sliwinski, Roubos, Zoet, Van Boekel, & Wouters, 2003), while α-La adsorbs preferentially at lower pH (Hunt & Dalgleish, 1996). The adsorbed BSA, on the other hand, was observed to stay in a compact and

native-like conformation in a dense thin film at the air-water interface (Eaglesham, Herrington, & Penfold, 1992).

2.2.7 Heat-induced gelation of whey proteins

Aguilera (1995) and Schmidt (1981) suggested that the gelation of proteins induced by heating consists of four steps. Step one: the native protein molecules unfold; step two: the unfolded protein molecules aggregate; step three: the protein aggregates form strands; step four: the strands associate together and eventually form a space-spanning network.

Native whey proteins exist as monomers and dimers in solution when the pH is neutral. Upon heating, the dimers would dissociate into monomers, thus change the structure of the proteins and the system becomes more flexible. The free thiol groups as well as the hydrophobic groups that were shielded in native state would unfold, expose and interact with other molecules. The intermolecular interactions between the protein molecules would result in forming the oligomers (mainly dimers and trimers). When the concentration of the oligomers surpasses certain value, the oligomers would cross-link into larger aggregates. When the concentration reaches a critical value, the aggregates would crosslink into the formation of a three-dimensional network. The critical concentration for gelation would increase when the pH increases and / or the ionic strength decreases. Co-aggregation was found between the different proteins presented in whey (Nicolai, Britten, & Schmitt, 2011).

Depending on the strength of the electrostatic interactions between protein molecules, different types of gels are formed (Mehalebi, Nicolai, & Durand, 2008; Pouzot, Nicolai, Visschers, & Weijers, 2005). At relatively low ionic strength (i.e. salt concentration < 50 mM) or pH values far from pI, the electrostatic repulsion between protein molecules would be relatively high. Therefore, it is more difficult for protein molecules to form

bonds with each other. However, bonds can still be formed through the attractive forces such as hydrophobic interactions between the non-polar patches from two protein molecules. As a result, the protein molecules appear to associate in a 'head to tail' manner and form a filament structure. This indicates that the protein molecules approached each other at fixed sites on the opposing ends, forming the fine-stranded gel.

At relatively high ionic strength (salt concentration > 50-100 mM) or pH values close to pI, the electrostatic repulsion is screened down. Therefore, the protein molecules can associate with each other at random spots on their surfaces, forming rather large aggregates in spherical shapes. The large and spherical aggregates would randomly associate into a three-dimensional network, forming the particulate gel. A mixture of fine-stranded gel and particulate gel is formed at intermediate ionic strength and pH conditions (Mehalebi et al., 2008).

Heat-induced protein gelation results from the protein aggregation process, at protein concentrations above the critical gelation concentration (Verheul & Roefs, 1998). Whey proteins denature in an irreversible way upon heating, and cross-link into a space-spanning network. The heat-induced gel network often comprises of fine strands with a diameter in the order of nanometers in size (Ako, Durand, Nicolai, & Becu, 2009a; Ikeda & Morris, 2002; Pouzot et al., 2005). With the shifting of pH towards the pI, or increasing salt concentrations, the intermolecular repulsion would decrease, larger aggregates with a diameter in the order of micrometers would form, and the gel networks become coarser (Ikeda & Morris, 2002; Mehalebi et al., 2008; Pouzot, Durand, & Nicolai, 2004). These changes in structure can be observed in the macroscopic properties of the gels. The fine-stranded gels are semi-transparent with good water-holding capacity, while the particulate gels are not transparent. Fine-stranded gels formed at neutral pH are often

elastic. At acidic pH far from pI, it is more difficult to form disulphide bonds between protein molecules because the thiol groups are stable at acidic pH (i.e. pH 2); the fine-stranded gels formed at acidic pH are often brittle. On the other hand, fracture happens normally at a small strain for particulate gels, with a much higher fracture stress required (Ikeda & Foegeding, 1999; Ikeda, Foegeding, & Hagiwara, 1999).

2.3 Tween 80

Tween 80, or polysorbate 80, is a non-ionic small molecule surfactant often used in food and cosmetic applications. This compound is synthesized by the esterification of oleic acids to sorbitol molecules; polyoxyethylene groups are also attached to the sorbitol molecules in order to make the sorbitan esters water soluble (Goff, 1997). Its full chemical name is polyoxyethylene (20) sorbitan monooleate, or (x)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl), with a chemical formula of $C_{64}H_{124}O_{26}$ and a molecular weight of 1310 g/mol. It is a water-soluble, viscous yellow liquid. In solution, Tween 80 either exists as monomers or forms micelles depending on various factors such as Tween 80 concentration, buffer composition, temperature, etc. It is reported to have a critical micelle concentration in pure water of 0.012 mM (Wan & Lee, 1974).

Tween 80 is amphiphilic with an HLB (hydrophilic-lipophilic balance) value of 15 (Rabišková & Valášková, 1998). The hydrophobic nature comes from its hydrocarbon chains (fatty acid tail) and the hydrophilic nature comes from its polyoxyethylene groups. Tween 80 is often used as the emulsifier in the food industries. During homogenization or whipping, Tween 80 in solution quickly moves to and adsorbs on oil-water / air-water interfaces, and reduces interfacial tension (Bezelgues, Serieye, Crosset-Perrotin, & Leser, 2008). It is well elucidated in literature that Tween 80 can reduce the interfacial tension to a larger extent than whey proteins (Bezelgues et al., 2008; Dalgleish, 2003; Gomes,

Costa, & Cunha, 2018), because the Tween 80 molecules move faster in solution, adsorb strongly to the interface and have few steric constraints in preventing them from packing closely (El-Nokaly & Cornell, 1991). The competitive adsorption at the interface is often observed in systems presented with both Tween 80 and whey proteins, and that the adsorbed whey proteins can be displaced by Tween 80. The extent of displacement also depends on a few factors, including the age of the protein interface, the concentration of Tween 80, the addition of Tween 80 before or after the interface formation, etc (Bezelgues et al., 2008; Chen & Dickinson, 1995; Dalgleish, 1997; Gomes et al., 2018; MacKie, Wilde, Wilson, & Clark, 1993; Wilde & Clark, 1993).

2.4 Emulsions

An emulsion is a colloidal dispersion where the discrete droplets are suspended in a continuous phase in which they are immiscible. There is an interfacial layer between the two phases where essential surfactant material is adsorbed. A liquid where oil is the dispersed phase and water is the continuous phase is an oil-in-water (or O/W) emulsion (such as milk, salad dressing); a liquid where water is the dispersed phase and oil is the continuous phase is a water-in-oil (or W/O) emulsion (such as butter, margarine). There is another form of emulsions named double emulsions in which the water droplets are encapsulated in oil droplets and then dispersed in continuous water phase (water-in-oil-in-water or W/O/W emulsions) or oil droplets are encapsulated in water droplets and then dispersed in continuous oil phase (oil-in-water-in-oil or O/W/O emulsions) (Dalgleish, 2003). Double emulsions have been investigated for their potential applications in food products, for instance, the implantation of W/O/W emulsions in mayonnaises and ice creams for fat reduction while maintaining the same sensory properties (Oppermann, Verkaaik, Stieger, & Scholten, 2017; Tekin, Sahin, & Sumnu, 2017; Yildirim, Sumnu,

& Sahin, 2016), or the use of O/W/O emulsions in spreads for encapsulating and protecting the oxidative compounds such as oils rich in polyunsaturated fatty acids side chains (O' Dwyer, O' Beirne, Ní Eidhin, Hennessy, & O' Kennedy, 2013a; O'Dwyer, O'Beirne, Ní Eidhin, Hannon, & O'Kennedy, 2013b).

Emulsions can be separated into two types depending on their thermodynamic stabilities: micro-emulsions and conventional emulsions. Conventional emulsions are thermodynamically unstable and will eventually detach into an oil layer on the top and a water layer below. They require energy input during emulsion formation. Micro-emulsions, on the other hand, are thermodynamically stable and can form spontaneously when the conditions are favourable (Danielsson & Lindman, 1981; McClements, 2012). Only conventional O/W emulsions are discussed here.

2.4.1 Emulsion formation

Emulsions are formed by mixing oil with water at a certain ratio, then supply energy into the system to disrupt the oil or water phase to create small droplets. The energy usually comes from mechanical shearing or sonication. Emulsifiers are added in and adsorbed at interface during emulsion formation to reduce the interfacial tension and form a protective layer of the droplets in order to stabilize and prevent them from merging with each other.

Emulsifier is defined as a group of amphiphilic substances that can adsorb to the interface and reduce interfacial tension between air-water or oil-water, therefore enhance emulsification and increase emulsion stability (Krog & Vang Sparsø, 2003). They can be separated into two types depending on their molecular weights: 1) small-molecule surfactants (such as polysorbates, sugar esters, monoglycerides); 2) proteins (such as casein, β-lactoglobulin, etc.). Small-molecule surfactants can easily adsorb to the

interface during emulsification, and their hydrophobic parts will stay in oil while their hydrophilic parts stay in water. Based on the Bancroft's rule, the phase where the surfactant is more soluble would be the continuous phase. HLB (hydrophilic-lipophilic balance) value is used to characterize small-molecule surfactants, which can range from 3 (more soluble in oil) to more than 10 (more soluble in water) (Dalgleish, 2003).

The emulsifying mechanisms of proteins, on the other hand, are more complicated. Native proteins are usually soluble in water at pH values far away from their isoelectric points. For instance, native β-lactoglobulin is soluble at neutral pH, and the hydrophobic parts of β-lactoglobulin are hidden inside its globular structure while the hydrophilic parts are exposed to water. However, during emulsification, β-lactoglobulin changes its conformation to expose its hydrophobic parts during and/or shortly after adsorption (Haynes & Norde, 1994, 1995; MacRitchie & Owens, 1969). Therefore, adsorbed proteins are partly denatured. Their stabilization mechanisms after adsorption are usually through electrostatic repulsion and/or steric repulsion.

Protein adsorption to the interface, however, is reversible and can be replaced by small-molecule surfactants when they present at sufficient concentrations (Dalgleish, 1997). This is due to the facts that generally, small-molecule surfactants can lower interfacial tension to a larger degree than proteins, and they adsorb to the interface much easier and faster. The extent to which the proteins are desorbed and displaced by small-molecule surfactants depends on the surfactant/protein ratio.

2.4.2 Emulsion stability

Emulsion destabilizes through various mechanisms such as creaming, sedimentation, flocculation, coalescence, Oswald ripening and phase inversion. They act individually and can also affect each other (McClements, 2009). The changes in temperature, pH,

ionic strength or with other compounds such as polysaccharides presenting in the system can induce emulsion destabilization.

Creaming or sedimentation occurs when continuous phase and dispersed phase have a density difference. The velocity of droplets moving up or down depends on the differences between gravitational, buoyancy and friction forces, calculated by the Stoke's law equation:

$$v_{droplet} = \frac{2R^2 g}{9\mu^c}(\rho_c - \rho_d) \qquad \textbf{Equation 2-1}$$

Where, $v_{droplet}$ is the velocity of sedimentation or creaming; R is the radius of the oil droplet; ρ_c and ρ_d are the densities of the continuous and dispersed phase respectively; μ_c is the viscosity of the continuous phase; g is the gravitational acceleration. According to Equation 2-1, it is easy to learn that creaming or sedimentation can be decelerated by decreasing emulsion droplet size, and/or increasing the viscosity of the continuous phase, and/or decreasing the density difference between continuous and dispersed phase.

Flocculation is the procedure where two or more droplets are aggregated into larger units but not merged into bigger droplets (Tadros, 2013). Its mechanism is explained by droplet-droplet interactions. Evans and Wennerström (1994) established an equation to depict flocculation in colloidal systems containing monodispersed spherical particles:

$$\frac{dn_t}{d_t} = -\frac{1}{2}EF \qquad \textbf{Equation 2-2}$$

Where, n_t is the total number of particles per unit volume; t is the time; dn_t/d_t is the flocculation rate; E is the collision efficiency; F is the collision frequency, expressed as the total number of droplets encounter per unit volume per time, which depends mainly on the Brownian motion. The collision efficiency, expressed as the fraction or possibility

of those encounters leading to droplet flocculation, depends on the nature of the interfacial films and the forces (attractive vs. repulsive) between droplets. The attractive forces come from Van der Waals interaction, depletion interaction, hydrophobic interaction, hydrogen bond and electronic interactions between oppositely charged ionic groups. The repulsive forces come from electrostatic repulsion, steric repulsion and Born repulsion (McClements, 2015). By manipulating droplet-droplet interactions, flocculation can be prevented.

Flocculation is separated mainly into three types depending on their mechanisms: depletion flocculation, bridging flocculation and charge annihilation flocculation (Fellows & Doherty, 2006). Only depletion flocculation is discussed here. Depletion interaction often happens in a mixed system of small and large objects. In emulsion systems, the big objects are likely to be oil droplets and the small objects could be proteins, polysaccharides, etc. As shown in Figure 2-3, when the distance between two oil droplets (yellow colour) is smaller than the diameter of the small objects (blue colour), it will form an exclusion zone where the small objects cannot enter. Therefore, the concentration of the small object will be higher in region B than in region A, which causes an osmotic pressure difference in region A and B. Consequently, the pressure difference is the driving force that causes aggregation of the oil droplets.

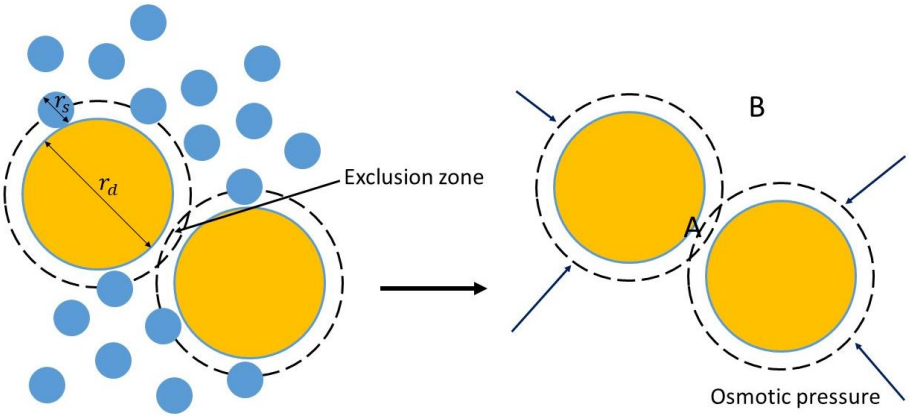


Figure 2-3 Schematic presentation of depletion flocculation

The depletion attractive force can be calculated based on Equation 2-3 (McClements, 2000):

$$\omega_{dep} = -\frac{3kT}{2}\frac{cR_v}{\rho}\left(1 + \frac{1}{2}\frac{cR_v}{\rho}\right)\left(\frac{r_d}{r_s} + \frac{2}{3}\right) \qquad \textbf{Equation 2-3}$$

Where, $\boldsymbol{\omega_{dep}}$ represents depletion attractive force; c represents the concentration of the small object in aqueous phase; ρ represents the density of the small object; r_d represents the radius of the big droplet and r_s represents the radius of the small object. R_v can be calculated based on Equation 2-4:

$$R_v = \frac{4\pi r_s \rho N_A}{3M} \qquad \textbf{Equation 2-4}$$

Where, M is the molecular weight of the small object and N_A is the Avogadro number. According to Equation 2-3, depletion flocculation can be prevented by reducing the size of the big object or decreasing the aqueous concentration of the small object.

2.5 Emulsion gels

An emulsion gel denotes a complex colloidal system which exists as both an emulsion and a gel (Dickinson, 2012). Therefore, it requires both emulsifiers and gelling or thickening agents during formation. They can be either the same or different components, and this has a large impact on emulsion gel properties. Proteins, especially whey proteins as gelling agent are discussed here.

Emulsion gels can be separated into two types ideally, depending on their structural arrangements: 1) emulsion-filled protein gel (Fig. 2-4 a); 2) protein-stabilized emulsion gel (Fig. 2-4 b) (Dickinson, 2012). In reality, an emulsion gel is usually a mixture of those two types. In emulsion-filled protein gels (Fig. 2-4 a), emulsion oil droplets are embedded in protein gel matrix, thus their solid-like rheological properties

predominantly depends on protein gel matrix properties. However, oil droplet size has a large impact on gel strength: if oil droplets are small, they can be incorporated in protein gel network, therefore the gel becomes stronger (Fig. 2-5 a); on the other hand, if oil droplets are large, they act as default to the gel network, therefore the gel becomes weaker (Fig. 2-5 b).

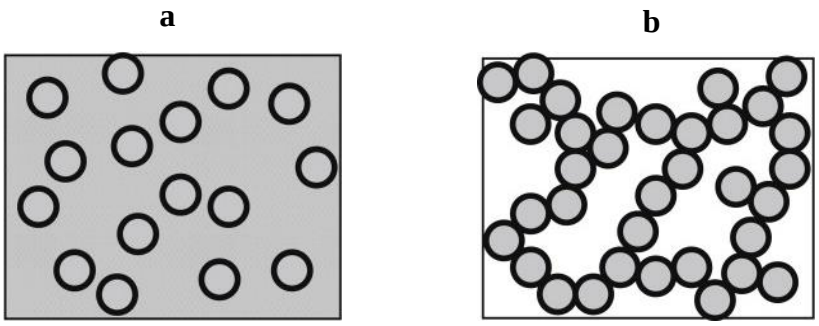


Figure 2-4 Schematic presentations of two idealized models of an emulsion gel: a) emulsion-filled protein gel; b) protein-stabilized emulsion gel (Dickinson, 2012)

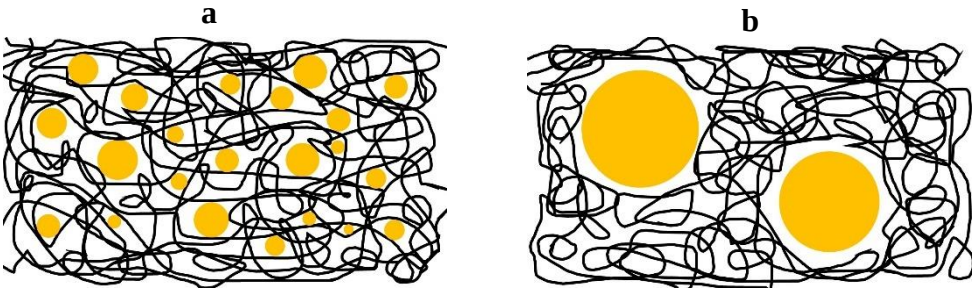


Figure 2-5 Schematic presentations of emulsion-filled protein gel with: a) small oil droplets incorporated in protein gel network; b) large oil droplets not incorporated in protein gel network

In protein-stabilized emulsion gels, protein-coated oil droplets are aggregated either randomly or at a certain angle, therefore form a space-spanning network (Fig. 2-4 b). As a type of particulate gel, their rheological properties mainly depend on the network properties of aggregated oil droplets (Dickinson, 2012). For instance, the shifts in pH or ionic strength will change the surface charge of protein-adsorbed oil droplets. As a consequence, it will affect how oil droplets approach each other and therefore affect the

network thickness and strength: when the surface charge is low, electrostatic repulsion between oil droplets is low, so they can approach each other easily at a random angle, the network is closely packed and strong; on the other hand, when the surface charge is high, electrostatic repulsion between oil droplets is high, and they can only approach each other at a certain angle, the network is weaker as there is less oil droplets aggregated per unit volume (Fig. 2-6).

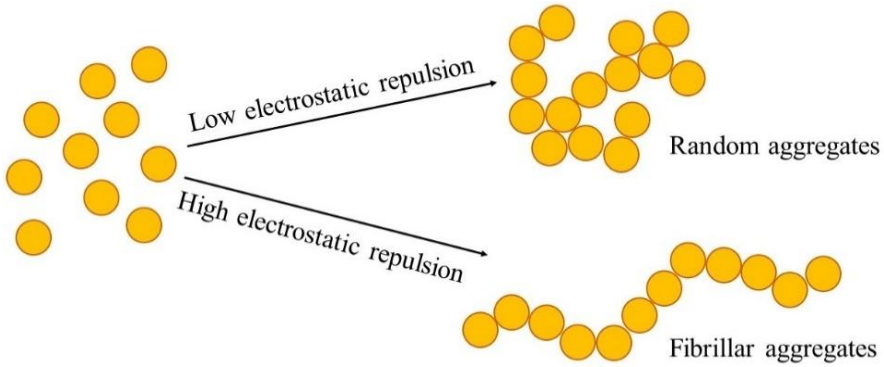


Figure 2-6 Schematic presentations of protein-stabilized emulsion gel formed with low/high electrostatic repulsion between protein-coated oil droplets

2.5.1 Emulsion gel formation

The first step of emulsion gel formation is preparing a liquid emulsion, usually by high-pressure homogenization or high-shear mixing. A few factors are considered to characterize an emulsion: oil content, droplet size distribution, emulsifier type and concentration (Dickinson, 2012). The emulsifier can be proteins (e.g. sodium caseinate, whey protein isolate), or small-molecule surfactants (e.g. monoglycerides, polysorbates and sugar esters).

Then, the emulsion gel is generated by either gelling the continuous phase or aggregating the oil droplets or acting through both (Dickinson, 2012). Even by concentrating the emulsion oil droplets by centrifugation could form a dense protein-

stabilized emulsion with solid-like properties (Dimitrova & Leal-Calderon, 2001). When whey protein isolate is presented as gelling agent, the methods for gel formation are usually through heat treatment (McClements, Monahan, & Kinsella, 1993; Yost & Kinsella, 1992, 1993), acidification (Boutin, Giroux, Paquin, & Britten, 2007; Ye & Taylor, 2009), enzyme treatment (Dickinson & Yamamoto, 1996; Yamamoto & Dickinson, 2004) or addition of calcium salts (Liu & Tang, 2011; Sok Line, Remondetto, & Subirade, 2005).

2.5.2 Rheological properties of emulsion gels

An emulsion gel is a soft solid that predominantly has an elastic response to an applied force, it can store energy and won't yield immediately (Walstra, 2002). Therefore, by rheological definition, an emulsion gel has a loss tangent value smaller than 1 (i.e. tan $\delta = G''/G' < 1$; G' denotes the shear storage modulus, G'' denotes the shear loss modulus).

The rheological properties are important in production, storage and consumption of an emulsion gel (such as yogurt, cheese, jelly, etc.). For instance, during gel formation, both shear storage and shear loss moduli don't stay invariably. Starting from a liquid emulsion, G' is lower than G''; after gel formation, G' is higher than G''. There is a point where it turned from a viscous liquid into a soft solid. Therefore, to have a closer understanding on the transaction mechanisms, it is essential to measure the values of both moduli as a function of time (by small strain oscillatory shear rheometry). By manipulating the speed towards the transaction and the final G' values, it is able to generate products with controlled rheological properties.

Apart from small deformation properties, large deformation properties play an important role as well, especially during consumption, on sensory perceptions and oral

processing behaviours (van Vliet, van Aken, de Jongh, & Hamer, 2009). Emulsion gel samples are usually compressed uni-axially and several parameters are measured. These mechanical properties can then represent or predict sensory texture properties. For instance, young's modulus represents the stiffness, fracture stress represents the strength, fracture strain represents the elasticity, and specific work of fracture represents the toughness of the sample.

2.5.3 Active vs. inactive fillers

In emulsion-filled protein gel systems, oil droplets are embedded in protein gel matrix (Fig 2-4 a). There are a few ways of how the filler particles (i.e. oil droplets) influence the rheological properties of the emulsion gel: the concentration of the filler particles, the mechanical properties of the filler particles and the nature of the filler-matrix interaction (Luo, Ye, Wolber, & Singh, 2020; Tolstoguzov & Braudo, 1983; van Vliet, 1988). Accordingly, it is possible to categorize the filler particles as either active or inactive based on their contribution to network rheology (Ring & Stainsby, 1985). Active filler particles have strong interactions with gel matrix and contribute to gel strength; inactive filler particles have no interactions with gel matrix and act as defects to the network leading to monotonically decreased gel strength (Jampen, Britt, Yada, & Tung, 2001).

Chen and his co-authors have investigated the effects of active versus inactive filler particles on the rheological properties of the emulsion gels (Chen & Dickinson, 1998a, 1999; Chen, Dickinson, & Edwards, 1999; Chen, Dickinson, Langton, & Hermansson, 2000; Dickinson & Chen, 1999). The results indicate that the filler particle- matrix interaction was an important factor determining gel strength. When whey proteins were used as both emulsifying and gelling agent, the proteins covering the oil droplets have strong interactions with protein gel matrix through hydrophobic interactions, hydrogen

bonds and disulphide bonds. The protein-coated oil droplets are considered as active filler particles and promote gel strength (Fig. 2-7 a). When small-molecule surfactant Tween 20 and whey proteins were both present (surfactant / protein molar ratio = 15), oil droplets will be covered by non-ionic Tween 20, thus have no interactions with protein gel matrix. Therefore, Tween 20-coated oil droplets would act as 'slippery' materials in gel network and energy would be dissipated during shearing, thus decrease gel strength significantly (Fig. 2-7 b) (Chen & Dickinson, 1999).

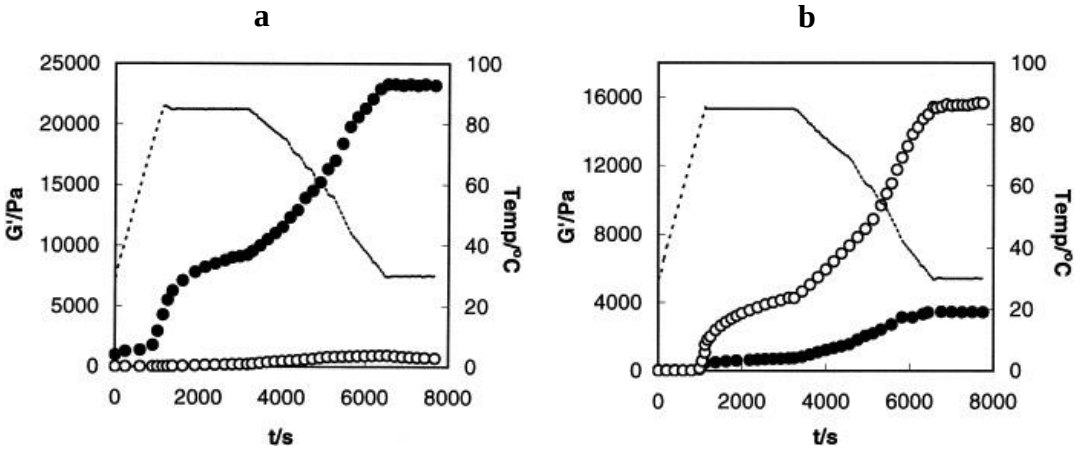


Figure 2-7 Development of elasticity of pure protein gel (○) and emulsion gel (●) during thermal processing. a) Protein gel: 9 wt% WPI, pH 7; emulsion gel: 9 wt% WPI in aqueous phase, 45 vol.% triolein oil, pH 7. b) Protein gel: 7 wt% WPI + 7 wt% Tween 20, pH 7; emulsion gel: 7 wt% WPI + 7 wt% Tween 20 in aqueous phase, 45 vol.% triolein oil, pH 7. Storage modulus G' (1 Hz) plotted against time. Dashed line shows changing temperature (Chen & Dickinson, 1999)

Dickinson and Chen (1999) also reported the effect of oil volume fraction on elasticity of emulsion gels containing active or inactive filler particles (Fig. 2-8). The results indicate that when oil volume fraction increased, the impacts of active fillers on enhancing gel strength or inactive fillers on reducing gel strength become greater.

With the development of confocal laser scanning microscopy technology in recent decades, Kerstens, Murray, and Dickinson (2006) were able to observe flocculation of β-

lactoglobulin-stabilized emulsions with the presence of non-ionic surfactant Tween 20 or excess free protein in aqueous phase. Tween 20 was not able to replace adsorbed protein at room temperature; however, during heating (from 25 to 85 ℃) Tween 20 displaced protein from the interface.

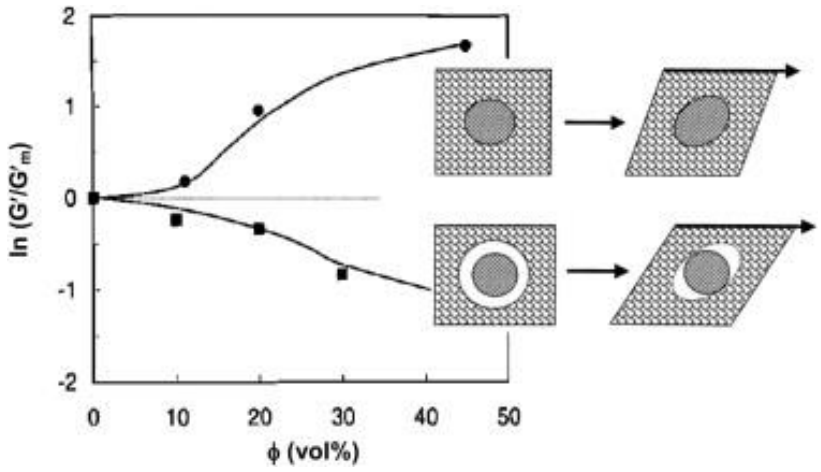


Figure 2-8 Effects of active fillers (●) and inactive fillers (■) on elasticity of heat-set emulsion gels. The logarithm of G'/G'$_m$ is plotted against oil volume fraction, where G' is the shear storage modulus of the emulsion gel and G'$_m$ is the shear storage modulus of the gel matrix. Active filler: 12 wt% WPI in aqueous phase, pH 7; inactive filler: 6 wt% WPI + 6 wt% Tween 20 in aqueous phase, pH 7 (Dickinson, 2012)

2.6 Upper gastrointestinal tract

2.6.1 Mouth

2.6.1.1 Oral cavity

Figure 2-9 shows the anatomic diagram of a human oral cavity. The general features and functionalities of the human mouth are the same; however, the differences among individuals should not be underrated. Factors including race, age, sex, health status and so on would make unneglectable variations for individuals (Chen, 2009). These variations are also part of the reasons why it is difficult to universally quantify food

texture perceptions (Chen, 2009). For instance, the size of the oral cavity differs significantly for individuals, therefore oral capacities in taking foods also differ. Medicis and Hiiemae (1998) indicated that adult males and females could take a normal mouthful of 30.5 ± 10.2 g and 25.2 ± 8.1 g of water, respectively.

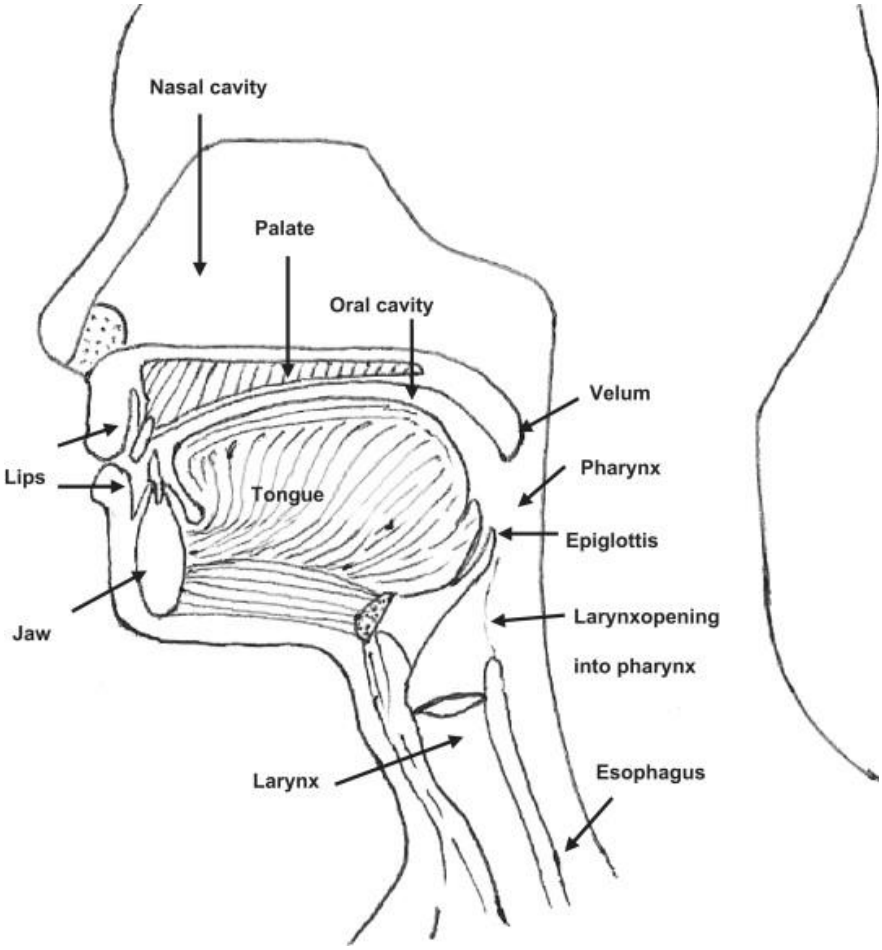


Figure 2-9 The human oral organ (Chen, 2009)

2.6.1.2 Teeth

There are 32 teeth in the mouth of a healthy adult, divided evenly between two jaws. Depending on their locations and functions, teeth can be classified into incisors, canine and molars (Chen, 2009). The incisors are located at the front, mainly initiate the first bites from a food by cutting; food particles are further disintegrated by premolars located between posterior molars and canine (Fig. 2-10).

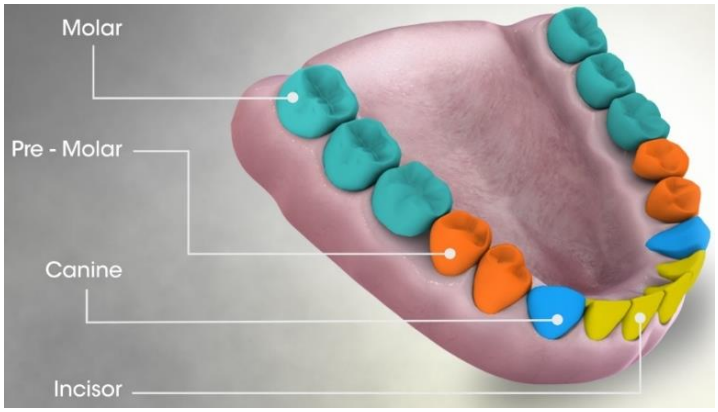


Figure 2-10 The teeth in human mouth (Wikimedia Commons, 2020a)

2.6.1.3 Oral surfaces

The oral surface consists of the surfaces of the tongue, lips, cheeks, palate and teeth, with a total average value of about 214.7 cm^2 for adults (Collins & Dawes, 1987). There is a mucous layer covering the oral surface, composing of 95 – 99.5% water and 0.5 – 5% mucins. The mucous layer has a thickness of around 40 μm (Wolff & Kleinberg, 1998), it functions as a protective barrier against harsh factors such as bacteria and pH, and lubricates the oral surfaces (van Aken, Vingerhoeds, & de Hoog, 2007). Taste buds are detected in the papillae of the tongue, which consist of clusters of receptor cells (Bear, Connors, & Paradiso, 2015). Four main types of papillae are identified: the papillae circumvallate, papillae fungiformis, papillae foliate, and papillae filiformis. They all contain taste buds except the papillae filiformis. The function of the papillae filiformis is to provide certain roughness required for food processing to the tongue surface.

2.6.1.4 Saliva

Saliva, a clear liquid comprising of 99.5 % water, 0.3% proteins and 0.2% inorganic and trace substances, serves a key role in lubricating the oral surfaces, breaking down and digesting the foods, as well as keeping general oral hygiene (Humphrey &

Williamson, 2001; van Nieuw Amerongen, Bolscher, & Veerman, 2004). More than 1050 proteins and peptides have been identified in salivary fluid, for instance, proteins including mucins, proline-rich glycoproteins; peptides including cystatins, statherin, histatins; and enzymes including α-amylase and lingual lipase. The inorganic substances of the salivary fluid contain common electrolytes such as potassium, sodium, bicarbonate and chloride (van Aken et al., 2007). Healthy human beings have pH value of the natural saliva ranging from 5.6 to 7.6, with an average pH of 6.75 (Jenkins, 1978). Engelen, Fontijn-Tekamp, and van der Bilt (2005) investigated the flow rate of the saliva with 266 human participants. They asked the participants to chew a slice of tasteless parafilm and reported the average flow rate to be 0.45 ± 0.25 ml/min under unstimulated state and 1.25 ± 0.67 ml/min under stimulated state. The flow rate of the saliva is also influenced by the time of day, the serving temperature as well as the type of food presented.

2.6.2 Stomach

2.6.2.1 Anatomy of human stomach

Figure 2-11 shows the anatomy of the J-shaped human stomach that connects the oesophagus to the duodenum. It is separated into four domains including the cardia, the fundus, the body and the pylorus. The top of the stomach that surrounds the opening is called the cardia; the fundus is located on the left of the cardia. The body is the large central area of the stomach. The pylorus is the area connecting the stomach to the duodenum. The pylorus consists of two sub-domains: the pyloric antrum connecting to the body and the pyloric canal connecting to the duodenum. The pylorus and the duodenum communicate through a muscle called the pyloric sphincter (the value), which modulates the emptying of food chyme from the stomach into the small intestine (Tortora & Derrickson, 2018). A three-dimensional model of the human stomach was established

by Ferrua and Singh (2010) for depicting the dimension of the stomach after a typical meal (Fig. 2-12). The maximum width is around 10 cm, the greater curvature is around 34 cm in length, and the pyloric sphincter has a diameter of around 1.2 cm. The maximum volume of food that the stomach can expand to accommodate is around 4 litres (Curtis & Barnes, 1994). The geometry of the stomach is dependent on various aspects, for instance, whether the body is in standing, sitting or lying down position, the type and amount of meal, etc (Liao et al., 2004; Schulze, 2006).

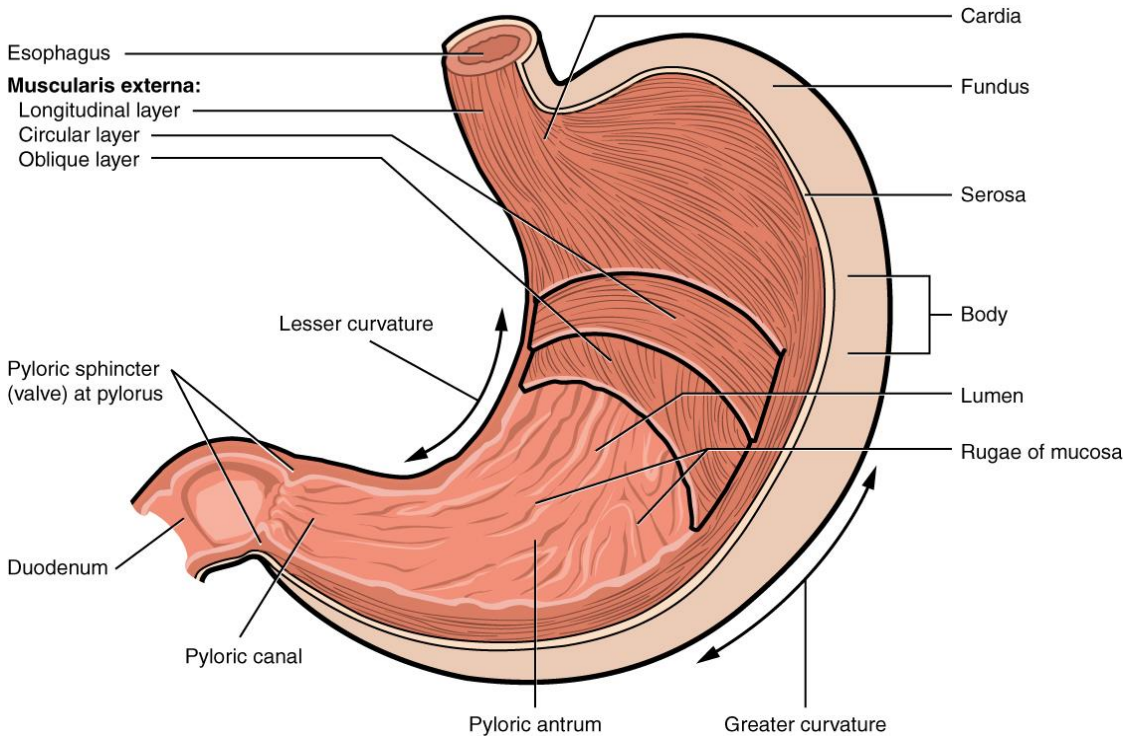


Figure 2-11 Anatomy of the human stomach (Wikimedia Commons, 2020c)

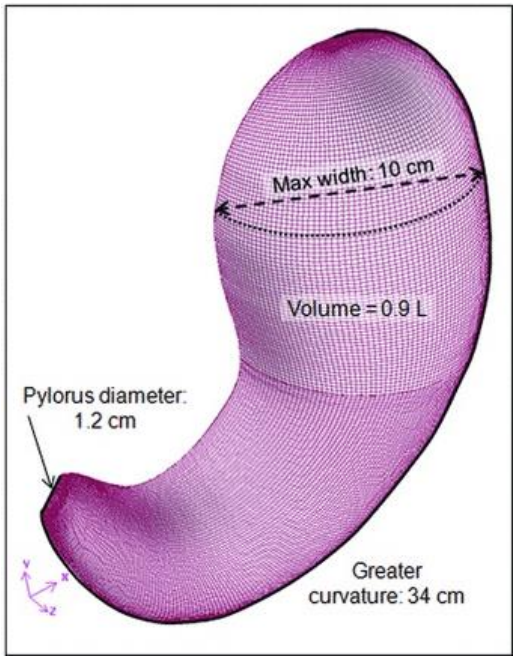


Figure 2-12 Three-dimensional model of an average human stomach (Ferrua & Singh, 2010)

2.6.2.2 Gastric motility and emptying

Once the food bolus goes into the stomach, the fundus and the upper part of the body of the stomach loosen up and hold the food while there is no obvious rise in pressure. In several minutes, the wall of the stomach generates peristaltic movements (i.e. antral contraction waves, ACWs) at a frequency of 3 cycles per minute. The ACWs blend, shear and move the food bolus to the pylorus (propulsion) (Ferrua & Singh, 2010; Ganong, 1995; Marciani et al., 2001c). Indentations of these waves become deeper when they get to the pylorus. The pyloric sphincter then allows certain amount of food particles with sizes smaller than 1 mm to cross and be delivered into the duodenum as the antral contraction waves progress over the middle of the antrum. The pylorus closes when the antral contraction waves reach it and the terminal antrum produces a retro contraction force to push the contents back into stomach (Bilecen, Scheffler, Seifritz, Bongartz, & Steinbrich, 2000; Malagelada & Azpiroz, 2010; Schulze, 2006; Tortora & Derrickson,

2018). The repeated propulsion / retropulsion movements grind the food particles and the functions of the digestive enzymes and acids further reduce the food particles into smaller pieces.

2.6.2.3 Gastric secretion

The gastric gland, as shown in Figure 2-13, has a daily secretion of about 2.5 L gastric juice. The parietal cells secrete hydrochloric acid. The chief cells secrete pepsinogen, which is the inactive precursor of pepsin. The G cells secrete gastrin. Gastrin is a hormone that stimulates the secretion of gastric juice and the growth of the mucosa of the GI tract (Ganong, 1995). The gastric pH in fasting state is about 1.5 – 2.0 (Arora, Ali, Ahuja, Khar, & Baboota, 2005; Ibekwe et al., 2008; Ulleberg et al., 2011). Fordtran and Walsh (1973) investigated the gastric acid production by 6 healthy human participants after consuming a normal meal which includes toasted bread (two pieces) with butter (one teaspoon), cooked steak (5 ounces) and water (360 ml). The quantity of acid required to maintain the gastric content at pH 5.5 was measured. They reported that the acid was secreted at a basal rate of 0.7 mmol/h. The maximum acid secretion rate, 31.8 mmol/h, was reached after 1.5 h of food ingestion. The rate decreased to 7.7 mmol/h at 4 h of food ingestion. The average rate of acid secretion was about 16.5 mmol/h during this 4 h of gastric digestion. Feldman, Cryer, McArthur, Huet, and Lee (1996) recruited 206 healthy human participants and investigated their gastric acid secretion provoked by subcutaneous injection of 6 μg/kg pentagastrin. They reported that the maximum gastric acid secretion rate was about 30 mmol/h for young (18-34 years old) and middle-aged (35-64 years old) adults and 19 mmol/h for the elderly (65-98 years old). The approximately 30% reduction in acid secretion for the elderly groups was attributed to

histology, infection of *Helicobactor pylori*, etc. Age did not affect acid secretion independently.

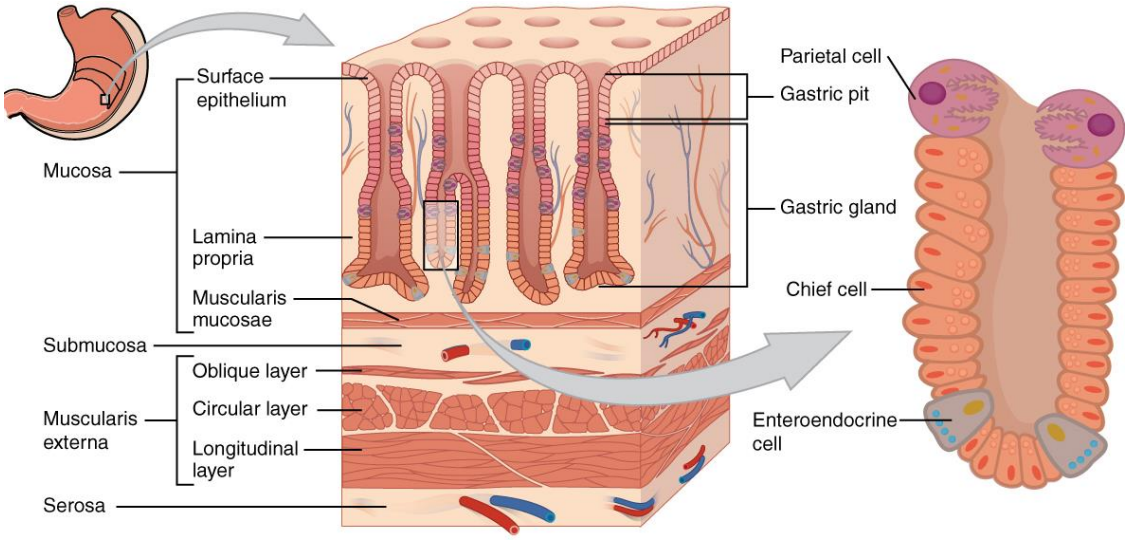


Figure 2-13 Histology of stomach (Wikimedia Commons, 2020b)

As for the secretion of pepsin, Feldman et al. (1996) reported the maximum pepsin secretion stimulated by administrating 6 µg/kg pentagastrin was around 68100 U/h for 186 young and middle-aged adults (6 µg/kg pentagastrin reached maximal effect). The gastric content was presumed to be 500 ml without emptying during the 4 h of digestion. With increasing digestion time, pepsin activity per ml of gastric content gradually increased; the pepsin activity per ml gastric content at 1, 2, 3 and 4 h of digestion was reported to be 136, 272, 408 and 544 U/ml respectively. There was a reduction of about 40% in pepsin output for the elderly groups. Age was a critical factor causing this reduction. The pepsin output in the human stomach usually reaches its maximum in 1 hour after food consumption with a value ranging ~ 180 – 420 mg/60 min, and diminishes after 120 min to the base level (~ 5 mg/60 min) (Malagelada, Go, & Summerskill, 1979; Malagelada, Longstreth, Summerskill, & Go, 1976). Kalantzi et al. (2006) investigated the evolution of pepsin concentration during 3.5 h of digestion by 20 healthy human

participants averaged at 25 years old (16 men and 4 women). After ingesting 500 ml of Ensure Plus®, the concentration of pepsin was found to be 0.26 mg/ml at 0.5 h and raised to 0.58 mg/ml at 3.5 h. øktedalen, Nesland, Opstad, and Berstad (1988) found the concentration of pepsin was around 0.3 mg/ml during fasting state. After consuming a meal containing protein (12 g), carbohydrate (62 g) and fat (21 g), the concentration of pepsin reduced to nearly zero in the first 20 min and then slowly raised to 0.55 mg/ml at 3 h. Tang, Wolf, Caputto, and Trucco (1959) collected fresh gastric juice from several human participants, lyophilized, re-dissolved in water at pH 2.0, and then measured the pepsin activity, which was found to be 843 U per mg protein. Ulleberg et al. (2011) also reported the pepsin activity to be 2220 U/mg measured from fresh gastric juice collected from 18 human participants. The unit used here for pepsin activity is called Anson unit, and the definition is that one unit will produce a ΔA_{280nm} of 0.001 per minute at pH 2.0 at 37 °C measured as TCA-soluble products using haemoglobin as substrate (Minekus et al., 2014).

2.6.3 Small intestine

Small intestine is the primary site for food digestion and nutrient absorption. It absorbs the majority of the liquids from diet intake and secretions during gastrointestinal digestion (9 L) and only permits 1 – 2 L to go through to the colon (Ganong, 1995). The small intestine is comprised of three distinct regions: the duodenum, jejunum and ileum. The duodenum is about 25 cm in length; the jejunum and ileum are about 260 cm in length (Ahrens Jr, Blankenhorn, & Hirsch, 1956). The inner wall, or mucosa, of the small intestine is covered with villi projecting into the intestinal cavity. Each villus has many microvilli projecting from its surface (Ganong, 1995). The villi and microvilli significantly enlarge the effective internal surface area of the small intestine.

The intestinal motility is comprised of three kinds of motions: segmentation contractions, peristaltic waves and longitudinal contractions. Segmentation contractions are initiated from the contractions of the circular muscle layer. They emerge frequently along the small intestine and then vanish and then are substituted by another contraction. Peristaltic waves, with a frequency of 8 – 12 cycles per minute, move the intestinal contents forward to the colon (Ganong, 1995). The longitudinal contractions are relatively prolonged contractions. They mainly resulted from the contraction and relaxation of the longitudinal muscle layer (Fullard, Lammers, Wake, & Ferrua, 2014). It has been reported that the segmentation contractions and the longitudinal contractions slow down the transit of intestinal contents.

The gastric chyme, once enters the duodenum, is immediately mixed with bile, pancreatic juice (pH 8.0) and intestinal juices (alkaline or neutral), and the pH of the mixture achieves ~ 6 to 7. The pH of the food chyme is nearly neutral and rarely alkaline when it reaches the jejunum (Ganong, 1995). Table 2-3 presents the principal enzymes in the pancreatic juice.

Table 2-3 Principal enzymes in the pancreatic juice (Ganong, 1995)

Enzyme	Substrate	Catalytic function or products
Trypsin	Proteins or polypeptides	Cleave peptide bonds on carboxyl sides of basic amino acids
Chymotrypsin	Proteins or polypeptides	Cleave peptide bonds on carboxyl side of aromatic amino acids
Elastase	Elastin, some other proteins	Cleave peptide bonds on carboxyl side of aliphatic amino acids
Carboxypeptidase A	Proteins or polypeptides	Cleave carboxyl terminal amino acids that have aromatic or branched aliphatic side chains
Carboxypeptidase B	Proteins or polypeptides	Cleave carboxyl terminal amino acids that have basic side chains
Colipase	Fat droplets	Facilitate the exposure of the active sites of pancreatic lipase
Pancreatic lipase	Triglycerides	Mono- or di-glycerides, fatty acids
Bile salt-acid lipase	Cholesteryl esters	Cholesterol
Pancreatic α-amylase	Starch	Hydrolyses 1:4 α linkages

Bile salts are essential biological surfactants, which are synthesized in the liver from cholesterol and kept in the gallbladder between meals (Hofmann & Roda, 1984; Verde & Frenkel, 2010). The basic structure of bile salts is a tetracyclic steroid that is hydrophobic and a bit curvy (Madenci & Egelhaaf, 2010); one acidic group and 1 to 3 hydroxyl groups are attached to the hydrophobic region, which form the hydrophilic face. Bile salts can be different in the position, number and spatial arrangement of the hydroxyl groups and in the conjugated amino acids. The major types are cholate, chenodexicholate and deoxycholate, which account for 50%, 30% and 15% of bile salts respectively (Ganong, 1995; Maldonado-Valderrama, Wilde, Macierzanka, & MacKie, 2011). Bile salts are able to self-assemble into micelles or aggregates with different shapes because of their amphiphilic nature, when they are presented in aqueous solution upon a critical micelle concentration (CMC) (Madenci & Egelhaaf, 2010; Mukhopadhyay & Maitra, 2004). The driving forces are hydrogen bonding and hydrophobic interactions for this self-assembly. The CMC depends on the type of the bile salt, for instance, facial arrangements of the hydrophobic and hydrophilic regions would form small micelles and display high CMCs (Madenci & Egelhaaf, 2010; Maldonado-Valderrama et al., 2011). The micelles can solubilize lipolytic products by storing them in the hydrophobic cores, and deliver them to the enterocytes, where they can be absorbed (Ganong, 1995). In practical, micelle formation in the small intestine is much more complicated because of the action of phospholipids and monoglycerides, where they can take part in and facilitate the formation of micelles as well.

2.7 Caco-2 cell line

The Caco-2 cell line, developed by Dr. Jorgen Fogh and his co-workers in the Sloan-Kettering Institute for Cancer Research in the 1970s, is a continuous line of

heterogeneous human epithelial colorectal adenocarcinoma cells (Fogh & Trempe, 1975). It is originated from a colon carcinoma, however, when cultured under specific conditions, the cells become differentiated and polarized and their phenotype resembles the enterocytes lining the small intestine, both morphologically and functionally (Hidalgo, Raub, & Borchardt, 1989; Pinto, Robine Leon, & Appay, 1983). Over the years, Caco-2 cell line has been widely used in different laboratories over the world and the characteristics of the cells have significantly diverged, which makes it hard to compare results from different labs (Sambuy et al., 2005). Instead of been used as individual cells, Caco-2 cells are commonly used as a confluent cell monolayer, where they are normally cultured on a cell culture insert filter (e.g. Transwell). The cells would differentiate to form a polarized epithelial cell monolayer that resembles the structure and function of the human small intestinal epithelium (Artursson, 1990; Hidalgo et al., 1989).

The renewal of intestinal epithelium involves two phases: 1) proliferation that occurs within crypts; 2) differentiation that occurs during migration of cells from the crypt to the villus tip (Delie & Rubas, 1997). Similar to that, the growth of Caco-2 cells on either plastic or permeable filter is characterized by the proliferation sequence, confluency, and differentiation. Pinto et al. (1983) reported that after seeding the cells at 4×10^4 cells / cm^2, proliferation started after a time lag of 48 h; confluency was reached after 5 days, but proliferation continued until after 9 days when a plateau was reached. According to morphological and functional differentiation, Caco-2 cells in culture can be discriminated into three states: 1) homogeneously undifferentiated (before confluency); 2) heterogeneously polarized and differentiated (day 0 to day 20 after confluency); 3) homogeneously polarized and differentiated (> day 30 after confluency) (Vachon & Beaulieu, 1992). Differentiation can be evidenced by the development of microvilli and tight junction formation that separates basolateral and apical domains. Simultaneously,

cells become polarized with specific expression of proteins and lipids to the basolateral and apical membranes. Enzymatic activities gradually increase during differentiation and reach maximum activities 15 to 21 days after confluency. Differentiation is completed after 25 – 30 days in culture (Delie & Rubas, 1997).

The progressive formation of the Caco-2 cell monolayer can be routinely checked by measuring the integrity of the tight junction, which is represented by the transepithelial electrical resistance (TEER); more reliably, by examining the permeability of paracellular markers like lucifer yellow, PEG 400, Dextran, inulin and mannitol (Mukherjee, Squillantea, Gillespieb, & Shao, 2004). The structure and function of the tight junction is influenced by the presence of substances such as pharmaceutical excipients, fatty acids, hormones and surfactants, and affects paracellular transport pathway of Caco-2 cell monolayer (Anderberg & Artursson, 1993; Chirayath et al., 1998; Lindmark, Kimura, & Artursson, 1998; Smith, Wood, & Dornish, 2004). It should be noted that the pore radius of the tight junction in Caco-2 cell monolayer is about 4.5 Å (Watson, Rowland, & Warhurst, 2001), which is smaller than that of the human intestine, which is about 8 – 13 Å (Fine, Santa Ana, Porter, & Fordtran, 1995).

For any substance to exert an effect on a living organism, it has to be delivered to its site of action. Oral administration is the most common way of administration; any ingested compound must make its way through the gastrointestinal tract to its final target in the body. For essential compounds that are required for maintaining normal functions of an organism, their absorption processes are highly modulated and the interaction of, for example, nutrients and transporter proteins occurs as a fact of life. However, for substances such as bioactive compounds, food additives, or pharmaceuticals, they are recognized as non-essential compounds and therefore normally have limited access to

their sites of action. The investigation of their absorption mechanism is critical and has drawn increasing interest (Nigsch, Klaffke, & Miret, 2007). Four routes have been proposed for the absorption of such compounds by the intestinal epithelium: the passive transcellular route, the passive paracellular route, the active carrier mediated route and the transcytosis route (Fig. 2-14) (Artursson, Palm, & Luthman, 2012). The Caco-2 cell monolayer has been used to study all four routes. For instance, Yu and Huang (2011) identified the absorption of solubilized curcumin using Caco-2 cell monolayers to be passive diffusion (route 1); Xu, Hong, Wu, and Yan (2019) reviewed the absorption mechanisms of various bioactive peptides derived from food proteins and the absorption by opening of tight junctions (route 2), transport proteins (route 3), and transcytosis (route 4) have been reported in literature (Gleeson, Brayden, & Ryan, 2017; Gleeson, Frías, Ryan, & Brayden, 2018; Hong, Tanaka, Koyanagi, Shen, & Matsui, 2016; Vij, Reddi, Kapila, & Kapila, 2016).

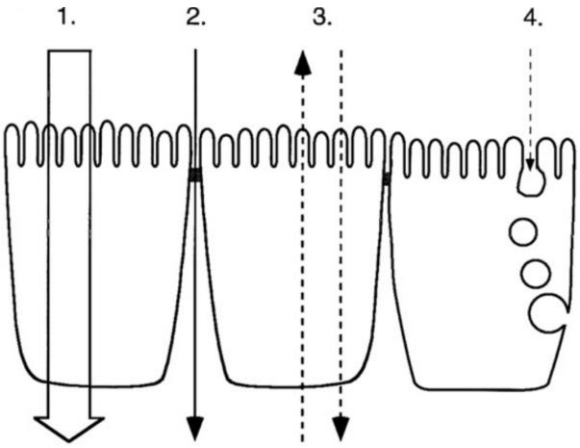


Figure 2-14 Schematic diagram of an intestinal epithelium and four routes of nutrient uptake: 1. the passive transcellular; 2. the passive paracellular; 3. the active carrier-mediated transcellular; 4. the transcytosis route (Artursson et al., 2012)

Apart from absorption studies, Caco-2 cell monolayers can also be used to test the effect of bioactive food compounds on intestinal inflammation (Iftikhar, Iftikhar, Zhang,

Gong, & Wang, 2020; Ponce de León-Rodríguez, Guyot, & Laurent-Babot, 2019). The intestinal epithelium, comprising of different cell types such as enterocytes, M cells, goblet cells, immune cells, etc. functions as a permeable barrier that plays an important role in modulating solute and fluid exchange and has direct influences on nutrient uptake and transportation. The mucosal integrity is challenged daily by external factors in the luminal environment, losing intestinal barrier function could result in nutritional and clinical consequences such as malnutrition and inflammation (Ponce de León-Rodríguez et al., 2019). Inflammation is a non-specific, harmonized reaction of the immune system, which can be stimulated by various internal (e.g. cell lysis) and external (e.g. microbial) factors (Iftikhar et al., 2020). The initial inflammatory responses include activation of macrophages, which results in the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL-1β), tumour necrosis factor α (TNF-α) or anti-inflammatory factors such as interleukin-10 (IL-10) (Kagnoff, 2014). The pro-inflammatory / anti-inflammatory effects of the bioactive food components on intestinal epithelium can then be measured in an *in vitro* model by quantification of these cytokines produced by the Caco-2 cell monolayers. For instance, Romier-Crouzet et al. (2009) studied the anti-inflammatory effect of various natural polyphenolic extracts (sugar cane, grape seed, cocoa, mangosteen, oak and pomegranate), and found that extracts from oak and pomegranate could reduce IL-8 synthesis in Caco-2 cells by 10.3 and 6.7-fold respectively.

2.8 Sensory evaluation techniques

As quoted from the book 'Sensory Evaluation Practices' (Stone, 2012), "*sensory evaluation is a scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by the*

senses of sight, smell, taste, touch and hearing." It is a young and Interdisciplinary science developed since 1950s, and has gradually established a formalized, structured, codified and credible methodology (Meilgaard, Civille, & Carr, 2007b).

Sensory tests are classified into three types based on their purposes: discriminative analysis, descriptive analysis and affective analysis (Meilgaard, Civille, & Carr, 2007e). Discriminative analysis measures the differences between samples or the thresholds value of certain attributes; descriptive analysis measures both qualitative and quantitative sensory aspects of a product thus build up the product flavour/texture profile; affective analysis measures consumer acceptability or preference of certain sensory attributes or products. The first two analyses are objective and require trained sensory panel only while affective analysis can use both trained and untrained panel. Only discriminative analysis is reviewed here.

Stevens (1957) developed the famous power law function (Equation 2-5) stating that the perceived sensation magnitude grows as a power function of the stimulus intensity:

$$R = kC^n \qquad \textbf{Equation 2-5}$$

Where, R is the subjective responses from human panels (i.e. perceived sweetness); C is the objective measurements (i.e. sugar concentration); n is the power factor that depends on the characteristics of the attribute being judged; k is a constant that replies on the units in which R and C are measured.

Based on Stevens' power law, a scaling technique called magnitude estimation is developed (Doty & Laing, 2003; ISO, 1999; Meilgaard & Reid, 1979; Moskowitz, 1977). The general rule using magnitude estimation is as following: panellists will firstly receive a reference sample with an assigned rating number of a target sensory attribute; then they

are asked to give all subsequent ratings of subsequent samples in proportion to the rating of the reference sample, and also they cannot give zero or negative ratings. For instance, if a test sample appears to be four times as strong as the reference, the assigned rating of this test sample should be four times the rating assigned to the reference (Meilgaard, Civille, & Carr, 2007c).

The advantages and disadvantages of magnitude estimation is discussed by Pangborn (1984). Data generated by magnitude estimation have ratio properties, similar to the standard measurements such as length, weight, etc. Also, magnitude estimation method is still credible for tests with 20 or less panellists. However, magnitude estimation is less suitable for scaling degree of preference (Pangborn, Guinard, & Meiselman, 1989). Therefore, when using magnitude estimation technique in discriminative analysis, it is essential to train panellists to think in ratio terms in order to produce reliable and reproducible results.

2.8.1 Selection and training of panellists

According to the guidelines provided by ASTM (1981) and ISO (1993) for selection and training of sensory panellists, several steps should be considered. First of all, potential participants should be recruited based on their interests, health conditions and availabilities on the time and location of the tests. Then the researchers should design necessary screening tests to be carried out by potential participants to determine their eligibilities. For discriminative analysis, four screening tests are commonly used including matching test, detection test, discrimination test and attribute intensity ranking test (Meilgaard, Civille, & Carr, 2007d). After screening, successful participants should have the ability to discriminate and describe a certain sensory attribute among a

complicated mixture of all kinds of sensory impressions, and the ability to identify the intensity differences of the same sensory attribute among different samples.

2.8.2 Sensory bias affecting the verdicts of the results

Gregson (1963) indicated that human sensation of the real world is not a passive, but an active and selective process. People tend to extract those elements that they can easily access and understand and ignore the others when they are in a complicated situation. However, in sensory analysis, participants must be objective and understand the characteristics that they are measuring instruments (Meilgaard, Civille, & Carr, 2007a). Therefore, in order to obtain reliable sensory results, it is essential for the researchers to understand physiological and psychological factors influencing sensory perceptions and avoid or minimize a number of sensory bias inherent in the handling of participants, the text of questionnaires and the presentation of samples (Amerine, Pangborn, & Roessler, 2013; Lawless & Heymann, 2010; Pangborn, 1979; Poste, Mackie, Butler, & Larmond, 1991).

2.8.2.1 Physiological factors

Four physiological factors influencing sensory verdicts are discussed here including adaptation, enhancement, suppression and synergy. According to the definitions given by Meilgaard et al. (2007a), adaptation is a decrease in sensitivity to a given stimulus caused by continuous exposure to that stimulus or a similar one; enhancement is the effect of the presence of one component enhancing the perceived intensity of another component; suppression is the effect of the presence of one component decreasing the perceived intensity of other components; synergy is the effect of the presence of one component enhancing the perceived combined intensity of two components, and that the

mixture is perceived with higher intensity than the sum of the intensities of the individual components.

It is essential to take these factors into consideration when designing sensory tests. For instance, adaptation effect could be avoided by having time intervals and/or carriers (zero set) between samples to allow recover from the previous sample (O'Mahony, 1986).

2.8.2.2 Psychological factors

Seven psychological factors are discussed here including subconscious bias, stimulus error, halo effect, logical error, order of sample presentations, mutual suggestion and lack of motivation (Meilgaard et al., 2007a).

Some errors can be easily avoided if the researchers design and carry out the tests carefully, such as subconscious bias, logical error, stimulus error and halo effect. Subconscious bias exists when samples are coded as '1, 2, 3…' or 'A, B, C…', especially in ranking test, panellists tend to rank samples in the order of the alphabet rather than perceived intensities. Stimulus error happens when irrelevant information is given to the panellists. For instance, panellists tend to give lower ratings to those wines that they have been told to be cheaper (Amerine et al., 2013). Logical error occurs when panellists associate one characteristics of the sample to another. For instance, panellists tend to perceive a yellow-coloured jelly to be lemon flavour or a darker beer to be more bitter, since they associated the colour of the sample to the perceived flavour. Halo effect occurs when two or more attributes of a sample need to be evaluated, the ratings of each attribute will then tend to influence each other. The above errors can be eliminated by using blind coding, giving only necessary information to the panellists, using coloured light (red, yellow, green or combination) in the sensory room to neutralize colour differences and

presenting separate sets of samples for evaluation for any particular variable (Meilgaard et al., 2007a).

On the other hand, some errors cannot be eliminated, but their effects can be reduced and controlled. These errors include bias caused by the order of sample presentations, mutual suggestion and lack of motivation. At least five types of bias are identified in the order of sample presentations: group effect, pattern effect, contrast effect, error of central tendency and positional bias. These effects can be minimized by using a randomized, balanced order of sample presentation. Mutual suggestion error exists when the experimental environment allows the panellists to influence each other. This can be minimized by separating panellists in individual sensory booths and avoiding unnecessary discussion between them. Lack of motivation is common when panellists are supplied with deficient information about the test, and/or when they lose interest, etc. This can be minimized by building a well-understood and well-defined test situation, running the test in an efficient and controlled manner and providing the panellists with a report of the results afterwards (Meilgaard et al., 2007a).

2.9 Disintegration and digestion of solid / semi-solid foods in the human body

2.9.1 Oral processing

2.9.1.1 Oral processing and mastication behaviour

Oral processing is the initial phase for food consumption, not only important for the perception and appreciation of food texture and flavour, but also essential for food digestion as it fragments solid/semi-solid foods into smaller particles safe for swallowing, lubricates foods with saliva and partially digests starch (Chen, 2009; van Vliet et al., 2009). Foods with different characteristics are processed differently. Liquid foods only

stay in the mouth for few seconds before swallowing and do not require chewing; while solid foods require mastication for several cycles for breakdown of foods, texture perception and flavour release. The solid foods are broken down sufficiently by each type of teeth (Fig. 2-10), the cheeks and tongue would also help hold and direct the food particles between molars for mastication (Xu & Bronlund, 2010). The chewing forces applied on the teeth depend on the type of foods being masticated. For single tooth, the force applied also differs from the total force being applied on all the contacting teeth during mastication. For instance, the applied force to any single tooth ranges from 70 N to 150 N when chewing foods like carrots, biscuits and cooked meats (Anderson, 1956); the chewing forces applied on all the contacting teeth fall in the range of 190 N to 260 N (Gibbs et al., 1981).

Oral processing has two major functions: to breakdown food into sufficiently fine particles and mix thoroughly with saliva in the formation of food bolus safe for swallowing (Hutchings & Lillford, 1988; Prinz & Lucas, 1995) and to have sensory perception and appreciation from the food (van der Bilt, 2012). Oral processing is an early step in the process of food particle size reduction and release small molecules available for absorption into the bloodstream. It usually decreases particle size by two to three orders of magnitude before swallowing (Bourne, 2004). Food oral processing is considered as a physiological procedure driven by the central nerve system, as well as a physical procedure regulated by the rheological and geometrical properties of food (Chen, 2009). The physiological variation can be attributed to the differences in age, sex, race, health status, etc. for individuals, which would lead to fluctuations in chewing behaviour. For instance, the size of the oral cavity differs significantly between individuals (Chen, 2009); large variations in biting force have also been reported between different race groups, genders and among people with same ethnicity (Bourne, 2002; Paphangkorakit

& Osborn, 1997). The physical variation can be explained and modulated by food characteristics (Woda, Mishellany, & Peyron, 2006). For instance, Medicis and Hiiemae (1998) investigated the effect of physical properties of foods on oral capacity. The results showed that the quantity of food intake for every normal mouthful significantly decreased from liquids to soft solids and further to hard solids. This could be explained by the fact that oral breakdown and manipulation has become more difficult for solid foods (Chen, 2009).

The process of solid/semi-solid foods in human mouth is divided into three phases: the first-bite, masticatory and residual phases (Brandt, Skinner, & Coleman, 1963; Hiiemae & Palmer, 1999; Lund, 1991). The first-bite, or initial phase usually has only one bite, and can be divided into three individual movements including opening, fast closing and slow closing (Schwartz, Enomoto, Valiquette, & Lund, 1989). After the initial bite, several food textural sensations can be received such as first-bite hardness, brittleness, springiness, cohesiveness, etc. (Brandt et al., 1963). The residual phase, which is swallowing, can be divided into three individual stages: an oral stage, a pharyngeal stage and an esophageal stage. The oral stage starts with the formation of food bolus and ends with the bolus passing to the back of the mouth. The pharyngeal phase and esophageal phase are both reflex processes and pass foods downwards much faster. Swallowing showed no evidence of flavour or texture perception, except the possibility of retronasal aroma stimulation (Buettner, Beer, Hannig, & Settles, 2001).

The masticatory or chewing phase is the main phase for consumption of solid/semi-solid foods. The chewing duration and number of chews vary significantly for foods with different rheological properties as well as for people with difference chewing habits (i.e. slow or fast eaters; not discussed here). Food with high hardness would in general need

more chewing cycles. For instance, Hiiemae et al. (1996) reported the chewing cycles of a mouthful of apple, banana and cookie to be 7, 16 and 19 respectively for the first bolus to form and swallow. Also, they observed the vertical movements of jaw has gradually decreased, which is probably attributed to the size reduction of food particles. However, the chewing speed does not change much for the same person consuming different foods. Wilson and Brown (1997), Fontijn-Tekamp, van der Bilt, Abbink, and Bosman (2004) and Engelen et al. (2005) established a positive correlation between the number of chews and food mechanical hardness. Mioche (2004) also confirmed that perceived tenderness of cooked meat was linearly correlated with muscle activity and mechanical hardness of the food. In addition, many studies have established a relationship between jaw muscle activity and food hardness that food with higher hardness would require enhanced activity of the jaw muscles and longer burst duration of the muscle activity (Agrawal et al., 1997; Foster, Woda, & Peyron, 2006; Peyron, Lassauzay, & Woda, 2002; van der Bilt, Engelen, Abbink, & Pereira, 2007).

2.9.1.2 Sensory perception during oral processing

There are two primary types of sensory perception involved during oral processing: flavour and tactile perceptions. The flavour perception senses the taste and smell of foods; the tactile perception provides sensations on attributes such as mouth-feel, texture, temperature and pain (Spence, 2012). According to the International Standards Organization (ISO 5492, 1992), flavour is defined as a ‘complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting’. The flavour may be affected by tactile, thermal, painful and/or kinaesthetic effects (Delwiche, 2004). In theory, the perceived taste intensity is positively correlated with the concentration of the sensory stimulus presented in the food and can be quite different among individuals

because of their variations in sensitivity/acceptability to the sensory stimulus. The perceived flavour intensity also closely relates to the physical properties of the food, such as hardness and viscosity. For instance, Christensen (1980) studied the effect of viscosity of solution on the perceived sweetness and saltiness and found that the taste perception decreased with increasing viscosity. This effect has both physicochemical and psychological origin. Delwiche (2004) explained at the physicochemical level that increasing viscosity would likely lead to reducing volatility at the air-food surface; from psychological point of view, it can be explained by the cross-modal integration theory (Driver & Spence, 2000), i.e., an increase in one attribute could lead to enhancement or suppression in another attribute. Cerf-Ducastel, van de Moortele, MacLeod, Le Bihan, and Faurion (2001) studied the interactions between gustatory, somatosensory and olfactory stimuli at the cortical level and provided neurological evidence that there was a clear convergent effect of the gustatory, somatosensory and olfactory stimulations, which together constitute the neural substrate for a multimodal representation of sensory information. Because the sensory pathways widely overlap from the very periphery, the gustatory nerves may respond to both taste and temperature or tactile stimulations and the same stimulus may elicit both gustatory and somatosensory responses. Therefore, when a food with a multimodal percept is presented, various sensory stimuli would give signals that share common neural pathways. The signals would be integrated and interpreted by the brain through a logical process; however, the signals would interact and the perception from one stimulus would be affected by that from another (Luo, Ye, Wolber, & Singh, 2019).

Texture is defined as the functional and sensory representation of the mechanical, structural and surface properties of foods perceived by the senses of touch, hearing, vision and kinesthetics. This definition delivers four major concepts: 1) texture is a

sensory property and therefore, it can only be perceived and described by human beings; 2) it is an attribute with multiple parameters; 3) it is derived from the structure of food at the molecular, microscopic and macroscopic levels; 4) it is detected by a few senses, the most vital ones are the senses of pressure and touch (Bourne, 2002; Szczesniak, 1963, 2002). Although texture is a sensory property, it is ultimately dependent on the structure of food. Therefore, it is possible to predict the sensory texture perception of a food by its instrumental measurements and establish correlations among the sensory terms and the mechanical properties. These mechanical or rheological tests are categorized as either fundamental or empirical. The advantage of using fundamental tests such as failure test, dynamic test, etc. to investigate the mechanical aspects of texture is that they are related to theories that elucidate at the molecular and microstructural levels of texture. The mechanical properties may be used to predict variations happening during oral processing (Foegeding, 2007; Foegeding & Drake, 2007).

The interrelationships between food oral processing, rheological properties and sensory perception are well established (Brandt et al., 1963; Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015a; Devezeaux de Lavergne, van Delft, van de Velde, van Boekel, & Stieger, 2015b; Hutchings & Lillford, 1988; Stieger & van de Velde, 2013; Wilson & Brown, 1997; Woda et al., 2006). The rheological properties of food will modify how food is processed in mouth as well as provide texture and sensory appreciations. For instance, an increase in gel strength and melting point resulted in longer chewing duration, lower intensity but more prolonged flavour perception of banana-flavoured gelatine gels (Wilson & Brown, 1997). Moreover, as oral processing is a dynamic process, the physical properties of food and sensory perceptions are not invariable as well.

2.9.2 Food disintegration in human stomach

There are four major roles of the stomach: store food, blend food with gastric juice, grind food particles into smaller sizes and empty food into the duodenum (Davenport, 2010). With both biochemical and mechanical processes, the food bolus further disintegrates into finer particles small enough to be emptied into the small intestine. Mechanical properties of a food product significantly affect food disintegration behaviour during digestion (Lucas et al., 2002). The breakdown of solid/semi-solid foods during gastric digestion is affected by various factors: acid effect, swelling effect, ion effect, enzyme effect, gastric motility effect and food structural effect.

2.9.2.1 Acid effect

Hydrochloric acid is able to hydrolyse proteins or peptides by breaking the peptide bonds. It catalyses the reaction between water and the amide. However, the rate of reaction is fairly low at 37 °C because the access of the hydrogen ions to the peptide bonds is not easy because of the electrostatic repulsion and the steric repulsion of the chain, and the nature of the bond formed between amino acids (Harris, Cole, & Pon, 1956; Zhong, Marcus, & Li, 2005). Synge (1945) reported the half period of several dipeptides under acid hydrolysis (volume ratio 1:1 of glacial acetic acid and 10 N HCl) to be in the range of 2.8 to 190 days. The acid hydrolysis of proteins and peptides is much lower under physiological conditions (i.e. pH range from 1.5 to 7; at 37 °C). Starch acid hydrolysis is much faster as compared to protein acid hydrolysis (Hoover, 2000; Wang, Truong, & Wang, 2003).

2.9.2.2 Swelling effect

Based on the Flory-Huggins theory, there are three factors contributing to the change of Gibbs free energy of a system: elastic retraction of the network, network-solvent mixing, and the difference in the concentrations of the mobile ions inside and outside of the network when an ionic network is placed in a swelling agent (Flory, 1953; Quesada-Pérez, Maroto-Centeno, Forcada, & Hidalgo-Alvarez, 2011). The change in Gibbs free energy can be expressed as:

$$\Delta G = \Delta G_{elastic} + \Delta G_{mix} + \Delta G_{ion} \qquad \textbf{Equation 2-6}$$

These factors that determine the osmotic pressure outside and inside the network would decide the level of swelling. A food particle is considered as an open system, which is mainly composed of polysaccharides, proteins and lipids. It is regarded as a complex, three-dimensional polymer gel. Many studies have reported the swelling of food particles during gastric digestion. For instance, Kong, Oztop, Singh, and McCarthy (2013) investigated the impact of various processing patterns on the swelling of peanuts during simulated gastric digestion and found that raw peanuts had the highest swelling ratio, as compared to peanuts that have been boiled, roasted or fried. By the use of time domain NMR relaxometry, Bordoni et al. (2014) observed the diffusion of gastric fluid into the meat matrix and reported the occurrence of swelling during simulated gastric digestion. Marciani et al. (2001b) also reported that significant swelling (i.e. dilution and volume expansion) of viscous locust bean gum appeared after 36 min of gastric digestion in human participants, examined by magnetic resonance imaging (MRI). Studies have reported a noticeable growth in the swelling of carrot tissue under microscopy under both *in vitro* and *in vivo* gastric conditions (Tydeman et al., 2010a; Tydeman et al., 2010b).

The mechanical properties of food particles are greatly influenced by their swelling equilibrium in gastric juice during digestion. Tanaka et al. (1987) observed that crease generated at the surfaces of the polyacrylamide gels when they were immersed into water. The crease was fine at the beginning, however, with increasing time, the crease coalesced and eventually vanished when the gel reached swelling equilibrium. The authors also tested under a specific condition where the bottom of the gel piece was fixated to a rigid film while the top was free to expand or swell, they recognized the elastic origin of the mechanical instability. The surface would swell first, and this swollen layer is restrained by its inner side that remained not swollen. This opposing behaviour resulted in crease formation during gel swelling, which was reported by others as well (Onuki, 1989; Tanaka & Sigehuzi, 1994; Tanaka, Tomita, Takasu, Hayashi, & Nishi, 1992). The diffusion or penetration of external solvent from out layer into inner layer is a kinetic process, and it indicates the diffusion process of gastric juice into food particles during digestion. This progressive diffusion of gastric juice from outer layer into inner layer was reported in the gastric digestion of various foods (e.g. locust bean gum, peanuts and carrots) under both *in intro* and *in vivo* conditions, using techniques such as MRI (Kong et al., 2013; Kong & Singh, 2009; Marciani et al., 2001b). The mechanical instability could facilitate the disintegration of food particles, especially with the effects of mechanical shearing and enzymatic reaction. This is how food is softened during gastric digestion.

2.9.2.3 Ion effect

The gastric juice starts to mix with the food bolus particles when they enter the stomach, and gradually diffuses from the surface of the food particles towards the centre as digestion moves along. Solid and semi-solid foods are complex systems with

heterogenous structures and are mainly comprised of the three macronutrients: carbohydrates, proteins and lipids. Carbohydrates have four sub-groups: polysaccharide, oligosaccharide, disaccharide and monosaccharide. All carbon atoms except the carbonyl carbon in monosaccharides have a hydroxyl group attached (e.g. fructose, galactose and glucose). For monosaccharides with charged carboxyl groups, one hydroxyl group is attached to each carbon atom (e.g. galacturonic acid, glucuronic acid). Monosaccharides synthesize into disaccharides, oligosaccharides and polysaccharides by glycosidic linkages. Starch is a polysaccharide and non-ionic. Polysaccharides such as pectin, gum arabic, alginic acid, xanthan gum are anionic. Chitosan is cationic polysaccharide. Proteins are charged due to the existence of polar basic amino acids (i.e. histidine, lysine and arginine), polar acidic amino acids (i.e. glutamic acid and aspartic acid), hydroxyl and carboxyl groups. Lipids are hydrophobic, some of which has a charged R group (e.g. sulfolipid, phospholipid). But when lipids are emulsified into oil droplets, they can have charges depending on the emulsifiers. The ion equilibrium between the food particle and the gastric juice in the stomach is similar to the Donnan membrane equilibrium (Donnan, 1924; Flory, 1953).

Driven by the electrical gradient as well as the ion concentration gradient, the mobile ions and counterions would move out or into the food particle until balance is achieved. Food particles behave like semi-permeable membranes that prevent the fixed charged substituents to diffuse out. At equilibrium, the concentrations of the mobile ions would always be higher inside the food particles than outside due to the electrostatic attraction force of the fixed charges presented inside the food particles. It usually requires longer time to achieve ion equilibrium outside and inside of food particles. Vardhanabhuti, Khayankan, and Foegeding (2010) investigated the alternation of pH of a whey protein gel (15% protein) by soaking the gel cubes (1.9 cm x 1.9 cm x 15.3 cm) in acids at

ambient temperature and reported a drastic pH reduction in the first 16 h of soaking and the extent of this pH reduction increased with increasing acid concentration. Longer equilibrium time decreased the pH of the gels only slightly. Ion exchange among gastric juice and food particles is probably faster inside the stomach because of the mechanical shearing, enzymatic degradation and food fragmentation. Ions such as Na^+, Cl^- and H^+, etc. would diffuse into the food particles and significantly change the physicochemical conditions in the food particles, and therefore, influence the disintegration of food particles. Ions diffusing into food particles would shield the charges inside the particles, which may lead to obstruction in the expansion and disintegration of the food particles. On the other hand, the diffusion of H^+ into food particles would result in the ionization of NH_2 to NH_3^+, which means there would be more charges, leading to higher electrostatic repulsion inside the food particles, and therefore leads to the relaxation of the network (Guo, 2015).

2.9.2.4 Enzyme effect

Pepsin, a charged macromolecule, is able to hydrolyse proteins by cleaving peptide bonds, especially the bonds next to hydrophobic and aromatic amino acids (Ganong, 1995). Pepsin hydrolysing of proteins contributes largely to the breakdown of protein-containing food particles during gastric digestion. Based on Donnan law, the diffusion of pepsin into food particles is free if the water channels inside the food particles are sufficiently large (Donnan, 1924). Protein hydrolysis by pepsin facilitates the breakdown of food particles by disrupting their inner structure.

Kerrigan, Mangnall, Read, and Johnson (1991) investigated the effect of acid-pepsin secretion on gastric emptying of solid foods in humans. They used cimetidine to inhibit acid-pepsin secretion and found that the gastric emptying rate was significantly delayed.

This delay was linked to the change in the slope of the emptying profile, rather than the prolonging of the lag phase. Cimetidine was able to reduce pepsin activity and acid secretion, and therefore the solid foods were broken down much slower in stomach and delayed emptying. Similarly, Guo et al. (2015) investigated the effect of pepsin on the disintegration of whey protein emulsion gels during simulated gastric digestion using a human gastric simulator. They also found that the breakdown of emulsion gels without pepsin was much slower, indicating that pepsin plays a vital role in the disintegration of solid/ semi-solid foods during gastric digestion, specifically for those protein-containing foods.

The effect of gastric lipase on the breakdown of foods was also investigated. Shani Levi, Goldstein, Portmann, and Lesmes (2017) studied the effect of gastric lipase on the *in vitro* gastric digestion of liquid emulsion and found that gastric lipase accelerated the degradation of the emulsion and led to significant coalescence after 120 min. With the absence of gastric lipase, smaller droplets were maintained during the initial stages of gastric digestion. The smaller droplets were then emptied into the small intestine and can be effectively digested due to their smaller sizes. On the contrary, in the presence of gastric lipase, droplet sizes rapidly increase, which led to less degradation by bile and pancreatic lipase during intestinal digestion. However, gastric lipase only accounts for a small proportion of dietary lipid digestion, due to a phenomenon called product inhibition (Wilde & Chu, 2011), which means that because the lypolytic products of gastric lipase (i.e. fatty acids and diglycerides) are more surface active than the neutral triglycerides, they accummulate at the interface, sometimes forming solid or crystalline phases (Patton & Carey, 1979) and prevent lipase from gaining access to the interface (Pafumi et al., 2002) and thus inhibiting lipase activity. Therefore, the effect of gastric lipase on the

disintegration of foods has not been widely studied and often neglected in *in vitro* gastric digestion studies.

2.9.2.5 Gastric motility effect

The grinding of food particles in the stomach mainly happens in the antrum and pyloric regions, because the shear forces and flow are at maximum level. Camilleri and Prather (1994) carried out a human study where the participants were asked to swallow a balloon (1.8 cm in diameter) together with either solid or liquid meals. They reported that over a 30 min period, the balloon received an accumulated force of 22 N from antral contraction for emptying solid meals and 6 N for liquid meals. Marciani et al. (2001a) reported that agar gel beads (1.27 cm in diameter) with high fracture strength (> 0.65 N) were disintegrated much slower in the human stomach than the beads with low fracture strength (0.15 – 0.65 N). However, this study did not take into account the impact of oral processing on the breakdown of the beads prior to entering the stomach.

2.9.2.6 Food structural effect

The type of foods, food composition, and processing parameters are the principal factors considered by researchers when carrying out gastric digestion studies. Both *in vitro* and *in vivo* studies have been widely used, as well as the correlation and modelling between *in vitro* and *in vivo* conditions. The importance of carrying out *in vivo* studies are to gather information on what happens in human body, to correlate with *in vitro* conditions and to make sure that *in vitro* studies are indeed representing *in vivo* conditions. The advantages of using *in vitro* studies are that it is able to demonstrate in a more intuitive way of how foods are broken down and digested at each digestion time point, and therefore study the mechanisms; *in vitro* studies are easier to carry out; it is easier to collect and analyse samples, and they are, in general, cheaper. By using *in vitro*

studies, it is able to clearly show how food structure influences its digestion and disintegration behaviour in the stomach.

The effect of food structure on the breakdown behaviour during gastric digestion has drawn increasing interest recently. Both *in vivo* and *in vitro* studies have demonstrated that the disintegration of the food during gastric digestion is greatly affected by its structure. Oral processing of solid/semi-solid foods largely changes the food structure; the food is fragmented into smaller pieces, mixed with saliva and swallowed in the form of a bolus. Bolus properties including rheological properties, particle size, pH, etc. have significant effects on subsequent gastric digestion. Yet, not many studies have taken the impact of oral processing into consideration on the gastric digestion of foods. Nonetheless, the studies where they ignored the effect of oral processing still provided useful information on the structural effect of food on gastric digestion. Kong and Singh (2009) studied the disintegration of various foods representing different food type, composition and structure, including ham, carrot, beef jerky, fried dough, peanut and almond, under simulated gastric conditions, by the mass loss method. Their simulated stomach model was able to mimic the biochemical conditions and mechanical shearing of the human stomach. In general, the rate of breakdown increased with decreasing hardness of the food. For instance, ham (i.e. food with very low hardness) lost half of the weight during the first 5 min of gastric digestion, while peanut (i.e. food with high hardness) required 715 min to loss 50% weight. Siegel et al. (1988) also reported similar findings that foods with different characteristics vary in the rate of disintegration during gastric digestion. Processing of foods also greatly affects their behaviour during gastric digestion. For instance, the process of boiling, frying or roasting significantly changed the structural properties of peanuts. The rate of disintegration of peanuts, which was denoted by the time required for the food particles to loss 50% weight during gastric

digestion, differed among different processing techniques. The time required for 50% weight loss under gastric digestion was 3.6 h, 6.7 h, 8.3 h and 10.7 h for fried, roasted, boiled and raw peanuts respectively (Kong et al., 2013). Kong and Singh (2011) also reported that when the boiling time increased, the rate of disintegration of carrots during simulated gastric digestion gradually increased due to decreasing hardness.

Bornhorst and Singh (2013) investigated the impact of the properties of masticated bread bolus on its disintegration during simulated gastric digestion. The masticated bread bolus was simulated by grinding and then mixed with simulated salivary fluid. It was clear that the original structure of the breads affected the breakdown of the bread bolus during simulated gastric digestion significantly, through modifying the water adsorption ability and the cohesive force of the bread bolus. Barbé et al. (2013) conducted an animal study with six mini-pigs and found that the heated rennet milk protein gel was emptied much slower than the raw rennet milk protein gel. This indicates that a denser microstructure of the heated rennet milk protein gel was able to slow down gel disintegration under gastric digestion.

Apart from above where the food structural effect on its disintegration behaviour during gastric digestion at a macroscopic level is discussed, the impact at molecular level is also the key for understanding food gastric digestion. The rate of protein hydrolysis and the peptide species generated during gastric digestion are determined by the structural properties of the protein aggregates or the protein 3D network. Heated whey proteins treated below pI were less susceptible to gastric digestion than those treated above pI. This was attributed to the fact that protein unfolding and aggregation at close to neutral pH could expose more hydrophobic residues and peptide bonds accessible for enzymatic reaction (Zhang & Vardhanabhuti, 2014). Nyemb et al. (2014) provided an

evidence at a molecular scale that food structure affects digestion. They prepared ovalbumin aggregates with different morphologies: spherical (30 μm), spherical agglomerated (80 μm), linear (~ 33 nm) and linear-branched (~ 16 nm), by heating at pH / ionic strength of 7 / 0.3 M, 5 / 0.3 M, 9 / 0.03 M and 7 / 0.03 M respectively. Their results indicated that the spherical aggregates were hydrolysed much slower than linear aggregates during simulated gastric digestion. Moreover, the peptide bonds seemed to be cleaved at specific sites according to different morphologies of the aggregates. The possible mechanisms are that different cross-linked patterns of proteins were formed during aggregation and also the differences in surface area of the aggregates with different morphologies.

Understanding food disintegration during gastric digestion is very important from nutritional aspects; it is also crucial for food structure design. Yet, there is a knowledge gap on food disintegration in the human body because there are only few techniques available in monitoring the changes in the physicochemical properties of food in the human body. Therefore, *in vitro* studies are very helpful in probing the disintegration mechanisms of food under gastric digestion, especially for solid and semi-solid foods, where the mechanical shearing and grinding are vital.

2.9.3 Gastric emptying

2.9.3.1 Pyloric sieving

Gastric emptying into the duodenum is modulated by the pyloric sphincter, which functions as a valve that only allows liquids and sufficiently small food particles to pass through. The properties of the gastric chyme leaving the stomach, such as the particle size distribution, the solid content, the composition, etc. are vital for food digestion and nutrient uptake in the small intestine. Meyer and his co-workers have done substantial

pioneering work on exploring the particle size of the gastric chyme emptied from the stomach (Meyer, Dressman, Fink, & Amidon, 1985; Meyer, MacGregor, Gueller, Martin, & Cavalieri, 1976; Meyer, Ohashi, Jehn, & Thomson, 1981; Meyer, Thomson, Cohen, Shadchehr, & Mandiola, 1979). In an animal study where they cannulated the dogs, the food chyme was obtained from the duodenal cannula for analysis of particle size distribution after the dogs were fed with a standard meal which contains liver, beef steak and water. They found that particles bigger than 2 mm could not be emptied out from the stomach; about 70% of the emptied chyme were smaller than 0.06 mm and about 97% were smaller than 1 mm (Meyer et al., 1985; Meyer et al., 1979). In human studies where participants were fed with ^{99m}Tc-chicken liver in 1 mm or 10 mm cubes (30 g), steak (60 g) and water (100 ml), the rate at which particles smaller than 1.0 mm from proximal jejunum passed the aspiration tube was compared with the rate of gastric emptying of ^{99m}Tc captured by a gamma camera. The results indicate that there was no significant difference between these two rates and that most of the particles emptied out from the stomach were smaller than 1.0 mm, which was close to the gastric sieving of dogs (Meyer et al., 1981).

2.9.3.2 Pyloric trituration

Pyloric trituration reduces food particle size as well as favours the gastric emptying. The disintegration kinetics of food bolus is vital during gastric digestion; however, limited information is available in literature on how disintegration kinetics of food affect gastric emptying. Meyer et al. (1985) fed a standard test meal (steak, ^{99m}Tc-labeled chicken liver and water) and nondigestible spheres of different sizes and densities to six dogs. The results indicated that with the spheres at the density of 1, when the diameter of the spheres decreased from 5 mm to 1mm, gastric emptying was progressively faster;

but spheres with diameter of 0.015 mm emptied at approximately the same rate as those with diameter of 1 mm. Spheres with densities smaller or bigger than 1 empty slower than those with a density of 1. Their findings indicated that both the size and density of food particles would affect gastric emptying. Food trituration promotes gastric emptying by reducing particle size.

2.9.3.3 Masticated bolus particle size

The masticated bolus particle size going into the stomach have a significant impact on gastric emptying due to pyloric trituration is rate-limited and gastric sieving. Generally speaking, a smaller bolus particle size entering the stomach would lead to a greater gastric emptying rate. Pera et al. (2002) investigated the influence of oral processing on gastric emptying rate and reported that sufficient mastication would lead to significantly increased gastric emptying rate. Urbain et al. (1989) also investigated the impact of particle size of food bolus on gastric emptying. They prepared egg meals where the eggs were cut into cubes of 2.5 mm or 5 mm. Participants were asked to swallow the meal without chewing, and their results showed that increasing meal particle size led to decreasing gastric emptying rate. The impact of bolus particle size on gastric emptying have been evidenced in other studies as well (Holt, Reid, Taylor, Tothill, & Heading, 1982; Olausson et al., 2008).

2.9.3.4 Food characteristics

Gastric emptying is greatly affected by food characteristics, such as food composition, calorie content, and rheological properties. Cecil, Francis, and Read (1999) investigated the effect of food composition on gastric emptying by feeding human subjects with soups containing either high fat or high carbohydrate with the same calorie content and volume. Their results showed that high fat soup was emptied significantly slower than high

carbohydrate soup. The possible mechanism could be the secretion of a hormone called cholecystokinin (CCK) from the proximal small intestine after contact with digested fat and protein but not carbohydrate (Himeno et al., 1983), and CCK is known to slow down gastric emptying (Liddle, Green, Conrad, & Williams, 1986). Also, food components such as minerals, monosaccharides and disaccharides, could cause high osmotic pressure of chyme entering the duodenum and supresses gastric emptying (Shafer, Levine, Marlette, & Morley, 1985).

The effect of food calorie content on gastric emptying is independent from the effect of food composition (Velchik, Reynolds, & Alavi, 1989). With increasing food calorie content or calorie density, the rate of gastric emptying decreases, and this is because of the negative feedback mechanism monitored by the receptors located in duodenum (e.g. the release of cholecystokinin) (Liddle et al., 1986; Moran & McHugh, 1982). The gastric emptying rate is negatively correlated with food calorie content (Calbet & MacLean, 1997; Hunt & Stubbs, 1975). In general, the human stomach delivers about 150 kcal / h into the duodenum (Collins, Horowitz, Cook, Harding, & Shearman, 1983; Hunt & Stubbs, 1975). However, the acceptance of energy by the intestine would increase with increasing meal calorie content and volume (Brener, Hendrix, & McHugh, 1983; Hunt & Stubbs, 1975).

Rheological properties of foods and food bolus are a vital aspect in understanding food digestion in the human body. A food particle usually possesses both viscous and elastic characteristics. Liquid foods exhibit more viscosity while solid foods exhibit more elasticity. Solid foods have higher contribution to satiety than liquid foods with same compositions (Leidy, Apolzan, Mattes, & Campbell, 2010; Mattes & Campbell, 2009; Stull, Apolzan, Thalacker-Mercer, Iglay, & Campbell, 2008), and this would lead to

slower rate of gastric emptying. By increasing the viscosity of meals through adding different fibers such as guar gum, pectin, locust bean gum, etc. was also able to delay gastric emptying probably due to increased satiety with higher viscosity (Benini et al., 1995; Burton-Freeman, 2000; Darwiche, Björgell, & Almér, 2003; Di Lorenzo, Williams, Hajnal, & Valenzuela, 1988; French & Read, 1994; Leclère et al., 1994; Marciani et al., 2001b; Zhu, Hsu, & Hollis, 2013).

2.9.4 Food breakdown in the small intestine

Food digestion in the human body is a dynamic and continuous process. Food chyme is gradually emptied from the stomach, enters the duodenum, and digestion continues. The digested products are mainly absorbed in the small intestine into the body. In the small intestine, the digestion processes of lipids, proteins and carbohydrates continue by the actions of pancreatic lipase, trypsin and pancreatic amylase respectively. The mechanical motility of the small intestine, the diffusion of pancreatic juice as well as the characteristics of the food affect the food breakdown in the small intestine. Mechanical motility of the small intestine mix food chyme with pancreatic juice (Ganong, 1995). Enzyme diffuses into food particles and further degrades the food structure retained after gastric digestion (Benmoussa, Suhendra, Aboubacar, & Hamaker, 2006; Snow & O'Dea, 1981). Food structure influences disintegration of food in the small intestine. For instance, though starch digestion initiated in the human mouth, starch digestibility in the small intestine is determined by its molecular structure. Starch with amorphous structure (e.g. boiled hot potato) is quickly digested in the first 20 min of simulated intestinal digestion; starch with amorphous/crystalline structure (e.g. boiled millet) is slowly digested during intestinal digestion in the range of 20 to 120 min; starch with crystalline structure (e.g. raw potato starch) is resistant to digestion in the small intestine (> 120 min) (Englyst,

Kingman, & Cummings, 1992; Faisant, BuléOn, Colonna, & Champ, 1995; Hoover & Zhou, 2003; Weurding, Veldman, Veen, van der Aar, & Verstegen, 2001). Protein aggregates with dense and compact structure are digested slower than fine-stranded protein aggregates (Macierzanka et al., 2012; Nyemb et al., 2014). However, there is limited information on the impact of characteristics of gastric chyme on intestinal digestion, especially for solid and semi-solid foods.

2.10 Lipid digestion in the intestine

Lipid absorption in the small intestine is very effective, with only about 4% of the indigested fat escaping into the faces (Carey, Small, & Bliss, 1983). The dietary lipids, in spite of the source, are composed of hundreds and thousands of different triacyl glycerides (TAGs) (Small, 1991). There are three carbon atoms in a TAG, labelled as sn-1, sn-2 and sn-3. Lipid digestion is an interfacial process. Oil is in an emulsified state by proteins, peptides, polar lipids, phosphoric acids, bile salts, monoglycerides, etc. during digestion in the oral, gastric and intestinal phase, where, the small intestine is the primary site for lipid digestion. It is a complicated process. Pancreatic lipase catalyses the hydrolysis of TAG at sn-1 and sn-3 positions. The enzyme must adsorb onto the oil-water interface to hydrolyse the TAGs. Figure 2-15 shows the process of pancreatic lipase and co-lipase adsorbing to the oil-water interface with the aid from bile salts adsorbed at the interface.

Lipolytic products such as fatty acids, monoglycerides and diglycerides can precipitate and accumulate at the interface of the undigested oil, which would inhibit the access of pancreatic lipase and colipase to the interface of the oil droplets (Devraj et al., 2013; Reis et al., 2008a; Reis et al., 2008b). Bile salts are able to take the lipolytic products away from the interface and solubilize them by the formation of mixed micelles

and vesicles (Carey et al., 1983), and therefore, free the interface for lipase to access. It is reported that the presence of bile salts in lipid digestion in the small intestine would promote lipolysis and release more free fatty acids (Mun, Decker, & McClements, 2007; Sarkar, Horne, & Singh, 2010).

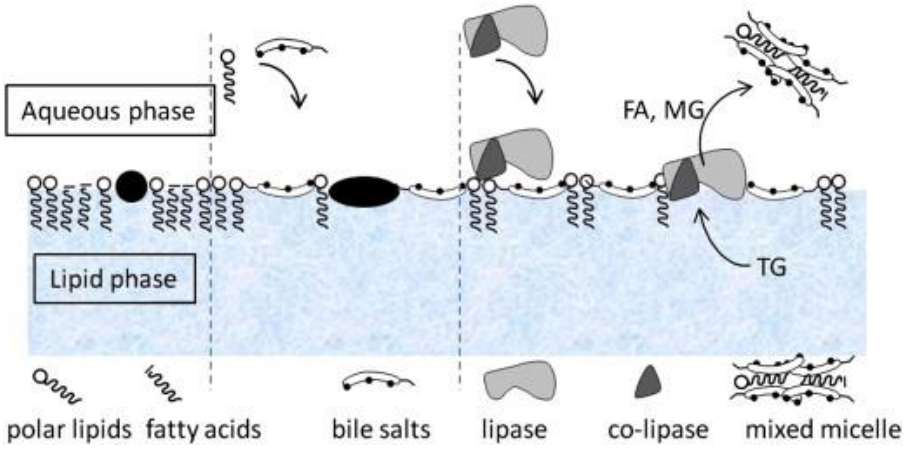


Figure 2-15 Interfacial processes during lipid digestion in the small intestine (Wilde & Chu, 2011)

2.11 Conclusions

Capsaicinoids, the lipophilic bioactive compounds present in many peppers from the genus *Capsicum*, are responsible for causing the sensory responses of burning, pain and irritation when peppers are consumed. The beneficial activities of capsaicinoids include analgesic, anti-carcinogenic and anti-inflammatory, and capsaicinoids have beneficial influences on the gastrointestinal system (Srinivasan, 2016). However, their low water solubility and their acute burning sensation / irritation have limited their use in many food, nutraceutical and pharmaceutical applications (Luo et al., 2020). Some studies have investigated the use of liquid emulsions as delivery systems for capsaicinoids (Lu et al., 2016; Popescu et al., 2013) and found that emulsion-based systems can reduce irritation and increase bioaccessibility. However, limited information was available on solid/semi-solid delivery systems and the release behaviour of capsaicinoids during oral processing

and subsequent gastrointestinal digestion (Luo et al., 2019). The sensory, disintegration and digestion behaviours of solid and semi-solid foods are much more complicated than liquid foods, as they require mastication in the mouth and further breakdown in the stomach and small intestine. By studying the disintegration behaviour of solid/semi-solid foods from mouth to intestine would fill the gap of the relationship between food structure and food digestion in the human body.

Emulsion gel, as a model system for solid and semi-solid foods, has drawn increasing interests in investigating its structure, rheology, breakdown behaviour in the human mouth and subsequent gastrointestinal digestion (Chen & Dickinson, 1998a, 1998b, 1999; Guo et al., 2014a, 2014b, 2016; Gwartney et al., 2004; Ye & Taylor, 2009). Lipophilic bioactive compounds such as capsaicinoids can be incorporated in the oil droplets of the emulsion gel for the purposes of increased incorporation and bioaccessibility. By controlling the structure and rheology of the emulsion gel, it is possible to obtain gels with tailor-made structures that would have different disintegration and digestion behaviours in the human mouth and gastrointestinal tract; the gels would be broken down in a controlled manner (Luo et al., 2020), therefore, it is possible to adjust the rate and position of release, and the bioaccessibility of capsaicinoids for targeted purposes, e.g. reduced irritation in the mouth and stomach, and increased bioaccessibility in the small intestine. However, there are limited *in vitro* and *in vivo* studies investigating the effect of food structure, especially the solid and semi-solid foods, on the release behaviour of bioactive compounds during digestion.

In addition, food digestion is a continuous and sequential process from mouth to intestine. *In vivo* studies have their limitations such as ethics, cost, technical difficulties and complexity. On the other hand, many *in vitro* studies have only focused on one part

of the whole digestion (either mouth, stomach or small intestine), especially for studies on solid and semi-solid systems, the oral processing part is often neglected. Therefore, it is necessary to fill the gap to study the whole digestion process and the impact of food structure on its digestion behaviour and the release behaviour of the incorporated bioactive compounds.

Chapter 3: Materials and methods

3.1 Materials

3.1.1 Capsaicinoids

Powdered capsaicinoids (CAP) was purchased from Wuxi AccoBio Biotech Inc., Wuxi, Jiangsu, China. The total CAP content was 95.7%: the capsaicin content was 61.23%, dihydrocapsaicin content was 31.96%, other capsaicinoids content was 2.51%.

3.1.2 Whey protein isolate

Whey protein isolate 895 instantized (WPI 895) was purchased from Fonterra Co-operative Group Limited, Auckland, New Zealand. It consists of 93% protein (N × 6.38), 4.8% moisture, 1.0% fat, 0.3% carbohydrate and 1.7% ash.

3.1.3 Tween 80

Food-grade polysorbate 80 (or Tween 80) was purchased from Hawkins Watts Ltd, Auckland, New Zealand.

3.1.4 Soybean oil

Refined soybean oil was purchased from Davis Trading Company, Palmerston North, New Zealand and used without further purification.

3.1.5 Enzymes

Pepsin from porcine gastric mucosa (#P7000: ≥ 250 units/mg solid), Pancreatin from porcine pancreas (#P7545: 8 × USP), amano lipase A from *Aspergillus niger* (#534781:

≥ 12 000 U/g) and bile bovine (#B3883) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.1.6 Fluorescent dyes

Fast green and Nile red were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nile red was dissolved in acetone at 0.1 w/v%; fast green was dissolved in Milli-Q water at 1.0 w/v%. Dyes were stored at 4 °C.

3.1.7 Other chemicals

Milli-Q water (Milli-pore Corp., Bedford, MA, USA) was used for all experiments, except for *in vivo* oral processing and sensory studies, where food-grade tap water was used. Food-grade iodized table salt was purchased from Cerebos Gregg's Limited (Auckland, New Zealand) for the *in vivo* oral processing and sensory studies. HPLC grade methanol, ethanol and acetonitrile (Thermo Fisher Scientific Inc., USA) were used for HPLC analysis. Pefabloc® SC (#76307) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid and sodium chloride were purchased from Merck Millipore (Darmstadt, Germany). Sodium hydroxide, sodium bicarbonate, sodium phosphate monobasic and potassium chloride were purchased from Thermal Fisher Scientific New Zealand Ltd (Albany, North Shore City, New Zealand). Acetone was purchased from Thermal Fisher Scientific Ltd (Fair Lawn, NJ, USA). Potassium thiocyanate, β-mercaptoethanol, sodium dodecyl sulphate (SDS), and gel electrophoresis reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemical reagents used were of analytical grade (except for *in vivo* oral processing and sensory studies) and were used without further modification, unless otherwise specified.

3.1.8 Simulated digestion fluids

Simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared following the instructions from Minekus et al. (2014) with slight modifications. The 1.25 × concentrates of simulated digestion fluids were prepared first and referred as stock simulated digestion fluids. The stock simulated digestion fluids were then combined with either water or enzyme solutions to form the simulated digestion fluids. The concentrations of the electrolytes in the final simulated digestion fluids and stock simulated digestion fluids (1.25 ×) are presented in Table 3-1. A stock $CaCl_2$ solution of 0.3 mol/L (0.3 M) was prepared separately. The stock simulated digestion fluids were magnetically stirred at room temperature for 2 h to allow complete dissolution. The pH of stock SSF (1.25 ×), stock SGF (1.25 ×) and stock SIF (1.25 ×) were adjusted to 7.0, 1.5 and 7.0 respectively by 6 M HCl / 10 M NaOH. Stock simulated digestion fluids and stock $CaCl_2$ (0.3 M) were stored at 4 °C, warmed up to room temperature (20 °C) in water bath before use and used within one month after preparation.

3.1.9 Caco-2 cell line

Minimum Essential Medium Eagle (MEM, #M2279), thiazolyl blue tetrazolium bromide (MTT, #M5655) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum, penicillin streptomycin glutamine (100× concentrates) and trypsin-EDTA (0.05%) were purchased from Gibco Life Technologies (New York, USA). Caco-2 [Caco2] (ATCC® HTB-37™) was obtained from American Type Culture Collection (Rockville, MD, USA).

Table 3-1 Concentrations of electrolytes in the final simulated digestion fluids and stock simulated digestion fluids (1.25 ×).

Constituent	Conc. in SSF* (mmol/L)	Conc. in stock SSF (mmol/L)	Conc. in SGF** (mmol/L)	Conc. in stock SGF (mmol/L)	Conc. in SIF*** (mmol/L)	Conc. in stock SIF (mmol/L)
KCl	15.1	18.875	6.9	8.625	6.8	8.5
KH_2PO_4	3.7	4.625	0.9	1.125	0.8	1
$NaHCO_3$	13.6	17	25	31.25	85	106.25
NaCl	\	\	47.2	59	38.4	48
$MgCl_2(H_2O)_6$	0.15	0.1875	0.1	0.125	0.33	0.4125
$(NH_4)_2CO_3$	0.06	0.075	0.5	0.625	\	\
$CaCl_2$	1.5	\	0.10875	\	0.6	\

*SSF: simulated salivary fluid.

**SGF: simulated gastric fluid.

***SIF: simulated intestinal fluid.

3.2 Methods

3.2.1 Preparation of CAP-loaded whey protein emulsions

Powdered CAP and WPI were added to soybean oil and water respectively. The solutions were magnetically stirred for 4 h at room temperature to allow complete dissolution.

Coarse CAP-loaded whey protein emulsions containing 0.02 wt% CAP, 19.98 wt% soybean oil and 10 wt% WPI were prepared using a high shear mixer (L4RT, Silverson, East Longmeadow, MA, USA) at 8000 rev/min for 5 min at room temperature. The average oil droplet size $d_{4,3}$ of the coarse emulsions was about 10 μm. The coarse emulsions were then homogenized by passing four times through a two-stage valve homogenizer (Homolab 2, FBF ITALIA SRL, Sala Baganza, Parma, Italy) at different first stage / second stage pressures of 0/10 bars, 100/20 bars and 250/20 bars to generate average oil droplet sizes ($d_{4,3}$) of ~ 4 ± 0.2, 1 ± 0.1 and 0.5 ± 0.05 μm. Emulsions with a $d_{4,3}$ of ~ 0.20 ± 0.03 μm were formed by passing four times through a micro-fluidizer (M-110P, Microfluidics, Newton, MA, USA) at 10,000 psi (~ 690 bars). The CAP-loaded whey protein emulsions with $d_{4,3}$ values of ~ 4 ± 0.2, 1 ± 0.1, 0.5 ± 0.05 and 0.20 ± 0.03 μm were named W_4, W_1, $W_{0.5}$ and $W_{0.2}$, respectively.

3.2.2 Preparation of CAP-loaded Tween 80 emulsions

Powdered CAP was added to soybean oil; Tween 80 and WPI were added to water respectively. The solutions were magnetically stirred in glass beakers covered with parafilm for 4 h at room temperature (20 °C) to allow complete dissolution.

Coarse CAP-loaded Tween 80 stock emulsions containing 0.03 wt% CAP, 29.97 wt% soybean oil and 3 wt% Tween 80 were prepared using a high shear mixer (L4RT) at 8000

rev/min for 5 min. The average oil droplet size $d_{4,3}$ of the coarse stock Tween 80 emulsions was about 10 μm. Coarse CAP-loaded Tween 80 stock emulsions with average oil droplet sizes ($d_{4,3}$) of ~ 1 ± 0.1, 0.5 ± 0.05 and 0.20 ± 0.03 μm were generated under the same conditions as described in Section 3.2.1. The CAP-loaded Tween 80 stock emulsions were then mixed with WPI stock solution (30 wt% WPI) at a mass ratio of 2:1 to achieve CAP-loaded Tween 80 emulsions with final concentrations of 0.02 wt% CAP, 19.98 wt% soybean oil, 2 wt% Tween 80 and 10 wt% WPI. The whey proteins were added after emulsification to ensure that there was no protein adsorption at the oil–water interface and that the whey proteins were present as a gelling agent only. The CAP-loaded Tween 80 emulsions with $d_{4,3}$ values of ~ 1 ± 0.1, 0.5 ± 0.05 and 0.20 ± 0.03 μm were named T_1, $T_{0.5}$ and $T_{0.2}$, respectively.

3.2.3 Formation of CAP-loaded emulsion gels

The required quantities of NaCl were added to the CAP-loaded whey protein emulsions and Tween 80 emulsions to give a final concentration of 10 mM, 100 mM or 200 mM NaCl. The emulsions were gently stirred for 1 h to allow complete dissolution of the NaCl. The emulsions were then poured into plastic tubes (inner diameter, 25 mm; capacity, 35 mL), sealed, heated in a water bath from 30 to 90 °C for 10 min and held at 90 °C for 20 min. The plastic tubes were immersed in an ice bath immediately after heating and then stored at 4 °C overnight until further use. The CAP-loaded whey protein emulsion gels with $d_{4,3}$ values of ~ 4 ± 0.2, 1 ± 0.1, 0.5 ± 0.05 and 0.20 ± 0.03 μm at 10 mM or 100 mM NaCl were named W_4-10, W_1-10, $W_{0.5}$-10, $W_{0.2}$-10 (10 mM NaCl) and W_4-100, W_1-100, $W_{0.5}$-100, $W_{0.2}$-100 (100 mM NaCl), respectively. The CAP-loaded whey protein emulsion gel with $d_{4,3}$ of ~ 0.5 ± 0.05 μm at 200 mM NaCl was named $W_{0.5}$-200. The CAP-loaded Tween 80 emulsion gels with $d_{4,3}$ values of ~ 1 ± 0.1, 0.5 ±

0.05 and 0.20 ± 0.03 μm at 100 mM NaCl were named T_1-100, $T_{0.5}$-100, $T_{0.2}$-100 respectively.

3.2.4 Small strain oscillatory rheological measurements

Small strain oscillatory rheological properties of CAP-loaded whey protein emulsion gels were measured using a rheometer (MCR 301, Anton Paar GmbH, Ostfildern, Germany) with a cylinder (CC27) geometry. CAP-loaded whey protein emulsions were carefully poured into the loading cell and covered with a thin layer of mineral oil to prevent evaporation. Heat-set gelation was induced *in situ* following the method described by Guo, Ye, Lad, Dalgleish, and Singh (2013): (1) heating the sample at a constant rate of 3 °C/min from 30 to 90 °C; (2) holding at 90 °C for 30 min; (3) cooling at a constant rate of 1 °C/min from 90 to 30 °C; (4) holding at 30 °C for 20 min. The shear storage moduli G′ and shear loss moduli G′′ were recorded as a function of time (minutes). All measurements were made in the linear viscoelastic region (0.5% strain) at a constant frequency of 1 Hz. Experiments were performed with three replicates from three independent samples.

3.2.5 Large deformation properties

A texture analyser (TA.XTPlus, Stable Micro Systems Ltd, Godalming, Surrey, England) with a plate–plate geometry was used to perform uniaxial compression tests on cylindrical emulsion gel samples (20 mm in height and 25 mm in diameter). Image of a representative cylindrical emulsion gel sample is shown in Figure 3-1. Both the top and the bottom of the sample were lubricated with a thin layer of mineral oil to prevent friction. The experiments were conducted as follows. Measurements were made with a constant compression and decompression speed of 10 mm/s to a compression strain of

85% to determine Young's modulus, fracture stress, fracture strain, fracture modulus and fracture energy. True stress (σ) and Hencky strain (ε) were calculated as described in Equation 3-1 and 3-2 respectively (Joyner & Daubert, 2017):

$$\sigma = \frac{F \times L}{A_0 \times L_0} \qquad \textbf{Equation 3-1}$$

$$\varepsilon = -ln\left(\frac{L}{L_0}\right) \qquad \textbf{Equation 3-2}$$

where A_0 is the initial cross-sectional area of the sample, L_0 is the initial sample height, F is the force recorded and L is the actual sample height during compression and decompression. Young's modulus was calculated as the slope of the strain–stress curve in the linear region. The fracture modulus was calculated as the fracture stress divided by the fracture strain. The fracture energy was calculated as the energy required to compress the sample to fracture point. The automatic trigger force was set to 0.049 N for all measurements. All measurements were conducted at room temperature (20 °C) with at least six replicates from six independent samples.

Figure 3-1 Representative cylindrical emulsion gel sample.

3.2.6 *In vivo* oral processing and sensory procedure

3.2.6.1 Selection and training of panellists

This human study was reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 16/48. Initially 53 potential candidates were recruited. They filled out two questionnaires; and went through five screening and training sessions, following the instructions described by Meilgaard, Civille, and Carr (2007f) (training for difference test). Eighteen subjects (4 males and 14 females) aged between 25 and 55 years were selected after screening and training tests. The detailed procedure is as follows.

Subject selection was based on strict dental criteria; successful candidates should neither be a regular smoker nor have significant tooth crowning; obvious tooth decay; pain or clicking during mastication; other known general or oral health problems that could influence chewing (Hutchings et al., 2011). All subjects were identified to be regular chili consumers (Lawless et al., 1985; Lawless et al., 2000), with no allergies to the ingredients used during the test. They were screened and trained over five sessions.

The first questionnaire (Appendix A) was to screen out non-frequent chili consumers. Successful candidates should be regular chili consumers with a score of 18 or higher (Lawless et al., 1985; Lawless et al., 2000). Secondly, candidates filled out a questionnaire on their dental health conditions (Appendix B).

Then, candidates took five screening and training sessions. In the first session, candidates performed a matching test. Successful candidates had the ability to differentiate and describe several sensory stimuli, such as sweet, sour, bitter, salty, mouth burn (pungent) and umami. These sensory stimuli were presented at well-above-

threshold intensities. An initial set of 4 unidentified and blind-coded samples was given to the candidates, followed by a second set of 6 unidentified and blind-coded samples of which a subset of 4 samples was identical to the initial set (Table 3-2). Samples were presented in random order. Candidates were asked to pick samples from the second set that matched the same sensory stimuli from the initial set and give the correct descriptors to each stimulus. Candidates scored fewer than 3 correct matches or fewer than 3 correct descriptors were rejected.

In the second session, candidates performed at least 3 triangle tests, where their abilities to detect differences between samples with similar levels of CAP were determined. Sequential triangle testing was used following the instructions described by Meilgaard et al. (2007d) for acceptance or rejection of candidates. Preliminary tests were carried out to determine appropriate CAP concentrations used (Lawless et al., 2000) and the correct order of presentation (Table 3-3). Carrier used for CAP was food-grade tap water. Three samples were presented in each triangle testing with two of the samples being identical (i.e. same CAP concentration) and an odd sample which has different CAP concentration to the other two samples. The candidates needed to pick out the odd sample. The level of difficulty of picking out the odd sample depends on the CAP concentration difference between the two identical samples and the odd sample, i.e. if the two identical samples had CAP concentration of 10 mg/L while the odd sample had CAP concentration of 0.316 mg/L or if the two identical samples had CAP concentration of 0.316 mg/L while the odd sample had CAP concentration of 10 mg/L, then the level of difficulty for the candidates to pick out the odd sample is easy. The levels of difficulty and the corresponding CAP concentrations used are presented in Table 3-3.

Table 3-2 Stimuli used for matching test in the first session

Flavour	Stimulus	Concentration (g/L)[a]
Sweet	Sucrose[d]	20[b]
Sour	Citric acid[d]	0.5[b]
Salty	Sodium chloride[d]	2.0[b]
Pungent	CAP[d]	0.003[c]
Bitter	Caffeine	1.0[b]
Umami	Monosodium glutamate	1.0

[a]Stimuli were dissolved in tap water at ambient temperature.

[b]Concentrations suggested by Meilgaard et al. (2007e).

[c]Concentration suggested by Lawless et al. (2000).

[d]Stimulus used in the initial set.

Table 3-3 Levels of difficulty in picking out the odd sample in triangle testing and the corresponding CAP concentrations used for the two identical samples and the odd sample; or the corresponding NaCl concentrations used to form whey protein gels.

	Levels of difficulty					
	Easy		Moderate		Moderately difficult	
CAP concentrations (mg/L)	0.316	10	0.316	3.16	1	3.16
NaCl concentrations (mg/L)	25	75	50	75	125	75

In the third session, candidates repeated sequential triangle testing with another sensory attribute: mouth-feel firmness. Whey protein gels (10 wt% WPI) with different gel hardness were used to give different mouth-feel firmness. The whey protein gels with different gel hardness were formed with different NaCl concentrations (McClements et al., 1993). The levels of difficulty in picking out the odd sample and the corresponding NaCl concentrations used to form whey protein gels are presented in Table 3-3. For

example, if the two identical whey protein gel samples were formed with NaCl concentration of 125 mM while the odd whey protein gel sample was formed with NaCl concentration of 75 mM or if the two identical whey protein gel samples were formed with NaCl concentration of 75 mM while the odd whey protein gel sample was formed with NaCl concentration of 125 mM, because the gels formed with either 75 mM or 125 mM NaCl had similar gel mechanical hardness (McClements et al., 1993), therefore the mouth-feel firmness would be similar and therefore the level of difficulty of picking out the odd sample was moderately difficult.

In sequential triangle testing, candidates were either accepted or rejected following Figure 3-2, where three regions were formed by two lines (Equation 3-3 and 3-4) and were identified as the acceptance region, rejection region and continue testing region. Appropriate values were chosen for test sensitivity parameters (α=0.1, β=0.1, p0=0.33, p1= 0.78) to reach moderate test sensitivity. α is the probability of stating that a difference occurs when it does not; β is the probability of stating that no differences occurs when it does; p0 is the expected proportion of correct decisions when the samples are identical; p1 is the expected proportion of correct decisions when the odd sample is detected (other than by guess) on 67% of the total number of occasions (Meilgaard et al., 2007d). The number of triangle tests conducted (cumulative) was plotted on x axis and the number of correct tests (cumulative) was plotted on y axis. The coordinates (x, y) of each candidate all started from (0, 0). By conducting one triangle test, coordinate x increased by 1; if the answer was correct (i.e. the candidate successfully picked out the odd sample), coordinate y was increased by 1, if wrong then by 0. Accordingly, the coordinate of each candidate after each triangle test was plotted on Figure 3-2, until the point where it reached or crossed one of the lines indicating the boarder of the regions, and the conclusion was drawn whether to accept or reject the candidate.

Lower line: $y_0 = \frac{\log\beta - \log(1-\alpha) - x\log(1-p_1) + x\log(1-p_0)}{\log p_1 - \log p_0 - \log(1-p_1) + \log(1-p_0)}$ **Equation 3-3**

Upper line: $y_1 = \frac{\log(1-\beta) - \log\alpha - x\log(1-p_1) + x\log(1-p_0)}{\log p_1 - \log p_0 - \log(1-p_1) + \log(1-p_0)}$ **Equation 3-4**

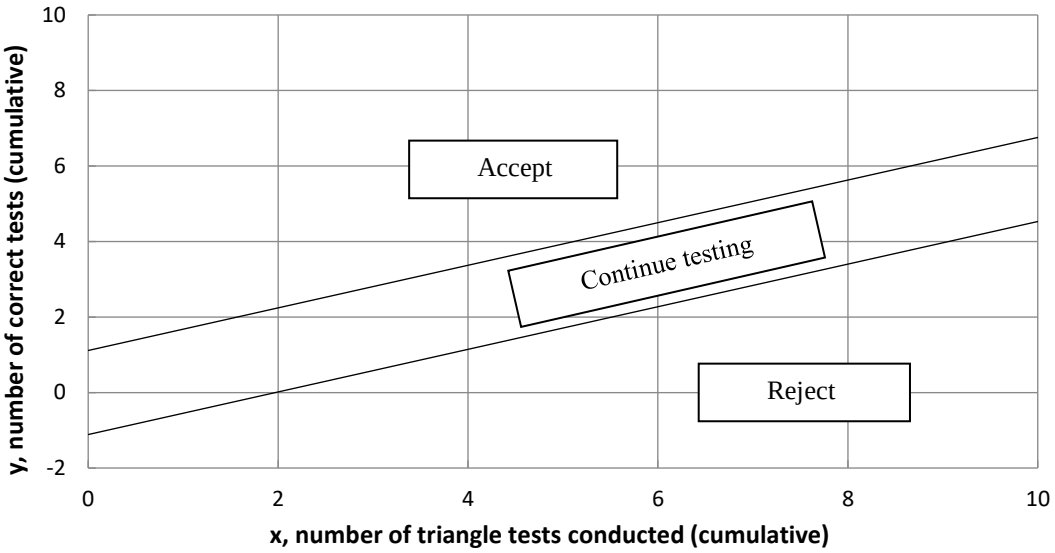


Figure 3-2 Selection of panellists by sequential triangle testing (Meilgaard et al., 2007d)

In the fourth session, candidates performed a ranking test where their abilities to differentiate and rank samples with gradient CAP levels were determined. CAP levels used were: 0.316 mg/L, 1 mg/L, 3.16 mg/L and 10 mg/L. Candidates were asked to rank the 4 blind-coded samples presented in random orders according to their mouth burn intensity. In between samples, candidates were asked to wait for at least 3 min to cool down the mouth burn carried over from the previous sample. Milk, water and crackers were provided to facilitate the cooling process.

In the fifth session, candidates performed a ranking test where their abilities to differentiate and rank samples with graded firmness levels were determined. Five whey protein gel samples (10 wt% WPI) containing 12.5 mM, 25 mM, 50 mM, 75 mM and 100 mM NaCl respectively were used. Candidates were asked to gargle their mouth with water in between samples.

In the fourth and fifth sessions, candidates with the exact correct rankings or only inverted the neighbouring samples were accepted.

3.2.6.2 In vivo oral processing procedure

After screening and training tests, successful candidates performed seven test sessions, with one session per week to avoid fatigue and memory effects. Panellists were asked to chew two gel samples (cube of ~ 1.5 cm in length, ~ 5 g in weight) in each session. Samples were blind coded and presented in random order. Prior to chewing, panellists were asked to gargle their mouth with water three times (3 × 30 mL). Then, they were asked to chew the gel samples one by one for as long as necessary, to spit the gel residues into a plastic container when they felt the intention to swallow, to rinse their mouths with 30 mL of water and to spit the debris into another plastic container. The number of chews and the chewing duration were recorded by a camera placed in front of the panellist. After chewing the first sample, panellists were asked to wait for at least 3 min to cool the burning sensation in their mouths before starting to chew the second sample. Room temperature water and cold milk (4 °C) were provided to facilitate the cooling process. Panellists were asked to gargle their mouths with water in between samples to clean the palate. Samples for analysis of the oil droplet size distribution, the degree of fragmentation of the masticated gel bolus and the oil droplet release from the protein matrix were collected.

3.2.6.3 Degree of fragmentation of masticated gel bolus

Samples of ~ 80 g of the masticated gel bolus (mixture of gel residues and debris) were taken, placed into pre-weighed and pre-dried aluminium pans and dried in an oven at 105 °C for 24 h to determine the dry matter content. Samples of ~ 100 g of the

masticated gel bolus were taken, and wet sieving analysis was conducted on each sample. Each sample was poured through a stack of six sieves with mesh sizes of 0.038, 0.425, 0.850, 1.40, 2.00 and 3.15 mm; the bolus particles retained in each sieve were gently washed for at least 2 min using mild running reverse osmosis water. Particles smaller than 0.038 mm were discarded because they were difficult to collect; this portion was calculated by subtracting the dry weight of the bolus particles retained in each sieve from the total dry weight of the masticated bolus being analysed. The fragments retained in each sieve were transferred to pre-weighed and pre-dried aluminium pans. The aluminium pans with gel fragments were dried in an oven at 105 °C for 24 h and then weighed. The dry weight of the masticated gel fragments retained in each sieve was expressed as a percentage of the total dry weight. The weight percentage of gel fragments passing through each sieve was then calculated and the cumulative particle size distribution of each gel type was also determined. The geometric mean diameter of the fragmented gel particles, d_{frag}, was calculated based on Equation 3-5 (American Society of Agricultural and Biological Engineers, 2008):

$$d_{frag} = log^{-1}\left[\frac{\sum_{i=1}^{n}(w_i log\overline{d_i})}{\sum_{i=1}^{n} w_i}\right] \qquad \textbf{Equation 3-5}$$

where $\overline{d_i}$ is the geometric mean diameter of two adjacent sieve sizes, $\overline{d_i} = \sqrt{d_i \times d_{i+1}}$, and w_i is the weight of the dried gel retained on each sieve. All experiments were conducted with three replicates.

3.2.6.4 Quantification of oil droplet release during mastication

Samples of ~ 35 g were taken from the masticated gel bolus and centrifuged at 3761 $\times g$ for 40 min at 4 °C (method from Guo et al. (2013) with slight modification). The centrifugation force (3761 $\times g$) was applied to separate the oil droplets that have already

been released from gel matrix during mastication from the solid masticated gel bolus particles. The solid bolus particles would sediment upon centrifugation, and it would not cause new release of oil droplets from the gel matrix under this relatively low centrifugation force. After centrifugation, the supernatants were taken, transferred into aluminium pans and dried in an oven at 105 °C for 24 h. The fat content of the dried samples was measured using the Soxhlet extraction method (AOAC method 920.39), petroleum ether was used as the extraction solvent. Measurements were conducted with samples from three independent digestion experiments, each conducted in duplicate. The percentage of oil droplets released from the protein matrix was calculated based on:

$$Percentage\ of\ oil\ droplet\ release = \frac{m}{m_0} \times 100\% \qquad \textbf{Equation 3-6}$$

where m_0 is the theoretical initial weight of oil in the emulsion gel and m is the measured weight of the fat content in the masticated gel bolus.

3.2.6.5 Sensory test procedure

The magnitude estimation method (Cardello, Matas, & Sweeney, 1982; Moskowitz, 1977) was used for sample rating. In each session, a sub-test for the perceived mouth burn intensity was carried out followed by a sub-test for the perceived mouth-feel firmness. In each sub-test, one control sample and two test samples were presented. From preliminary experiments, a whey protein emulsion gel containing CAP (0.02 wt% CAP, 19.98 wt% soybean oil and 10 wt% WPI) with an oil droplet size $d_{4,3}$ of 1 ± 0.1 μm at 10 mM NaCl (sample W_1-10) was chosen as the control sample. The control sample was assigned ratings of 100 for its perceived mouth burn intensity and its mouth-feel firmness; panellists were asked to give ratings to the test samples proportional to that of the control sample.

3.2.7 *In vitro* oral processing

A food processer (The Mini Wizz food chopper, BFP100, Breville Group Ltd, Sydney, Australia) was used to mimic oral breakdown of CAP-loaded emulsion gels and to produce *in vitro* masticated gel boluses that have similar bolus particle sizes to the *in vivo* masticated gel boluses. The food processer has three buttons on the panel for different grinding intensities: chop, whisk and grind. The emulsion gels were firstly cut into cylinders of 20 mm in height and 25 mm in diameter, then about 45 g of gel (seven cylindrical gel samples) was added into the processer. Different grinding patterns were carried out for different CAP-loaded emulsion gels, in order to obtain *in vitro* masticated gel boluses with similar bolus particle sizes to the *in vivo* masticated gel boluses. The grinding patterns for different CAP-loaded emulsion gels are presented in Table 3-4. For some of the emulsion gel samples, it was necessary to take out part of the gel fragments during grinding; the gel fragments collected during grinding and at the end were mixed together and the mixture was considered as the *in vitro* masticated gel bolus. The *in vitro* masticated gel boluses were analysed using wet sieving analysis as described previously in 3.2.6.3 to determine the degree of fragmentation.

Table 3-4 Grinding patterns for different CAP-loaded emulsion gels.

Characteristics of the CAP-loaded emulsion gels			Grinding patterns*
Oil droplet size ($d_{4,3}$, μm)	NaCl conc. (mM)	Emulsifier	
0.5	10	WPI	C → C → G → G → collect all
0.5	200	WPI	C → C → G → take 10 g out** → G → G → G → G → G → take 10 g out** → G → G → G → G → G → G → G → G → collect the remaining gel pieces and mix with previous
0.5	100	WPI	C → C → G → take 10 g out** → G → G → G → G → G → take 10 g out** → G → G → G → G → G → G → G → G → collect the remaining gel pieces and mix with previous
0.5	100	Tween 80	C → G → take 15 g out** → G → G → take 15 g out** → G → G → G → collect the remaining gel pieces and mix with previous

*The buttons C (chop) or G (grind) was pressed for about 1 second each time;

**10 g or 15 g of gel fragments were taken out during grinding and mixed with the gel fragments collected at the end; the mixture was considered as the *in vitro* masticated gel bolus.

3.2.8 *In vitro* gastric digestion

3.2.8.1 Human gastric simulator (HGS)

A human gastric simulator (HGS, as shown in Fig. 3-3), designed by Kong & Singh (2010), was used for *in vitro* gastric digestion. The mechanical drive system of the HGS, creating the peristaltic contractions during gastric digestion, is composed of 4 belts, 12 rollers, driving shafts and pulley system. A cone-shaped latex stomach chamber was vertically placed inside the machine, and a thin polyester mesh bag with pore size of 1 mm was placed inside the latex stomach chamber to mimic human gastric sieving as human stomach only allows particles < 1 mm to pass through to the duodenum (Meyer et al., 1976; Schulze, 2006). A plastic tube was connected to the tip of the stomach chamber at the bottom, leading to the outside of the machine. The tube can be closed by a clip and opened for periodic gastric emptying. The 4 belts were evenly distributed on four sides of the stomach chamber; each belt was 81 cm long, and 3 rollers were evenly installed onto one belt. Each roller was consisted of two side wheels of 9 mm thickness and the two wheels were 11 mm apart from each other. The rollers on the opposing belts were installed at the same heights, while the rollers on the neighbouring belts were installed with a 30 mm gap. To simulate the actual stomach contractions of 3 cycles per minute (Schulze, 2006), the driver system was set to create 3 contractions per minute on the latex chamber. A fan heater and a thermostat were placed inside the machine to keep the temperature at 37 °C.

Figure 3-3 The human gastric simulator and the latex stomach chamber. 1: SGF; 2: plastic tubes for secretion; 3: pump; 4: latex gastric 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 (Ye, Cui, Dalgleish, & Singh, 2016)

3.2.8.2 *In vitro* gastric digestion procedure

The pepsin and lipase activity in the final gastric digestion mixture was set at 1000 U/ml and 50 U/ml respectively. The Ca^{2+} concentration in the final gastric digestion mixture was set at 0.075 mmol/L. The enzyme solution used for *in vitro* gastric digestion was prepared by adding 2.72 g pepsin, 0.44 g amano lipase A and 0.29 ml stock $CaCl_2$ (0.3 M) in 159.71 ml Milli-Q water to reach concentrations of 17.02 g/L pepsin, 2.75 g/L amano lipase A and 0.54 mmol/L $CaCl_2$ in the enzyme solution.

The *in vitro* masticated gel bolus was prepared by adding 40 ml stock SSF, 0.5 ml stock $CaCl_2$ (0.3 M) and 9.5 ml Milli-Q water to 160 g grinded CAP-loaded emulsion gel. The volume of the *in vitro* masticated bolus was about 200 ml. The *in vitro*

masticated gel bolus was pre-warmed in 37 °C water bath for 2 min, and then added into the stomach chamber. 70 ml SGF (consisted of 56 ml stock SGF and 14 ml enzyme solution) was also added into the stomach chamber, to mimic the condition that the stomach contains a certain amount of gastric juice during fasting state (Camilleri, 2006). The gastric digestion time was 240 min. The gastric secretion rate was set at 2.5 ml/min (Hoebler et al., 2002). The stock SGF and enzyme solution were added in separately by two pumps; the secretion rates were 2 ml/min for stock SGF and 0.5 ml/min for enzyme solution. Gastric emptying started after 30 min on account of the lag phase of solid foods (Siegel et al., 1988; Urbain et al., 1989). For every 15 min, 45 ml of gastric digesta was collected from the bottom, corresponding to an emptying rate of 3 ml/min (Kong & Singh, 2010).

For gastric digesta collected at different digestion times, the pH and temperature were recorded; a portion of the emptied gastric digesta was dried in an oven at 105 °C for 24 h and then weighed to determine the solid content. The cumulative dry weight of gastric digesta emptied from the HGS at different digestion times was then calculated. The gel retention fraction at time t, denoted as y(t), was calculated using Equation 3-7:

$$y(t) = \frac{W_0 - W_t}{W_0} \times 100\% \qquad \textbf{Equation 3-7}$$

where w_0 was the total dry weight of solids (gels, salts and pepsin) entering the HGS; w_t was the cumulative dry weight of emptied gastric digesta at time t.

3.2.8.3 Protein hydrolysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions to determine the protein compositions of the gastric digesta emptied at different digestion times. Immediately after been emptied from the

HGS, 20 µl digesta sample was taken and mixed with 180 µl electrophoresis sample buffer (0.2 M Tris–HCl buffer, pH 6.8; 40% glycerol; 2% SDS; 0.04% Coomassie Brilliant Blue G-250), 10 µl of β-mercaptoethanol was added, and then the mixture was heated in boiling water for 10 min. After cooling to room temperature, the samples were centrifuged at 4200 g for 20 min and then 10 µl of the middle layer from each sample was loaded onto tricine gels previously prepared on a Mini PROTEIN II system (Bio-Rad Laboratories, Richmond, CA, USA). The resolving gel contained 16.0 w/v% acrylamide, made up in Tris–HCl buffer, and the stacking gel contained 4.0 w/v% acrylamide, made up in Tris–HCl buffer. The electrophoresis analysis was conducted at 125 V in a cold room (4 °C) for approximately 120 min. The gel was stained for 60 min with a Coomassie Brilliant Blue R-250 solution (0.003 w/v% Coomassie Brilliant Blue R-250, 10% acetic acid and 20% isopropanol) under gentle shaking. Then, the gel was firstly destained with a destaining solution of 10% acetic acid and 10% isopropanol for 1 h and then destained overnight in fresh destaining solution under gentle shaking. A Molecular Imager Gel Doc XR system (Bio-Rad Laboratories) was used to scan the gels.

3.2.9 *In vitro* intestinal digestion

3.2.9.1 *In vitro* intestinal digestion procedure and measurement of free fatty acid release

A pH-stat (TitraLab 856; Radiometer Analytical, Villeurbanne, France) was used for *in vitro* intestinal digestion and to measure free fatty acid release during *in vitro* intestinal digestion. The gastric digesta emptied at 60 min, 120 min and 240 min were used for *in vitro* intestinal digestion. The pH of the gastric digesta was adjusted to 7.0 using 10 M NaOH immediately after emptying from the HGS. 23 ml of the gastric digesta was mixed with 16.4 ml stock SIF (1.25 ×) and added into the temperature-controlled chamber of

the pH-stat. 46 mg pancreatin from porcine pancreas (8 × USP) was dissolved in 2 ml stock SIF, then 46 µl stock $CaCl_2$ (0.3 M) was added. 0.18795 g bile bovine was dissolved in 4.6 ml Milli-Q water. The pancreatin solution and the bile bovine solution were added into the chamber at the same time and the intestinal digestion started. The initial volume of the digestion mixture was 46 ml. The concentrations of bile bovine and $CaCl_2$ were 10 mM and 0.3 mM respectively in the initial digestion mixture. The concentration of pancreatin was 1 mg/ml in the digestion mixture, based on trypsin activity at 100 U/ml. The intestinal digestion was carried out at 37.0 °C for 120 min with a constant stirring rate of 100 rev/min. The titration was done with 0.05 M NaOH and the endpoint pH was set at 7.0. The quantity of free fatty acid released per ml of digestion mixture (Q, µmol/ml) was calculated using Equation 3-8:

$$Q = \frac{Volume_{NaOH} \times C_{NaOH}}{Volume_{reaction\ mixture}} \qquad \textbf{Equation 3-8}$$

where C_{NaOH} was the molar concentration of the NaOH titrant, i.e. 0.05 M. The initial lipolysis rate (µmol·ml^{-1}·min^{-1}) was calculated as the mol amount of free fatty acid released per ml of reaction mixture per minute during the initial 2 min of reaction.

The fat content of the gastric digesta was measured by Mojonnier method (AOAC method 989.05). Certain amounts of the intestinal digesta at 10 min, 30 min, 60 min, 90 min and 120 min of intestinal digestion were collected for measurements of particle size distribution and oil droplet size distribution. The small intestinal digestion was performed in triplicate with samples from three independent experiments.

3.2.9.2 Determination of bioaccessibility of CAP after *in vitro* intestinal digestion

The bioaccessibility of CAP was defined as the fraction of CAP which was released from the food matrix in the GI tract after digestion and becomes available for intestinal

absorption (Heaney, 2001). At the end of intestinal digestion, part of the intestinal digesta was collected and mixed with 1mM Pefabloc solution at volume ratio of 8:1, to cease the enzymatic reaction. Then, the digesta was centrifuged at 17,000 g for 40 min at 4 °C. After centrifugation, the clear middle layer (i.e. the mixed micelle layer) was taken and filtered through 0.22 µm. After filtration, the filtrate was considered as the bioaccessible fraction. The concentration of CAP in the bioaccessible fraction as well as the digestion mixture was determined by high-performance liquid chromatography (Section 3.2.14). The bioaccessibility (%) of CAP was calculated using Equation 3-9:

$$Bioaccessibility\ (\%) = \frac{CAP\ in\ bioaccessible\ fraction}{Total\ CAP\ in\ digestion\ mixture} \times 100\% \qquad \textbf{Equation 3-9}$$

3.2.10 Caco-2 cell experiments

3.2.10.1 Cell cultures

Caco-2 cells were grown and maintained in MEM (Minimum Essential Medium), supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin streptomycin glutamine (referred as "growth medium") in a temperature-controlled humidified incubator at 37 °C with 5% atmospheric CO_2 (Thermo Scientific Forma® Direct Heat CO_2 Incubator, Thermo Fisher Scientific Inc., USA). The cells were seeded at 2×10^6 cells per 20 ml in Corning® U-shaped cell culture flasks (capacity: 275 ml, 75 cm^2 cell growth area; Fisher Scientific, USA) and the growth medium was changed every 3 days. When the cell monolayer reached 70–90% confluency, cells were harvested with trypsin-EDTA, washed and passaged into new flasks or into assay plates. The cells were used between passage 16 and 40.

3.2.10.2 *In vitro* cytotoxicity of CAP in growth medium sample and gastric digesta of CAP-loaded emulsion gels obtained from *in vitro* gastric digestion

3.2.10.2.1 Preparation of CAP in growth medium

CAP in growth medium was prepared as a control sample. Powdered CAP was dissolved in growth medium at 50 µg/ml, stored at 4 °C and used within 3 days after preparation.

3.2.10.2.2 *In vitro* cytotoxicity test procedure

The *in vitro* cytotoxicity of CAP in growth medium and the *in vitro* gastric digesta from CAP-loaded emulsion gels emptied at different digestion times was determined by measuring the cell viability of Caco-2 cells using MTT assay. The cells were harvested and seeded in 96-well plates with 100 µl per well at a density of 2×10^5 cells / ml. The cells were incubated at 37 °C with 5% CO_2 for 48 h for the cells to settle, adhere to the bottom and reproduce. After 48 h of incubation of the cells, CAP in growth medium and *in vitro* gastric digesta were transferred into autoclaved 1.5 ml Eppendorf tubes and sterilized under ultraviolet (UV) light for 30 min. Then, the samples were diluted 1:10 in growth medium. 100 µl of the diluted samples / growth medium / Milli-Q water was added to the wells. The growth medium and Milli-Q water were used as negative and positive control respectively. The cells were incubated for another 24 h at 37 °C with 5% CO_2, then 100 µl was taken out from each well. 10 µl of MTT in phosphate buffer saline (5 mg/ml, sterilised through 0.22 µm syringe filter) was added to each well and incubated for 3.5 h at 37 °C with 5% CO_2. After 3.5 h incubation, the formation of purple crystals was verified visually under 100× magnification on an optical microscope. Then, 100 µl of 100% DMSO was added into each well to dissolve the formazan crystals. The plates

were analysed by a microplate reader (ELx808 Ultra Microplate Reader, Bio-Tek Instruments Inc., Winooski, VT, USA) at wavelength of 550 nm. The number of metabolically cells was calculated as a percentage relative to the positive control group, which was normalized to 100%. The cytotoxicity of CAP-loaded liquid emulsions and gastric digesta emptied at different digestion times was tested on two independent samples at least in triplicate wells for each sample and each dilution. The independent digesta samples were collected from independent digestion experiments; the independent emulsion samples were made on different day.

3.2.10.3 Quantification of human interleukin 8 (IL-8) production

3.2.10.3.1 Sample application and incubation on Caco-2 cells

During *in vitro* gastric digestion of CAP-loaded emulsion gels, the gels were gradually disintegrated and digested. The gastric digesta collected at different digestion times were applied on Caco-2 cells to test human IL-8 production by Caco-2 cells. Caco-2 cells were harvested when they reached about 70% confluency and seeded in 96-well plates with 100 µl per well at a density of 2×10^5 cells / ml. The cells were incubated at 37 °C with atmospheric 5% CO_2 for 5 days until they became confluent and ready for sample application and incubation. The growth medium was changed on day 3.

During the gastric digestion process, the pH of the gastric digesta gradually decreased. To investigate the effect of the pH of gastric digesta on IL-8 production, the pH of the gastric digesta was either adjusted to 7.0 or left unadjusted. Gastric digesta samples collected from *in vitro* gastric digestion of emulsion gels containing no CAP were also tested as blank controls. The freshly collected gastric digesta samples (pH either adjusted to 7.0 or not; emulsion gels either contained CAP or not) were transferred into autoclaved

1.5 ml Eppendorf tubes and sterilized under UV light for 30 min. Then, the samples were diluted 1:10 in growth medium.

On day 5 of cell culture on the 96-well plates, the cells became confluent. The growth medium was gently taken out and then 200 µl diluted digesta samples / growth medium / interleulin-1β (Recombinant human IL-1 beta protein (active), #ab9617, Abcam, Cambridge, UK; prepared at 2 ng/ml in growth medium) were added into each well. The growth medium and interleukin-1β (IL-1β) were used as negative and positive control respectively. The cells were then incubated with samples for 24 h at 37 °C with 5% atmospheric CO_2.

The quantification of human IL-8 production by Caco-2 cells after 24 h incubation with gastric digesta samples was carried out with digesta samples collected from two independent digestion experiments (duplicate). In each trial, the samples were tested in triplicate.

3.2.10.3.2 Quantification of human IL-8 production by enzyme-linked immunosorbent assay (Elisa)

The enzyme-linked immunosorbent assays (ELISA) were carried out using the human IL-8 antibody pair kit (Catalog #CHC1303, Invitrogen, Thermo Fisher Scientific Inc., USA) following the protocol provided by the manufacturer. The detailed protocol is as follows.

To prepare the ELISA plates, 96-well plates were coated with 100 µl of coating solution (1 µg/ml of coating antibody in coating buffer A) per well. The plates were covered with aluminium foil to avoid light and incubated for 12–18 h at 4 °C. After incubation, the plates were washed once using a microplate washer with the wash buffer

provided in the buffer kit (#CNB0011, Invitrogen, Thermo Fisher Scientific Inc., USA). Following wash, the plates were inverted and tapped on absorbent paper forcefully to remove excess liquid. Then, the plates were blocked with 300 µl of assay buffer per well for 1 h at room temperature. The plates were covered with aluminium foil to avoid light. After blocking the plates for 1 h, the liquids were removed by decantation followed by tapping the plates forcefully on absorbent paper.

After gastric digesta sample incubation on Caco-2 cells for 24 h, these plates were centrifuged at 1500 rpm for 1 min, then 100 µl of supernatant was taken from each well and applied on the Elisa plates prepared as previously described. Human IL-8 standard solutions were prepared in assay buffer with a series of IL-8 concentrations of 0, 12.5, 25, 50, 100, 200, 400 and 800 pg/ml, following the instructions from the manufacturer. 100 µl of standards (in duplicate) were also added to the Elisa plates. Afterwards, 50 µl of the detection antibody solution (0.04 µg/ml of detection antibody in assay buffer) was immediately added into each well. The plates were then incubated for 2 h at room temperature (20 °C) with continual shaking at 700 rpm. Following that, the plates were washed with the wash buffer for 5 times using the plate washer. Then, 100 µl of streptavidin-HRP solution (0.04% (v/v) streptavidin-HRP in assay buffer) was added to each well and incubated for 30 min at room temperature with continual shaking at 700 rpm. After 30 min, the plates were washed with the wash buffer for 5 times using the plate washer. Then, 100 µl of the TMB substrate was added to each well and incubated for 30 min at room temperature with continual shaking at 700 rpm. After 30 min, 100 µl of stop solution was added to each well, and the plates were analysed using the microplate reader at wavelengths of 450 nm and 620 nm. The absorbance was measured within 30 min after adding the stop solution. A 4-parameter curve fit (4PL) was performed by the

software SkanIt Software 4.1 for Microplate Readers RE (ver. 4.1.0.43) to analyse the results.

3.2.11 Determination of oil droplet size distribution

A MasterSizer 2000 (Malvern Instruments Ltd, Malvern, UK) was used to measure the average diameters and the particle size distributions of the oil droplets in the CAP-loaded stock Tween 80 emulsions, the WPI and Tween 80 emulsions and emulsion gels, the *in vivo* and *in vitro* masticated gel boluses, the emptied gastric digesta and the intestinal digesta from *in vitro* gastrointestinal digestion. The refractive index for the oil droplets was set at 1.47. The weight-to-volume diameter $d_{4,3}$ (μm) was used to denote the average oil droplet size, which was calculated using Equation 3-10:

$$d_{4,3} = \sum \frac{n_i d_i^4}{n_i d_i^3} \qquad \textbf{Equation 3-10}$$

where n_i is the number of particles with a diameter of d_i.

For liquid emulsion samples, 2 ml emulsion was added to 10 ml of 5 wt% sodium dodecyl sulphate (SDS) solution and gently mixed for a few seconds before measurement. For the emulsion gels and the masticated gel boluses, 2 g of emulsion gel or 20 g of masticated gel bolus was added to 20 ml of a solution containing 5 wt% SDS and 50 mM β-mercaptoethanol; for the emptied gastric digesta and the intestinal digesta, 3 ml 5 wt% SDS solution and 20 μl β-mercaptoethanol were added to 2 ml digesta samples. The mixtures were then shaken overnight in a water bath at 25 °C until complete dissolution. The dissolved mixtures were used for oil droplet size measurements. All measurements were conducted at room temperature (20 °C) with at least three independent samples, each conducted in triplicate.

3.2.12 Determination of particle size distribution of gel fragments

A MasterSizer 2000 (Malvern Instruments Ltd, Malvern, UK) was used to measure the average diameters and the particle size distributions of the gel fragments of the *in vivo* and *in vitro* masticated gel boluses, the emptied gastric digesta and the intestinal digesta from *in vitro* gastrointestinal digestion. The refractive index for the gel fragments was set at 1.47. The weight-to-volume diameter $d_{4,3}$ (μm) was used to denote the average gel particle size. The samples were measured immediately after collection. All measurements were conducted at room temperature with at least three independent samples, each conducted in triplicate.

3.2.13 Confocal laser scanning microscopy (CLSM)

A confocal laser scanning microscope (Leica, Heidelberg, Germany) was used to observe the microstructure of the CAP-loaded stock Tween 80 emulsions, the WPI and Tween 80 emulsions and emulsion gels, the *in vivo* masticated gel boluses and the *in vitro* emptied gastric digesta. Nile Red (0.1 w/v%) was used to stain oil (argon laser with an excitation line at 488 nm) and Fast Green (1.0 w/v%) was used to stain protein (He–Ne laser with an excitation line at 633 nm). 10 μl of Nile Red and 10 μl of Fast Green were added to 100 μl liquid emulsion samples and mixed gently. Emulsion gel samples were cut into thin pieces and put into 1.5 ml Eppendorf tubes, 20 μL of Nile Red and 20 μL of Fast Green were added and the samples were stained overnight at 4 °C. For the *in vivo* masticated gel boluses and the *in vitro* emptied gastric digesta, 200 μl samples were put into 1.5 ml Eppendorf tubes and stained with 20 μL of Nile Red and 20 μL of Fast Green immediately after sample collection. Stained digesta samples were placed in ice bath before CLSM. All stained samples were placed on concave microscope slides and covered with cover slip for CLSM.

3.2.14 Quantification of CAP by high-performance liquid chromatography (HPLC)

The CAP content in the mixed micelle phase (i.e. the bioaccessible fraction) and the intestinal digesta after *in vitro* gastrointestinal digestion of CAP-loaded emulsion gels, the CAP-loaded liquid emulsions and the cell growth media collected from both apical and basolateral sides were quantified by reversed-phase high-performance liquid chromatography (HPLC). The HPLC was equipped with a UV-VIS photodiode array detector (SPD-20AV, Shimadzu Corporation, Kyoto, Japan). The column was a Synergi^{TM} 4 µm Hydro-RP 80 Å LC column with dimensions of 150 × 4.6 mm (Phenomenex Inc., Torrance, CA, USA). The mobile phase was composed of acetonitrile and Milli-Q water at the volume ratio of 50:50. The running temperature was set at 30 °C, with a flow rate of 1 ml/min and the sample injection volume of 5 µl. Two main compounds from CAP were detected: capsaicin and dihydrocapsaicin. The detection wavelength was set at 280 nm, with retention times of about 8 min for capsaicin and 11.3 min for dihydrocapsaicin.

Standard solutions of powdered CAP (capsaicin: 61.23%; dihydrocapsaicin: 31.96%; other capsaicinoids: 2.51%) were prepared in methanol with CAP concentrations of 5, 10, 20, 40, 60 80, and 100 µg/ml, i.e., the concentrations of capsaicin were 3.0615, 6.123, 12.246, 24.492, 36.738 and 48.984 µg/ml; and the concentrations of dihydrocapsaicin were 1.598, 3.196, 6.392, 12.784, 19.176, 25.568 and 31.96 µg/ml.

CAP extraction from samples was done by mixing sample and absolute ethanol at the volume ratio of 1:1. The mixtures were vortexed for 5 min, then stored overnight at 4 °C. The next day, the mixtures were centrifuged at 10,000 × g at 4 °C for 15 min, then the supernatants were filtered through 0.22 µm before injecting to the HPLC column.

3.2.15 Statistical analysis

Data collected from the mastication behaviour and sensory studies from *in vivo* oral processing studies were analysed by SAS 9.4 software to calculate mean values and standard errors. The mean values of different samples were compared by *t* test distribution at $P < 0.05$. Pearson correlations were performed using SAS 9.4 software to analyse the correlations among the rheological properties, the degree of fragmentation and the sensory perception. The results were expressed as the correlation coefficient *r* and the probability level *P*. $r = 1$ denotes a perfectly positive correlation; $r = 0$ denotes no correlation; $r = -1$ denotes a perfectly negative correlation. *P* denotes the significance of the correlation. Power-law fittings in Chapter 4, Chapter 5 and Chapter 6 were performed using Origin 2017 64Bit software. The coefficient of determination (R^2, value between 0 and 1) denoted the goodness of the fit, where a value close to 1 indicated a good fit.

Other data were analysed by one-way analysis of variance using IBM SPSS Statistics 24 software. Means were compared by Tukey tests at $P < 0.05$.

Chapter 4: Structure of whey protein emulsion gels containing capsaicinoids: Impact on in-mouth breakdown behaviour and sensory perception

4.1 Abstract

This work investigated the sensory perception of whey protein emulsion gels containing capsaicinoids, in relation to their structure and in-mouth breakdown behaviour. The gel structure was modified by varying the oil droplet size and the ionic strength. Eighteen human subjects were used to investigate oral processing behaviour and sensory perception. The gels were classified into three groups according to their rheological properties: semi-solid gel (10 mM NaCl with $d_{4,3}$ of ~ 0.2 μm); soft and elastic gels (10 mM NaCl with $d_{4,3}$ of ~ 4, 1 and 0.5 μm); hard and brittle gels (100 mM NaCl with $d_{4,3}$ of ~ 4, 1, 0.5 and 0.2 μm). A power-law relationship between gel hardness and masticated bolus particle size was established: $Y = 25.38X^{-0.29}$, with R^2 = 0.69, indicating that greater hardness led to smaller bolus particle size. A power-law relationship between mouth burn perception and bolus particle size was also established: $Y = 119.5X^{-0.25}$, with R^2 = 0.76, indicating that mouth burn perception was positively related to the degree of fragmentation. A higher degree of fragmentation led to greater surface exposure during mastication and, therefore, a greater possibility of capsaicinoid molecules being released. Moreover, an increase in gel hardness led to a lower diffusion rate of capsaicinoid molecules through the gelled particles and, therefore, to lower mouth burn perception. The effects of oil droplet release after mastication and saltiness perception were less important than the degree of fragmentation and gel hardness.

4.2 Introduction

Capsaicinoids, the pungent lipophilic bioactive components in various peppers from the genus *Capsicum*, have several biological activities such as anti-carcinogenic, anti-inflammatory, analgesic and beneficial influences on gastrointestinal systems (Srinivasan, 2016). The sensory responses to capsaicinoids include burning, irritation and pain. Studies have shown that the intensity of this sensation is influenced by many factors, such as capsaicinoid concentration, type of carrier (oil or water based), fat content, the presence of other compounds (e.g. milk proteins, alcohol) and serving temperature (Baron & Penfield, 1996; Lawless et al., 2000; Nasrawi & Pangborn, 1990). Moreover, some studies have investigated the use of liquid emulsions as delivery systems for capsaicinoid (Lu et al., 2016; Popescu et al., 2014) and found that emulsion-based systems can reduce irritation and increase bioaccessibility. However, limited information was available on solid/semi-solid delivery systems and the release behaviour of capsaicinoids during oral processing and subsequent digestion.

The structure and mechanical properties of a food product have a great impact on how it is processed in the human mouth (Chen et al., 2013; Devezeaux de Lavergne et al., 2015a; Devezeaux de Lavergne et al., 2015b; Guo et al., 2013; Hutchings & Lillford, 1988; Stieger & van de Velde, 2013). When a food product enters the human mouth, it goes through a wide range of physical and biochemical processes, which result in the formation of a food bolus that is safe for swallowing (Foegeding et al., 2011; Sarkar, Goh, & Singh, 2009). Solid and semi-solid foods are of particular interest because they require several chewing cycles during oral processing, which lead to particle size reduction and contribute to texture perception and the release of flavour from the food (Foegeding et al., 2011). The degree of particle size reduction of a solid or semi-solid

food depends critically on its structural and rheological properties (Agrawal et al., 1997; Chen et al., 2013; Lucas et al., 2002). Gwartney et al. (2004) studied the effect of the mechanical properties of whey protein emulsion gels on the perception of sensory texture and the particle size distribution of the bolus. They found that stranded gels were perceived as being smooth and slippery and were broken down into a large and non-homogeneous bolus, whereas particulate gels were perceived as being crumbly, and were broken down into a small and homogeneous bolus. However, in this study, the particle size of the bolus was determined after only one chew.

Soft solid systems have drawn increasing interest because of their potential applications in a series of industries from pharmaceuticals and cosmetics to food formulations (Chen & Dickinson, 1999). Many foods, such as puddings, dairy desserts, set yoghurt and sausages, are categorized as solid/semi-solid foods. All these foods have a structure that resembles an emulsion gel (Rosa et al., 2006). By controlling the structure and rheology of the emulsion gel, it is possible to obtain gels with tailor-made structures that would have different behaviours in the human mouth and thus to realize controlled targeted release of the incorporated components (Mao, Roos, Biliaderis, & Miao, 2017). According to Lee, Choi, and Moon (2006), Malone and Appelqvist (2003) and Mao, Roos, and Miao (2014), gelled emulsions were able to slow down the release of lipophilic flavour compounds during oral processing, because of lower mass transfer and diffusion rates of the flavour molecules through the gelled particles. Also, flavour release could be controlled when the gels were broken down in a controlled manner, either by physiological triggers such as melting or enzyme treatment, or by manipulating the gel structure. For example, Mao et al. (2014) studied the retention and release rate of a volatile compound incorporated into whey protein emulsion gels using gas chromatography headspace analysis. They found that a higher storage modulus led to a

lower release rate; however, they did not perform a sensory analysis and did not consider the effect of different gel breakdown behaviours in the human mouth. Also, since capsaicinoids are non-volatile trigeminal stimuli, the release behaviour during mastication could be different from a volatile compound. The effect of food structure and oral breakdown behaviour on the release behaviour of capsaicinoids during mastication and the sensory perception still remains unclear.

Therefore, the aim of this study was to investigate the effect of emulsion gel structure on oral breakdown behaviour and sensory perception of capsaicinoids. Heat-set whey protein emulsion gel was chosen as the delivery system for capsaicinoids, because capsaicinoids need an oil-based carrier for higher solubility and reduced irritation; also, whey protein emulsion gel has a simple composition and can mimic real food systems. The gel structure can be precisely designed through the modification of several parameters, such as ionic strength and oil droplet size (Ye & Taylor, 2009; Dickinson, 2012), to provide a simple and systematic way of determining the effect of structural factors on oral processing behaviour and sensory perception. In this chapter, CAP-loaded whey protein emulsion gels with different gel structures and rheological properties were formed by varying oil droplet sizes $d_{4,3}$ of ~ 0.2, 0.5, 1 or 4 μm and NaCl concentration: 10 mM or 100 mM. The interrelationships among structure and rheology, in-mouth breakdown properties and sensory properties were also determined.

4.3 Results and discussion

4.3.1 Rheological properties of CAP-loaded emulsion gels

Figure 4-1 shows the development of viscoelastic properties during the formation, the final storage moduli and the CLSM images of heat-set CAP-loaded emulsion gels containing 10 mM and 100 mM NaCl. At 10 mM NaCl (Fig. 4-1 A), storage moduli gradually increased during heating. Gel formation (i.e. the time point when G' = G'') started after 17 min except for $W_{0.2}$-10, which started after 30 min. The final storage modulus gradually increased with increasing oil droplet size from $W_{0.2}$-10 to W_1-10; there was no significant difference between W_4-10 and W_1-10. In the CLSM images (Fig. 4-2), emulsion gels W_4-10, W_1-10, $W_{0.5}$-10 and $W_{0.2}$-10 all showed continuous protein networks (green), and the oil droplets appeared to be flocculated (red).

At 100 mM NaCl (Fig. 4-1 B), storage moduli had steeper increases upon heating and gelation started after 15 min. The final storage modulus gradually decreased with increasing oil droplet size. In the CLSM images, emulsion gels W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100 all showed discontinuous protein networks (green) with pores (black), but the oil droplets appeared to be relatively evenly distributed in the protein matrix. At a given oil droplet size, the final storage moduli of the emulsion gels were always higher at 100 mM NaCl than at 10 mM NaCl, indicating harder gels at 100 mM NaCl, which is consistent with the findings of Ikeda et al. (1999) on the rheological properties of heat-set whey protein gels with different NaCl concentrations. The emulsion gels had essentially the same oil droplet size distribution as the parent emulsions (data not shown), confirming that there was no coalescence of the oil droplets during the preparation of the emulsion gels.

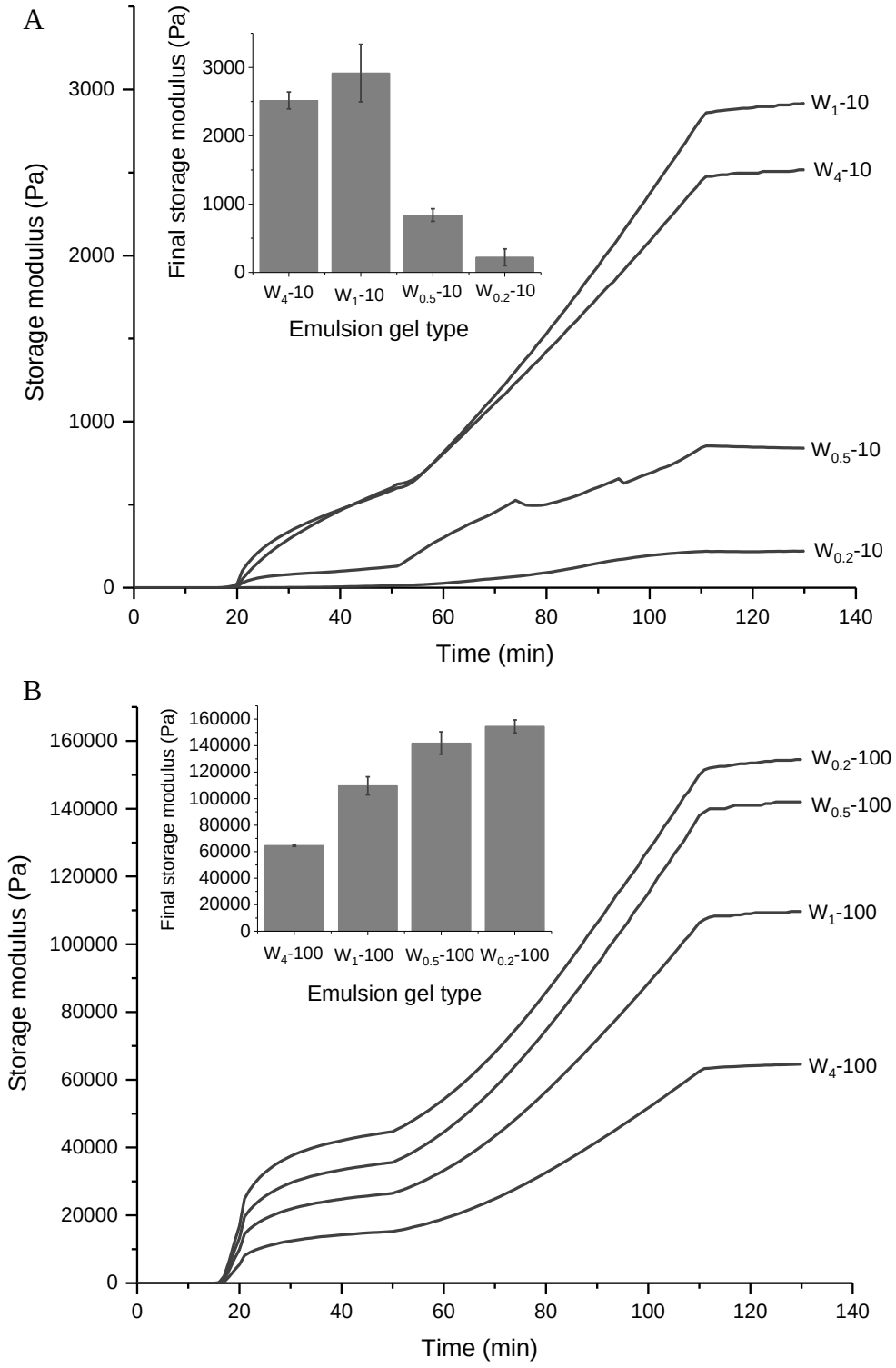


Figure 4-1 Development of viscoelastic properties during the formation and the final storage moduli of heat-set CAP-loaded emulsion gels: (A) emulsion gels containing 10 mM NaCl: W_4-10 ($d_{4,3}$ ~ 4 µm), W_1-10 ($d_{4,3}$ ~ 1 µm), $W_{0.5}$-10 ($d_{4,3}$ ~ 0.5 µm) and $W_{0.2}$-10 ($d_{4,3}$ ~ 0.2 µm); (B) emulsion gels containing 100 mM NaCl: W_4-100 ($d_{4,3}$ ~ 4 µm), W_1-100 ($d_{4,3}$ ~ 1 µm), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm) and $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm). Error bar: standard deviations of N = 3 independent experiments.

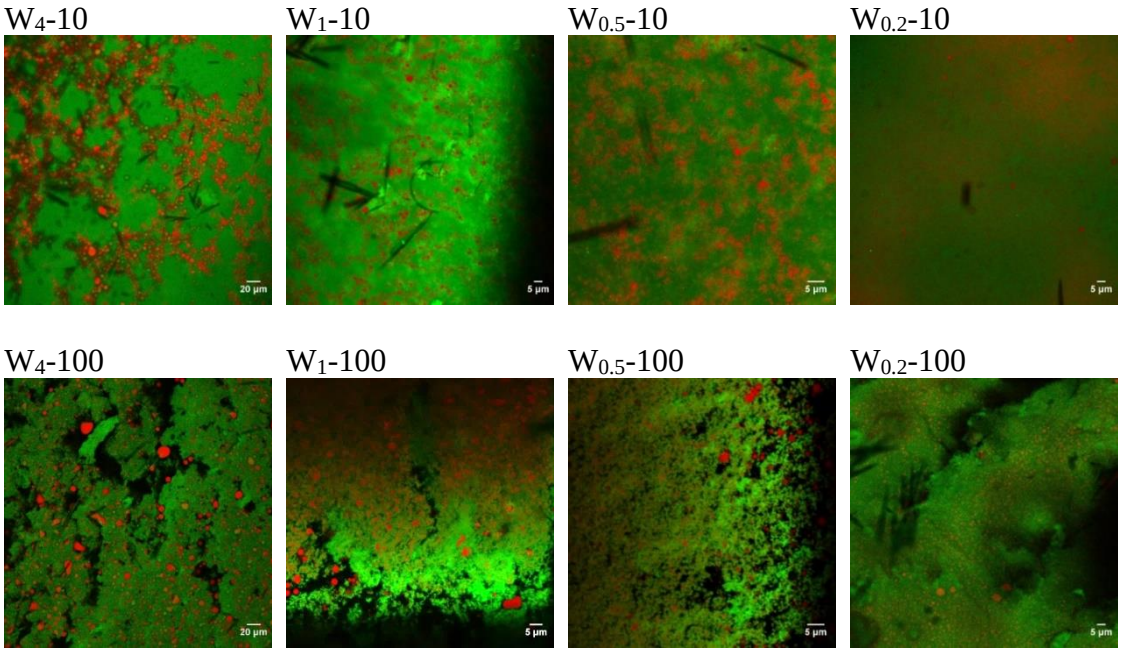


Figure 4-2 CLSM images of heat-set CAP-loaded emulsion gels: W_4-10 ($d_{4,3}$ ~ 4 µm with 10 mM NaCl), W_1-10 ($d_{4,3}$ ~ 1 µm with 10 mM NaCl), $W_{0.5}$-10 ($d_{4,3}$ ~ 0.5 µm with 10 mM NaCl), $W_{0.2}$-10 ($d_{4,3}$ ~ 0.2 µm with 10 mM NaCl), W_4-100 ($d_{4,3}$ ~ 4 µm with 100 mM NaCl), W_1-100 ($d_{4,3}$ ~ 1 µm with 100 mM NaCl), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm with 100 mM NaCl), $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm with 100 mM NaCl).

Table 4-1 shows the large deformation properties of CAP-loaded emulsion gels. Data for $W_{0.2}$-10 were not available; because it did not form a self-supporting gel, compression tests could not be performed. At 10 mM NaCl, Young's modulus, fracture stress and fracture modulus gradually decreased ($P < 0.05$) with decreasing oil droplet size from W_4-10 to $W_{0.5}$-10; there was no significant difference for fracture strain. Emulsion gel $W_{0.5}$-10 required significantly low fracture energy. At 100 mM NaCl, Young's modulus, fracture stress, fracture modulus and fracture energy gradually increased with decreasing oil droplet size from W_4-100 to $W_{0.2}$-100; there was no significant difference for fracture strain. At a given oil droplet size, emulsion gels at 10 mM NaCl always had lower Young's modulus, lower fracture stress, higher fracture strain and lower fracture modulus ($P < 0.05$); emulsion gels at 100 mM NaCl required higher fracture energy, except that there was no significant difference between W_4-10 and W_4-100.

From their rheological properties, emulsion gel $W_{0.2}$-10 was characterized as a semi-solid gel because it had the lowest final storage modulus; emulsion gels W_4-10, W_1-10 and $W_{0.5}$-10 were characterized as soft and elastic gels, because they had lower Young's modulus and higher fracture strain; emulsion gels W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100 were characterized as hard and brittle gels, because they had higher Young's modulus and lower fracture strain.

Table 4-1 Large deformation properties of CAP-loaded emulsion gels W_4-10 ($d_{4,3}$ ~ 4 µm with 10 mM NaCl), W_1-10 ($d_{4,3}$ ~ 1 µm with 10 mM NaCl), $W_{0.5}$-10 ($d_{4,3}$ ~ 0.5 µm with 10 mM NaCl), W_4-100 ($d_{4,3}$ ~ 4 µm with 100 mM NaCl), W_1-100 ($d_{4,3}$ ~ 1 µm with 100 mM NaCl), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm with 100 mM NaCl), $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm with 100 mM NaCl). Results are shown as mean ± standard deviation of N = 6 independent experiments.

Emulsion gel type	Young's modulus (kPa)	Fracture stress (kPa)	Fracture strain	Fracture modulus (kPa)	Fracture energy (mJ)
W_4-10	$9.3 \pm 0.3^{a,x}$	$44.6 \pm 4.0^{a,x}$	$1.86 \pm 0.02^{a,x}$	$23.9 \pm 2.1^{a,x}$	$351.9 \pm 20.2^{a,x}$
W_1-10	$8.8 \pm 0.2^{b,x}$	$49.4 \pm 2.4^{b,x}$	$1.88 \pm 0.00^{a,x}$	$26.3 \pm 1.3^{b,x}$	$354.4 \pm 15.8^{a,x}$
$W_{0.5}$-10	$3.8 \pm 0.1^{c,x}$	$36.1 \pm 2.1^{c,x}$	$1.87 \pm 0.00^{a,x}$	$19.3 \pm 1.1^{c,x}$	$192.6 \pm 12.4^{b,x}$
W_4-100	$83.8 \pm 0.8^{a,y}$	$71.9 \pm 9.3^{a,y}$	$0.90 \pm 0.04^{a,y}$	$79.3 \pm 7.5^{a,y}$	$345.0 \pm 49.3^{a,x}$
W_1-100	$106.8 \pm 1.9^{b,y}$	$88.0 \pm 2.9^{b,y}$	$0.87 \pm 0.04^{a,y}$	$101.6 \pm 6.2^{b,y}$	$417.7 \pm 20.2^{a,y}$
$W_{0.5}$-100	$137.3 \pm 5.9^{c,y}$	$121.7 \pm 10.0^{c,y}$	$0.90 \pm 0.04^{a,y}$	$135.9 \pm 8.7^{c,y}$	$617.2 \pm 60.9^{b,y}$
$W_{0.2}$-100	174.4 ± 3.3^{d}	152.3 ± 3.8^{d}	0.94 ± 0.05^{a}	161.4 ± 9.3^{d}	854.5 ± 55.5^{c}

$^{a–d}$Values with different letters within the same NaCl concentration group differ significantly ($P < 0.05$).

^{x,y}Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

Emulsion gel $W_{0.2}$-10 was not self-supporting, data not available because compression tests could not be performed.

4.3.2 Oral processing behaviour

4.3.2.1 Mastication parameters of CAP-loaded emulsion gels

The mastication parameters of the CAP-loaded emulsion gels are presented in Table 4-2. Emulsion gel $W_{0.2}$-10 had significantly lower number of chews, chewing duration and chewing frequency; there was no significant difference between W_4-10, W_1-10 and $W_{0.5}$-10. At 100 mM NaCl, there was an increasing trend in the number of chews and the chewing duration with decreasing oil droplet size from W_4-100 to $W_{0.2}$-100; there was no significant difference in chewing frequency.

At a given oil droplet size, there was no significant difference in the number of chews, the chewing duration and the chewing frequency between W_4-10 and W_4-100 or between W_1-10 and W_1-100. $W_{0.5}$-100 had a longer chewing duration than $W_{0.5}$-10 ($P < 0.05$). $W_{0.2}$-100 had a greater number of chews, a longer chewing duration and a greater chewing frequency than $W_{0.2}$-10 ($P < 0.05$).

Table 4-2 Mastication parameters, d_{frag} and oil droplet release after mastication of CAP-loaded emulsion gels W_4-10 ($d_{4,3}$ ~ 4 μm with 10 mM NaCl), W_1-10 ($d_{4,3}$ ~ 1 μm with 10 mM NaCl), $W_{0.5}$-10 ($d_{4,3}$ ~ 0.5 μm with 10 mM NaCl), $W_{0.2}$-10 ($d_{4,3}$ ~ 0.2 μm with 10 mM NaCl), W_4-100 ($d_{4,3}$ ~ 4 μm with 100 mM NaCl), W_1-100 ($d_{4,3}$ ~ 1 μm with 100 mM NaCl), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 μm with 100 mM NaCl), $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 μm with 100 mM NaCl). Results of number of chews, chewing duration and chewing frequency are shown as mean ± standard error obtained from 18 human participants. Results of d_{frag} and oil droplet release are shown as mean ± standard deviation of N = 3 replicates for d_{frag}; N = 2 replicates for oil droplet release after mastication.

Emulsion gel sample	Number of chews	Chewing duration (s)	Chewing frequency (s^{-1})	d_{frag} (mm)	Oil droplet release after mastication (%)
W_4-10	$29.2 \pm 4.2^{a,x}$	$18.8 \pm 1.7^{a,x}$	$1.55 \pm 0.08^{a,x}$	$2.50 \pm 0.06^{a,x}$	$8.2 \pm 0.1^{a,x}$
W_1-10	$28.1 \pm 3.8^{a,x}$	$17.6 \pm 1.8^{a,x}$	$1.60 \pm 0.09^{a,x}$	$1.97 \pm 0.16^{ab,x}$	$1.3 \pm 0.6^{b,x}$
$W_{0.5}$-10	$26.8 \pm 2.9^{a,x}$	$15.9 \pm 1.6^{a,x}$	$1.68 \pm 0.08^{a,x}$	$1.67 \pm 0.17^{b,x}$	$0.59 \pm 0.36^{b,x}$
$W_{0.2}$-10	$8.5 \pm 1.4^{b,x}$	$8.2 \pm 1.4^{b,x}$	$0.99 \pm 0.07^{b,x}$	$0.05 \pm 0.00^{c,x}$	$0.14 \pm 0.14^{b,x}$
W_4-100	$26.1 \pm 3.2^{a,x}$	$16.3 \pm 2.2^{a,x}$	$1.58 \pm 0.05^{a,x}$	$0.62 \pm 0.09^{a,y}$	$6.9 \pm 0.7^{a,x}$
W_1-100	$32.8 \pm 3.7^{a,x}$	$19.2 \pm 2.1^{a,x}$	$1.69 \pm 0.05^{a,x}$	$0.60 \pm 0.03^{a,y}$	$3.1 \pm 0.05^{b,y}$
$W_{0.5}$-100	$36.8 \pm 4.8^{ab,x}$	$22.2 \pm 2.1^{ab,y}$	$1.63 \pm 0.05^{a,x}$	$0.80 \pm 0.11^{a,x}$	$0.26 \pm 0.26^{c,x}$
$W_{0.2}$-100	$43.4 \pm 3.5^{b,y}$	$26.6 \pm 2.1^{b,y}$	$1.65 \pm 0.05^{a,y}$	$0.69 \pm 0.11^{a,y}$	$0.12 \pm 0.04^{c,x}$

^{a,b}Values with different letters within the same NaCl concentration group differ significantly ($P < 0.05$).

^{x,y}Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

4.3.2.2 Degree of fragmentation of masticated gel bolus

The cumulative dry mass percentages of masticated gel particles passing through sieves of given size (0.038, 0.425, 0.850, 1.4, 2.0 and 3.15 mm) are plotted in Figure 4-3. The curves can be separated into three groups according to their degree of fragmentation. The semi-solid gel ($W_{0.2}$-10) had the greatest degree of fragmentation, followed by the hard and brittle gels (W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100). The soft and elastic gels (W_4-10, W_1-10 and $W_{0.5}$-10) had the lowest degree of fragmentation.

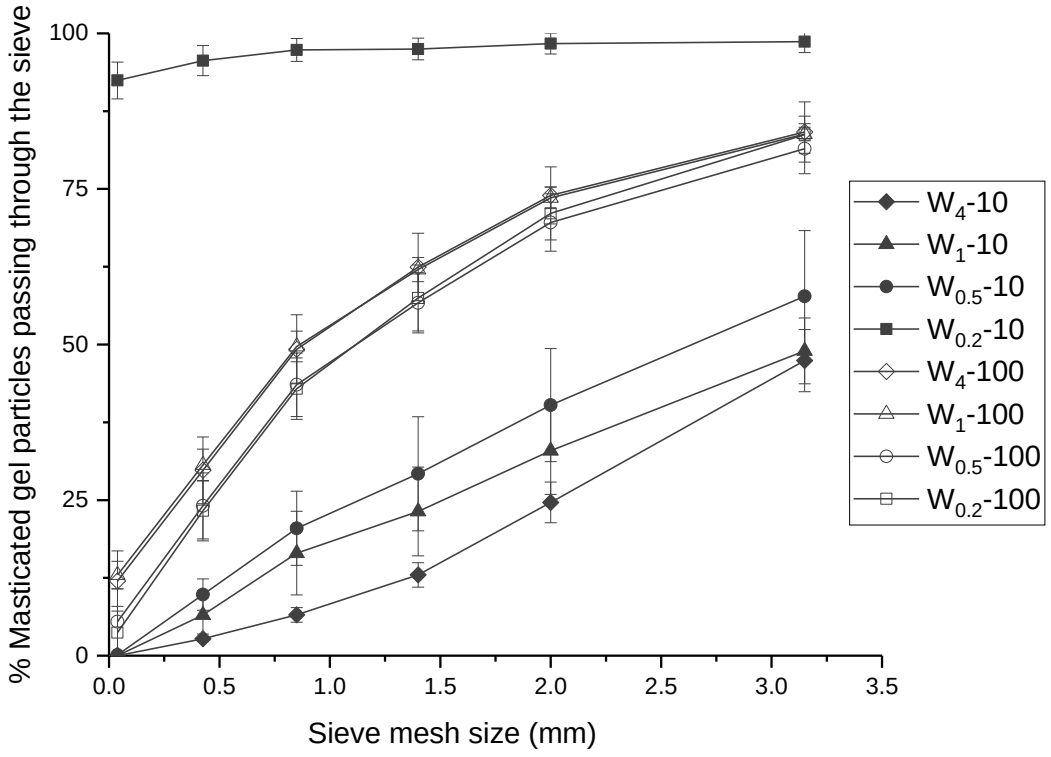


Figure 4-3 Cumulative dry mass percentage of masticated gel particles passing through sieves of given sizes (0.038, 0.425, 0.850, 1.4, 2.0 and 3.15 mm). Error bar: standard deviations of N=3 replicates.

The geometric mean diameters of the masticated gel boluses, d_{frag}, were calculated using Equation 3-5 and are presented in Table 4-2. A higher d_{frag} indicates a lower degree of fragmentation. At 10 mM NaCl, there was a decreasing trend in d_{frag} with decreasing oil droplet size from W_4-10 to $W_{0.2}$-10. $W_{0.2}$-10 had significantly lower d_{frag} because this

semi-solid sample could almost pass through the smallest sieve (value > 90%). At 100 mM NaCl, there was no significant difference in d_{frag} between W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100.

4.3.2.3 Oil droplet release after mastication

The percentage of oil droplets released from the protein matrix after mastication is presented in Table 4-2. At 10 mM NaCl, emulsion gel W_4-10 released a significantly greater amount of oil droplets after mastication (8.2 ± 0.1%); there was no significant difference between W_1-10, $W_{0.5}$-10 and $W_{0.2}$-10 (values < 2%). At 100 mM NaCl, emulsion gels W_4-100 and W_1-100 respectively released 6.9 ± 0.7% and 3.1 ± 0.04% of oil droplets after mastication; there was no significant difference between $W_{0.5}$-100 and $W_{0.2}$-100 (values < 0.5%).

These results can be attributed to the differences in gel structure caused by the oil droplet size. Smaller oil droplets were better incorporated into the gel structure; they were protected against mechanical shearing and compression by their thick protein coatings. In contrast, emulsion gels with larger oil droplets (i.e. W_4-10 and W_4-100) were more sensitive to shearing and mechanical forces, because they had a greater tendency to 'slip' under these forces. Similar results have been reported for WPI emulsion-filled gels with different oil droplet sizes (Guo et al., 2014a; Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009).

At a given oil droplet size, emulsion gel W_1-100 had significantly greater oil droplet release than W_1-10. This can be attributed to the difference in their gel structures, which was caused by ionic strength. At low ionic strength, W_1-10 had a continuous protein network, in which the oil droplets were interconnected and bound firmly with the protein matrix. At high ionic strength, W_1-100 had a porous protein network, with the oil droplets

embedded in the pores and therefore being more likely to be released during mastication. However, there was no significant difference in oil droplet release between W_4-10 and W_4-100, $W_{0.5}$-10 and $W_{0.5}$-100 or $W_{0.2}$-10 and $W_{0.2}$-100. This indicates that, when the oil droplet size was too big or too small, the difference caused by ionic strength would be eliminated. When the oil droplets were large (i.e. $d_{4,3}$ ~ 4 μm), the structure of the gel matrix was disrupted. The oil droplets acted like slippery defects in the gel matrix (Guo et al., 2014a; McClements et al., 1993), and the larger the oil droplets, the higher the tendency to slip during compression and mastication. During mastication, the large oil droplets had less protection from the gel matrix against the mechanical forces of chewing. Similarly, when the oil droplets were really small (i.e. $d_{4,3}$ ~ 0.5 and 0.2 μm), the oil droplets were well retained within the gel fragments after mastication.

The masticated gel bolus had essentially the same oil droplet size distributions as the parent emulsions (data not shown), confirming that there was no coalescence of the oil droplets during mastication.

4.3.3 Correlation between Young's modulus and d_{frag} of masticated gel boluses

Figure 4-4 presents the correlation between Young's modulus and d_{frag} of the masticated gel boluses. The hard and brittle gels (W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100) had smaller d_{frag}s whereas the soft and elastic gels (W_4-10, W_1-10 and $W_{0.5}$-10) had much larger d_{frag}s, indicating that mechanical properties have a significant impact on the in-mouth breakdown of these gels and bolus formation. A power-law fit between Young's modulus and d_{frag} was established, i.e. $Y = 25.38X^{-0.29}$, with R^2 = 0.69. Similarly, Chen et al. (2013) established a power-law relationship between food hardness and the median particle size d_{50} of food boluses, using the data obtained from seven different types of food materials and data from other twelve different types of foods from

literature (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Peyron, Mishellany, & Woda, 2004), i.e. $Y = 0.89X^{-0.80}$, with $R^2 = 0.59$. The differences in the fitting parameters in the functions can be attributed to differences in measurement: Chen et al. (2013) measured food hardness (with the unit of Newtons) using puncture tests and used d_{50} (i.e. the theoretical sieve that can pass 50 wt% of the bolus) to denote the particle size of the food bolus; in the present study, Young's modulus (with the unit of Pascals) was measured by uni-axial compression tests and the calculated geometric mean diameter (d_{frag}) was used to denote the particle size of the masticated gel bolus.

Based on the power-law relationship (Fig. 4-4), we drew the following conclusions with respect to bolus formation. Firstly, the hard and brittle gels produced much smaller and more homogeneous bolus particles, indicating that it is essential for hard gels to reach a small bolus particle size, aiming for safe swallowing. However, although gel hardness gradually increased from W_4-100 to $W_{0.2}$-100, there was no significant difference in bolus particle size. This indicates that, when the gel is sufficiently hard, the bolus particle size does not change much with an increase in hardness. In contrast, the number of chews and the chewing duration gradually increased (Table 4-2) and the fracture energy increased (Table 4-1) with the increase in gel hardness from W_4-100 to $W_{0.2}$-100, indicating that harder gels did require more chewing effort (i.e. more chewing cycles, higher fracture energy etc.) to obtain the bolus particle size that is acceptable for swallowing. Secondly, as the soft and elastic gels were slippery and easily deformable, the particle size of the bolus covered a wider range and was less crucial for safe swallowing. Thirdly, gels with intermediate hardness would be the most sensitive group, because a small increase in hardness could result in a steep decrease in bolus particle size.

In the present work, the whey protein emulsion gel was used as a delivery system for the bioactive compounds capsaicinoids, whereas, in previous studies, the whey protein emulsion gels contained no bioactive or flavour compounds (Guo et al., 2013, 2014a; Gwartney et al., 2004). Therefore, the burning sensation of the capsaicinoid may have caused the differences in the mastication behaviours and the degrees of breakdown. For example, in the work of Guo et al. (2013), the whey protein emulsion gels with $d_{4,3}$ ~ 0.5 μm and containing 10 mM or 100 mM NaCl had chewing cycles of 19.4 ± 2.1 and 24.8 ± 4.8 respectively and the d_{50} values of the masticated gel boluses were 4.00 and 1.05 mm respectively. However, in the present study, the CAP-loaded whey protein emulsion gels with $d_{4,3}$ ~ 0.5 μm and containing 10 mM or 100 mM NaCl had chewing cycles of 26.8 ± 2.9 and 36.8 ± 4.8 respectively and the d_{50} values of the masticated gel boluses were 2.64 and 1.12 mm respectively. The number of chews increased significantly when the gels contained capsaicinoids, which may have been because the CAP-loaded gels were able to provide a richer and more dynamic sensory perception, such that the panellists tended to chew for a longer time. However, the in-mouth breakdown behaviour of the CAP-loaded whey protein emulsion gels would also have had an impact on the release behaviour and the sensory perception of capsaicinoids.

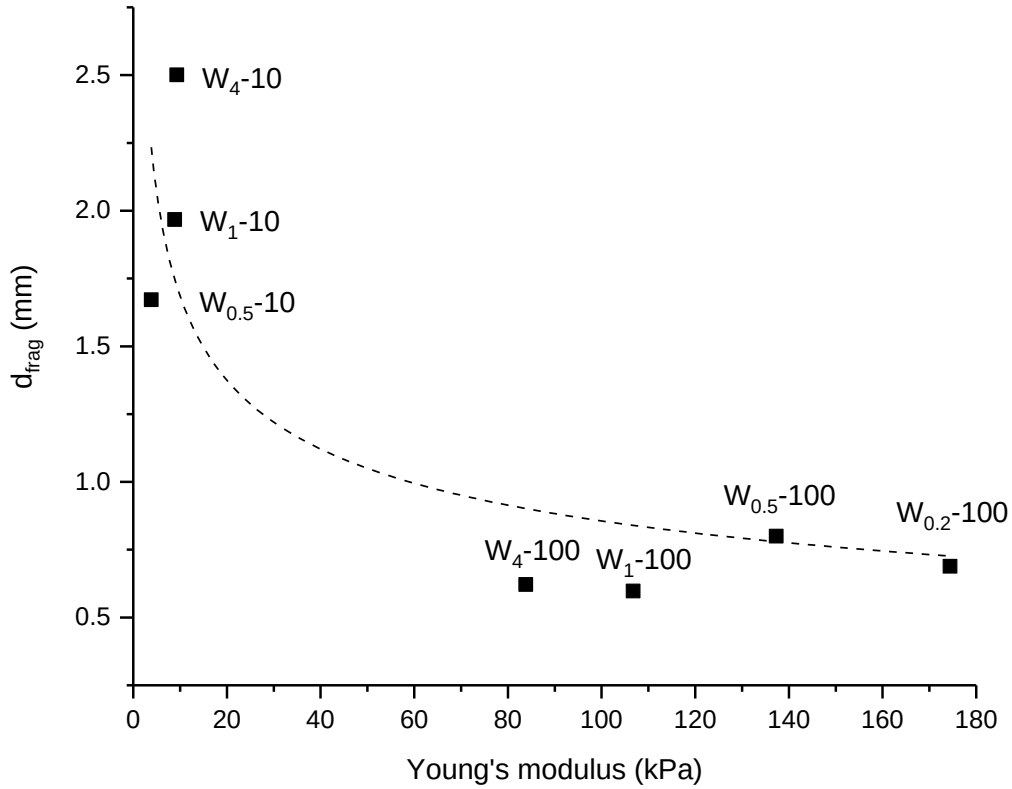


Figure 4-4 Correlation between Young's modulus and d_{frag} of masticated gel boluses.

4.3.4 Mouth-feel firmness ratings of CAP-loaded emulsion gels

Table 4-3 presents the perceived mouth-feel firmness ratings of the CAP-loaded emulsion gels that were obtained from the 18 human subjects. At 10 mM NaCl, the mouth-feel firmness gradually increased ($P < 0.05$) with increasing oil droplet size from $W_{0.2}$-10 to W_1-10; there was no significant difference between W_4-10 and W_1-10. At 100 mM NaCl, there was a decreasing trend in mouth-feel firmness with increasing oil droplet size from $W_{0.2}$-100 to W_4-100. At a given oil droplet size, the emulsion gels containing 100 mM NaCl always had higher mouth-feel firmness ratings than the emulsion gels containing 10 mM NaCl ($P < 0.05$).

Mouth-feel firmness was found to be positively correlated with the final storage modulus (r = 0.95, $P < 0.05$), Young's modulus (r = 0.98, $P < 0.05$), fracture stress (r = 0.99, $P < 0.05$), fracture modulus (r = 0.94, $P < 0.05$) and fracture energy (r = 0.98, $P < 0.05$) and to be negatively correlated with fracture strain (r = –0.82, $P < 0.05$). Similarly, Liu, Stieger, van der Linden, and van de Velde (2015) tested emulsion-filled gels that differed in fat content and reported that the mouth-feel attributes (i.e. MF-firm, MF-elastic etc.) were positively related to the physical properties.

4.3.5 Mouth burn intensity ratings of CAP-loaded emulsion gels

Table 4-3 presents the perceived mouth burn intensity ratings of the CAP-loaded emulsion gels that were obtained from the 18 human subjects. At 10 mM NaCl, $W_{0.2}$-10 had the highest mouth burn intensity ($P < 0.05$); there was no significant difference between W_4-10, W_1-10 and $W_{0.5}$-10. At 100 mM NaCl, the mouth burn perception gradually increased with increasing oil droplet size from $W_{0.2}$-100 to W_4-100. At a given oil droplet size, W_4-10 and W_1-10 had lower mouth burn perception than W_4-100 and W_1-100 respectively ($P < 0.05$), whereas $W_{0.2}$-10 had greater mouth burn perception than $W_{0.2}$-100 ($P < 0.05$); there was no significant difference between $W_{0.5}$-10 and $W_{0.5}$-100.

Table 4-3 Mouth-feel firmness and mouth burn perception ratings of CAP-loaded emulsion gels W_4-10 (d4,3 ~ 4 μm with 10 mM NaCl), W_1-10 (d4,3 ~ 1 μm with 10 mM NaCl), $W_{0.5}$-10 (d4,3 ~ 0.5 μm with 10 mM NaCl), $W_{0.2}$-10 (d4,3 ~ 0.2 μm with 10 mM NaCl), W_4-100 (d4,3 ~ 4 μm with 100 mM NaCl), W_1-100 (d4,3 ~ 1 μm with 100 mM NaCl), $W_{0.5}$-100 (d4,3 ~ 0.5 μm with 100 mM NaCl), $W_{0.2}$-100 (d4,3 ~ 0.2 μm with 100 mM NaCl). Results are shown as mean ± standard deviation obtained from 18 human participants.

Emulsion gel sample	Mouth-feel firmness	Mouth burn perception
W_4-10	$111.4 \pm 7.4^{a,x}$	$93.9 \pm 13.4^{a,x}$
W_1-10	$118.3 \pm 9.6^{a,x}$	$86.7 \pm 14.7^{a,x}$
$W_{0.5}$-10	$66.2 \pm 5.1^{b,x}$	$102.0 \pm 13.8^{a,x}$
$W_{0.2}$-10	$16.8 \pm 2.6^{c,x}$	$251.4 \pm 13.4^{b,x}$
W_4-100	$167.8 \pm 14.1^{a,y}$	$194.7 \pm 19.4^{ab,y}$
W_1-100	$218.1 \pm 23.0^{ab,y}$	$145.9 \pm 19.4^{bc,y}$
$W_{0.5}$-100	$270.3 \pm 27.6^{b,y}$	$120.0 \pm 19.4^{cd,x}$
$W_{0.2}$-100	$327.8 \pm 30.1^{b,y}$	$88.4 \pm 19.4^{d,y}$

[a-d]Values with different letters within the same NaCl concentration group differ significantly ($P < 0.05$).

[x,y]Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

4.3.6 Correlation between degree of fragmentation of the masticated gel bolus and the mouth burn perception of CAP-loaded emulsion gels

Figure 4-5 presents the correlation between the degree of fragmentation of the masticated gel bolus and the mouth burn perception ($r = -0.72$, $P = 0.04$). The degree of fragmentation, denoted as d_{frag} (Table 4-2), can be used to represent the surface area of the gel fragments exposed during mastication (i.e. the new surface area created by mastication and fragmentation). A power-law fit between d_{frag} and mouth burn perception was established, i.e. $Y = 119.5X^{-0.25}$, with $R^2 = 0.76$. This indicates that the increase in surface exposure during mastication would generally lead to an increase in mouth burn

perception. At higher degree of fragmentation (i.e. smaller d_{frag}), samples are more sensitive to changes in d_{frag} because a small increase in the degree of fragmentation may lead to a significant increase in the mouth burn perception. On the other hand, samples with lower degree of fragmentation (i.e. larger d_{frag}) are broken down mainly into large particles; the role of the degree of fragmentation in mouth burn perception becomes less critical, possibly because the differences caused by surface exposure are quite small and not detectable by human subjects.

Similarly, Hills and Harrison (1995) established the relationship between food fragmentation and flavour release based on the stagnant two-film theory; they reported that flavour release would increase exponentially with an increase in surface exposure. They used a simple food model – a boiled sweet with homogeneous composition and structure. They also used a theoretical exponential fragmentation model, in which a food cube of side L would fragment into eight cubes of side L/2 after one bite. However, real foods will have a more complicated composition, structure and fragmentation behaviour.

In this study, the semi-solid gel $W_{0.2}$-10 had the highest degree of fragmentation and gave the highest mouth burn perception score. The soft and elastic gels W_4-10, W_1-10 and $W_{0.5}$-10 had lower degree of fragmentation; d_{frag} decreased from W_4-10 to $W_{0.5}$-10, but presented similar scores for mouth burn perception, indicating that the differences caused by surface exposure were quite small and not detectable by human subjects. The hard and brittle gels W_4-100, W_1-100, $W_{0.5}$-100 and $W_{0.2}$-100 had intermediate degree of fragmentation. They had similar d_{frag}s, but presented decreasing mouth burn perception scores from W_4-100 to $W_{0.2}$-100. Emulsion gels W_4-100 and $W_{0.2}$-100 had started to deviate from the fitted curve. This indicates that mouth burn perception was not solely dependent on food fragmentation behaviour and surface exposure. The lipophilic

capsaicinoid molecules were probably heterogeneously distributed in the emulsion gel, i.e. mainly within the oil phase. This may have caused the deviation of emulsion gels W_4-100 and $W_{0.2}$-100 from the fitted curve.

It should be noted that the detection thresholds of capsaicin were 0.3 mg/L in water and 11.75 mg/L in vegetable oil (Lawless et al., 2000). Moreover, the solubility of capsaicinoids in water was 60 mg/L (Turgut et al., 2004). This indicates that, if capsaicinoid molecules were diffused into the aqueous phase, the mouth burn perception would be greatly magnified.

The release of capsaicinoids from these emulsion gels can be simplified into three sequential steps: (1) movement of capsaicinoid molecules across the oil–aqueous interface; (2) movement through the gelled protein aqueous phase towards the surface of the gel particles; (3) movement across the solid–air interface or the solid–liquid (i.e. saliva) interface (Mao et al., 2017). The driving force for the first step will be the non-equilibrium concentration gradient of capsaicinoid molecules between the different phases. The third step is positively related to the surface exposure during mastication, which can be represented by the d_{frag} of the masticated gel bolus. Therefore, with a lower d_{frag} (i.e. higher degree of fragmentation), the release rate of capsaicinoid molecules will increase because there is more surface exposure, which will increase the possibility of capsaicinoid molecules being released.

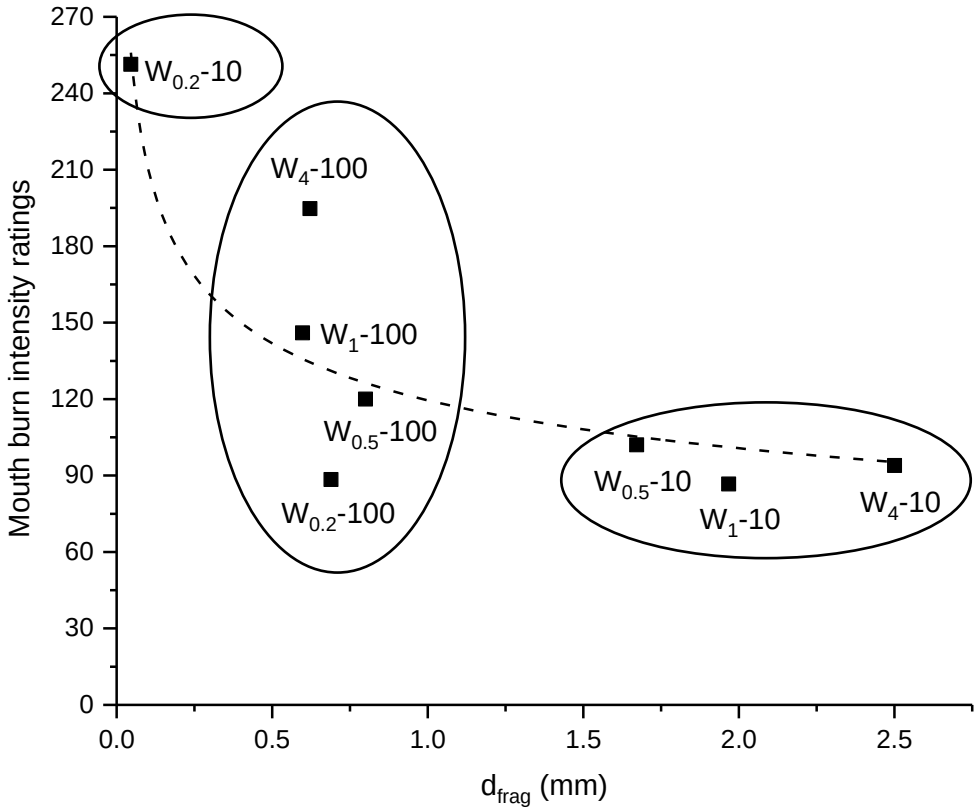


Figure 4-5 Correlation between d_{frag} of masticated gel boluses and mouth burn perception of CAP-loaded emulsion gels W_4-10 (d4,3 ~ 4 µm with 10 mM NaCl), W_1-10 (d4,3 ~ 1 µm with 10 mM NaCl), $W_{0.5}$-10 (d4,3 ~ 0.5 µm with 10 mM NaCl), $W_{0.2}$-10 (d4,3 ~ 0.2 µm with 10 mM NaCl), W_4-100 (d4,3 ~ 4 µm with 100 mM NaCl), W_1-100 (d4,3 ~ 1 µm with 100 mM NaCl), $W_{0.5}$-100 (d4,3 ~ 0.5 µm with 100 mM NaCl), $W_{0.2}$-100 (d4,3 ~ 0.2 µm with 100 mM NaCl).

4.3.7 Correlation between mouth burn perception and mouth-feel firmness of CAP-loaded emulsion gels

Figure 4-6 presents the correlation between mouth burn perception and mouth-feel firmness ratings of the CAP-loaded emulsion gels. At both 10 mM NaCl and 100 mM NaCl, mouth burn perception was negatively correlated with mouth-feel firmness ($r < -0.90$, $P < 0.10$). As mouth-feel firmness was positively correlated with the rheological properties, these results also indicate that mouth burn perception was negatively correlated with the rheological properties, such as the final storage modulus, Young's modulus and fracture modulus.

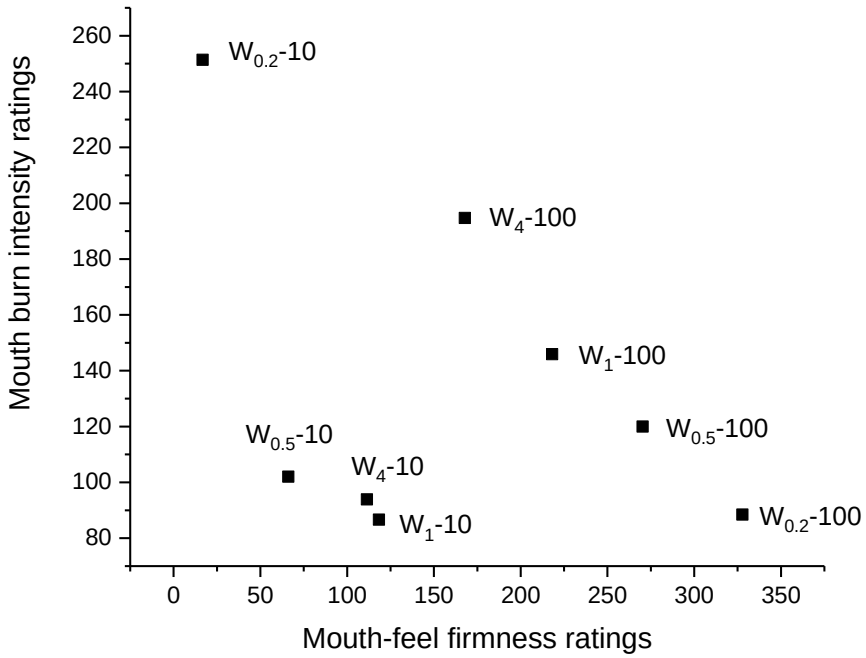


Figure 4-6 Correlation between mouth-feel firmness ratings and perceived mouth burn intensity ratings of CAP-loaded emulsion gels W_4-10 (d4,3 ~ 4 μm with 10 mM NaCl), W_1-10 (d4,3 ~ 1 μm with 10 mM NaCl), $W_{0.5}$-10 (d4,3 ~ 0.5 μm with 10 mM NaCl), $W_{0.2}$-10 (d4,3 ~ 0.2 μm with 10 mM NaCl), W_4-100 (d4,3 ~ 4 μm with 100 mM NaCl), W_1-100 (d4,3 ~ 1 μm with 100 mM NaCl), $W_{0.5}$-100 (d4,3 ~ 0.5 μm with 100 mM NaCl), $W_{0.2}$-100 (d4,3 ~ 0.2 μm with 100 mM NaCl).

The role of gel hardness in mouth burn perception can be explained by two mechanisms. The first mechanism is the diffusion of the capsaicinoid molecules. As mentioned above, the release of capsaicinoid molecules can be characterized into three sequential steps. The second step, which is the mass transfer or diffusion of capsaicinoid molecules through the gel matrix, is negatively related to the rheological properties of food and can be described by the Stokes-Einstein equation. The Stokes-Einstein equation is used with fluids with low Reynolds number (i.e. laminar flow is dominant) and for the diffusion of spherical particles. The equation can be written as Equation 4-1 (Dill & Bromberg, 2003):

$$D = \frac{k_B T}{6\pi\eta r} \qquad \textbf{Equation 4-1}$$

where D is the diffusion coefficient or 'diffusivity', k_B is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity and r is the radius of the spherical particle. The equation can be applied to the present work, even though the capsaicinoid molecules are not spherical. The diffusion coefficient D is solely dependent on the dynamic viscosity η, because all the other parameters are constant; the higher is the dynamic viscosity, the lower is the diffusivity. The dynamic viscosity η, also known as shear viscosity, describes the resistance of a fluid to shearing flows. It can be expressed as: $\eta = \frac{\tau}{\dot{\gamma}}$, where τ is the shear stress and $\dot{\gamma}$ is the shear rate. Soft solids, such as emulsion gels, respond partially viscously and partially elastically to an applied force. Table 4-4 presents the results for the final shear storage modulus G', final shear loss modulus G'' and final complex viscosity η^* of CAP-loaded emulsion gels under oscillatory shearing conditions in the linear viscoelastic region. The final dynamic viscosity η' and the elastic viscosity η'' can then be calculated based on the following Equations 4-2 to 4-4 (Angle,

Clarke, & Dabros, 2017; Barnes, Hutton, & Walters, 1989; Boger, 2009; Tadros, 1980; Zaman, 1998):

$$\eta' = \frac{G''}{\omega} \qquad \textbf{Equation 4-2}$$

$$\eta'' = \frac{G'}{\omega} \qquad \textbf{Equation 4-3}$$

$$|\eta^*| = \sqrt{\eta'^2 + \eta''^2} \qquad \textbf{Equation 4-4}$$

where ω is the angular frequency, which equals 6.28 rad/s because the frequency was set at 1 Hz for the present work ($\omega = 2\pi f$, f is the frequency in Hz). The calculated final η' and η'' are also presented in Table 4-4. The results show that the dynamic viscosity is positively correlated with the storage modulus (r = 1.00, P < 0.0001). Therefore, from Equation 4-1, it can be concluded that the diffusion rate of capsaicinoid molecules through the gel matrix is negatively proportional to the dynamic viscosity or the hardness of the gel. Similar results have been reported for polymer gels: the release rate of flavour compounds was slower with increasing gel hardness (Baek, Linforth, Blake, & Taylor, 1999; Bakker et al., 1996; Carr et al., 1996; Guinard & Marty, 1995; Hansson, Giannouli, & van Ruth, 2003).

The second mechanism of food hardness affecting mouth burn perception is the cross-modal integration theory from a neurological point of view, i.e. an increase in one attribute may lead to enhancement or suppression in another attribute (Driver & Spence, 2000). In the present work, an increase or decrease in mouth-feel firmness led to a decrease or increase in mouth burn perception of the CAP-loaded emulsion gels, or vice versa. Cerf-Ducastel et al. (2001) investigated the interactions of gustatory, somatosensory and olfactory stimuli at the cortical level and provided neurological evidence that there was a clear convergent effect of the gustatory, somatosensory and

olfactory stimulations, which together constitute the neural substrate for a multimodal representation of sensory information. As sensory pathways widely overlap from the very periphery, the gustatory nerves may respond to both taste and temperature or tactile stimulations and the same stimulus may elicit both gustatory and somatosensory responses. Therefore, when a food with a multimodal percept is presented, various sensory stimuli (in this work, both texture stimulus and trigeminal stimulus) would give signals that share common neural pathways. The signals would be integrated and interpreted by the brain through a logical process; however, the signals would interact and the perception from one stimulus would be influenced by that from another.

Table 4-4 Final shear storage modulus G', final shear loss modulus G'', final complex viscosity η^* measured by small strain oscillatory rheology, and calculated final dynamic viscosity η' and calculated final elastic viscosity η'' of CAP-loaded emulsion gels W_4-10 (d4,3 ~ 4 µm with 10 mM NaCl), W_1-10 (d4,3 ~ 1 µm with 10 mM NaCl), $W_{0.5}$-10 (d4,3 ~ 0.5 µm with 10 mM NaCl), $W_{0.2}$-10 (d4,3 ~ 0.2 µm with 10 mM NaCl), W_4-100 (d4,3 ~ 4 µm with 100 mM NaCl), W_1-100 (d4,3 ~ 1 µm with 100 mM NaCl), $W_{0.5}$-100 (d4,3 ~ 0.5 µm with 100 mM NaCl), $W_{0.2}$-100 (d4,3 ~ 0.2 µm with 100 mM NaCl). Results are shown as mean ± standard deviation of N = 3 independent experiments.

Emulsion gel type	Final G' (kPa)	Final G'' (kPa)	Final η^* (kPa·s)	Final η' (kPa·s)	Final η'' (kPa·s)
W_4-10	$2.52 \pm 0.12^{a,x}$	$0.26 \pm 0.01^{a,x}$	$0.40 \pm 0.02^{a,x}$	$0.04 \pm 0.00^{a,x}$	$0.40 \pm 0.02^{a,x}$
W_1-10	$2.92 \pm 0.42^{a,x}$	$0.34 \pm 0.02^{b,x}$	$0.47 \pm 0.07^{a,x}$	$0.05 \pm 0.00^{b,x}$	$0.46 \pm 0.07^{a,x}$
$W_{0.5}$-10	$0.84 \pm 0.09^{b,x}$	$0.18 \pm 0.00^{c,x}$	$0.14 \pm 0.01^{b,x}$	$0.03 \pm 0.00^{c,x}$	$0.13 \pm 0.01^{b,x}$
$W_{0.2}$-10	$0.22 \pm 0.12^{c,x}$	$0.03 \pm 0.02^{d,x}$	$0.04 \pm 0.02^{c,x}$	$0.005 \pm 0.00^{d,x}$	$0.04 \pm 0.02^{c,x}$
W_4-100	$64.60 \pm 0.62^{a,y}$	$7.51 \pm 0.07^{a,y}$	$10.37 \pm 0.12^{a,y}$	$1.20 \pm 0.01^{a,y}$	$10.28 \pm 0.10^{a,y}$
W_1-100	$109.67 \pm 6.81^{b,y}$	$12.50 \pm 0.72^{b,y}$	$17.60 \pm 1.08^{b,y}$	$1.99 \pm 0.12^{b,y}$	$17.45 \pm 1.08^{b,y}$
$W_{0.5}$-100	$142.00 \pm 8.48^{c,y}$	$15.80 \pm 0.99^{c,y}$	$22.75 \pm 1.34^{c,y}$	$2.52 \pm 0.16^{c,y}$	$22.60 \pm 1.35^{c,y}$
$W_{0.2}$-100	$154.50 \pm 4.93^{c,y}$	$16.92 \pm 0.67^{c,y}$	$24.75 \pm 0.82^{c,y}$	$2.69 \pm 0.11^{c,y}$	$24.59 \pm 0.78^{c,y}$

$^{a–d}$Values with different letters within the same NaCl concentration group differ significantly ($P < 0.05$).

^{x,y}Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

4.3.8 Other factors affecting mouth burn intensity

Figure 4-7 presents the correlations between the oil droplet release from the gel matrix during mastication and the perceived mouth burn intensity ratings of CAP-loaded emulsion gels. At 100 mM NaCl (Fig. 4-7 B), the mouth burn perception was positively correlated with oil droplet release during mastication ($r = 0.96$, $P = 0.04$). As capsaicinoid molecules are lipophilic and are dissolved mostly in the oil phase, when oil droplets were liberated from the gel matrix, the third step (i.e. movement of capsaicinoid molecules through the gelled protein aqueous phase towards the surface of the particles) would be omitted. Consequently, more oil droplets being released would increase the possibility of capsaicinoid molecules being released. In contrast, for emulsion gels at 10 mM NaCl (Fig. 4-7 A), the mouth burn perception was weakly negatively correlated with the oil droplet release ($r = -0.43$, $P = 0.57$). Only 0.14% of the oil droplets were released from emulsion gel $W_{0.2}$-10 after mastication; this indicates that, even though emulsion gel $W_{0.2}$-10 had a very high degree of fragmentation, the oil droplets with average droplet size $d_{4,3}$ of ~ 0.2 μm were bound strongly within the protein matrix after fragmentation, such that there was almost no oil droplet release during mastication. Therefore, in the present work, we were able to conclude that the effect of oil droplet release after mastication on mouth burn perception was much less critical than other factors such as the degree of fragmentation and the gel hardness.

At a given oil droplet size, emulsion gels W_4-100 and W_1-100 had higher mouth burn perception than emulsion gels W_4-10 and W_1-10 respectively ($P < 0.05$), which can be attributed to the differences in their salt concentration (i.e. differences in saltiness perception). Studies have shown that higher saltiness can lead to greater release of flavour compounds in emulsions (Benjamin, Silcock, Leus, & Everett, 2012; Bortnowska,

2012; Poll & Flink, 1984; Rabe, Krings, & Berger, 2003). This phenomenon, in which the addition of salts enhances the flavour perception, is termed 'salting-out'. The proposed mechanism is that salts can affect the interactions between flavour compounds and proteins (Pérez-Juan, Flores, & Toldrá, 2007). However, as the capsaicinoid molecules were dissolved mostly in the oil phase, the 'salting-out' effect was minor compared with other factors.

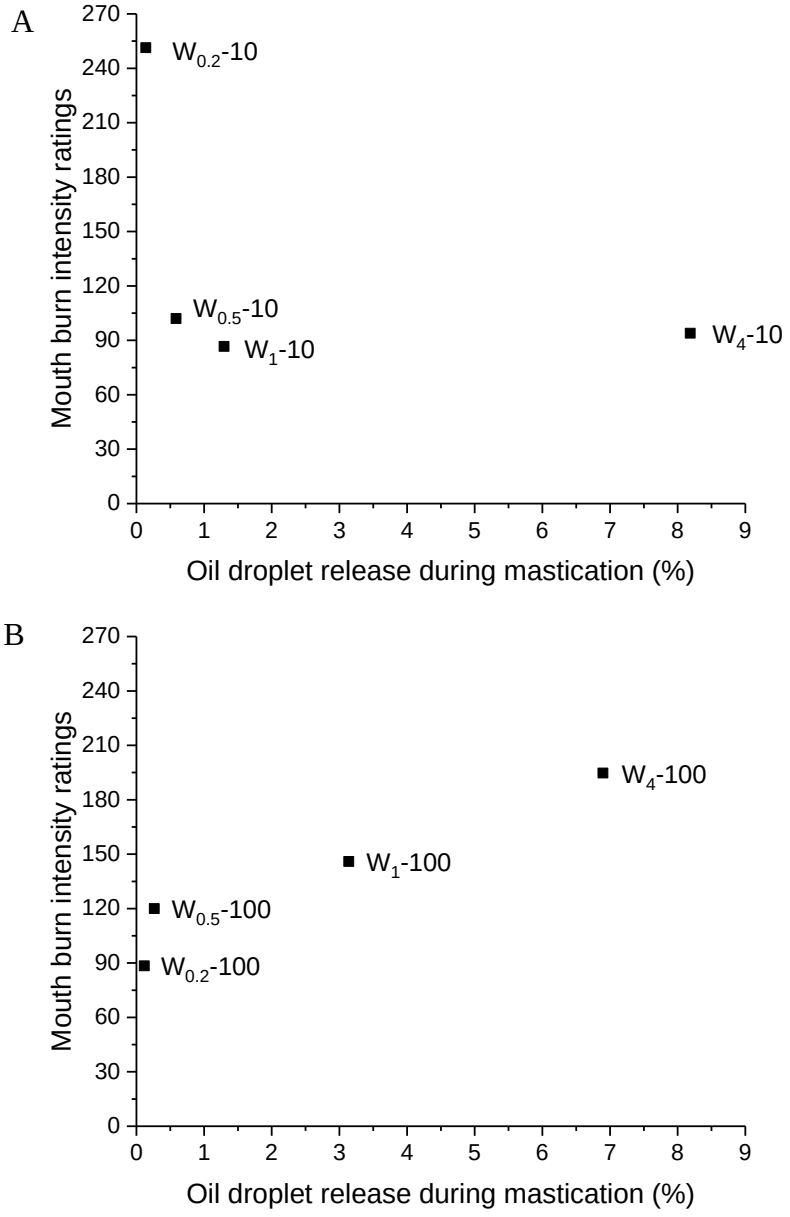


Figure 4-7 Correlation between oil droplet release from gel matrix after mastication and perceived mouth burn intensity ratings of CAP-loaded emulsion gels. (A) Emulsion gels containing 10 mM NaCl; (B) Emulsion gels containing 100 mM NaCl.

4.4 Conclusions

In this study, the oral processing behaviour and sensory perception of CAP-loaded whey protein emulsion gels with different structures and rheological properties was investigated. The results suggest that a greater hardness would generally lead to a lower particle size of the masticated gel bolus. The semi-solid emulsion gel was very soft and had the lowest bolus particle size. The soft and elastic gels were easy to deform and flow; they produced large bolus particles during mastication. The hard and brittle gels required greater chewing effort (i.e. higher fracture energy, more chewing cycles) and produced small bolus particles for easy flowability and safe swallowing. The relationship between gel hardness (represented by Young's modulus) and bolus particle size can be described by a power-law equation: $Y = 25.38X^{-0.29}$, with R^2 = 0.69, indicating that gel hardness is the critical factor for determining bolus particle size. The mouth-feel firmness was significantly positively correlated with the rheological properties, such as Young's modulus. A power-law relationship between bolus particle size and mouth burn perception was established: $Y = 119.5X^{-0.25}$, with R^2 = 0.76. A higher degree of fragmentation led to greater surface exposure (i.e. new surface area created by fragmentation) during mastication, and therefore to higher mouth burn perception. Furthermore, the mouth burn perception was negatively correlated with the rheological properties. The higher was the hardness, the lower was the diffusivity of capsaicinoid molecules through the gelled particles and the lower was the mouth burn perception. The effects of oil droplet release after mastication and saltiness perception on mouth burn perception were also considered; however, they were less critical than the degree of fragmentation and the food hardness. This work has provided useful information, such that, by manipulating the structure and rheology of food, the sensory perception of food can be tailored, through multiple mechanisms.

Chapter 5: In-mouth breakdown and sensory perception of emulsion gels containing active or inactive filler particles loaded with capsaicinoids

5.1 Abstract

This work investigated the effects of active versus inactive filler particles on the structure, rheological properties, *in vivo* oral breakdown behaviour and sensory perception of emulsion gels containing capsaicinoids (CAP). CAP-loaded Tween-80-coated emulsion droplets were not bound with the whey protein matrix (i.e. emulsion gels containing inactive filler particles) and appeared to be flocculated and inhomogeneously distributed in the gel matrix, which led to drastically decreased mechanical gel strength. CAP-loaded Tween 80 emulsion gels had significantly lower fracture stress and fracture stain than CAP-loaded whey protein emulsion gels (emulsion gels containing active filler particles), indicating that they were more easily broken down into small fragments in the mouth at small deformations with less chewing (i.e. lower fracture energy, fewer chewing cycles and shorter duration). During mastication, large amounts of oil droplets were released from the gel matrix of the CAP-loaded Tween 80 emulsion gels, which also contributed to their significantly smaller bolus particle size. The released oil droplets from the CAP-loaded Tween 80 emulsion gels facilitated bolus formation through lubrication and decreased the chewing cycles. A higher degree of fragmentation led to greater surface exposure (i.e. new surface area created) during mastication, and therefore to higher mouth burn perception. The large amounts of oil droplets released from the gel matrix during mastication and the inhomogeneous distribution of the oil droplets because of flocculation of the CAP-loaded Tween 80

emulsion gels also contributed to their higher mouth burn perception. The oral processing behaviour of the CAP-loaded emulsion gels and the sensory perception of capsaicinoids could be tuned by manipulating the droplet-matrix interactions and the gel structure.

5.2 Introduction

Capsaicinoids, the lipophilic bioactive compounds present in many peppers from the genus *Capsicum*, are responsible for causing the sensory responses of burning, pain and irritation when peppers are consumed. The beneficial activities of capsaicinoids include analgesic, anti-carcinogenic and anti-inflammatory, and capsaicinoids have beneficial influences on the gastrointestinal system (Srinivasan, 2016). However, their low water solubility and their acute burning sensation have limited their use in many food, nutraceutical and pharmaceutical applications. In this study, we used an emulsion gel as the delivery system for capsaicinoids, in which the capsaicinoids were dissolved in the emulsion droplets, to achieve increased solubility and reduced mouth burn perception (Lawless et al., 2000).

The use of an emulsion gel as a model system for solid/semi-solid foods has been extensively studied, with respect to its structure, rheology, breakdown behaviour in the human mouth and subsequent gastrointestinal digestion (Chen & Dickinson, 1998, 1999; Guo et al., 2014a, 2014b, 2016; Gwartney et al., 2004; Ye & Taylor, 2009). By controlling the structure and rheology of the emulsion gel, it is possible to obtain gels with tailor-made structures that would have different behaviours in the human mouth; the gels would be broken down in a controlled manner and thus realize controlled release of the incorporated components (Mao et al., 2017; Mao et al., 2014). An emulsion gel is a complex colloidal system that exists as both an emulsion and a gel (Dickinson, 2012). Both an emulsifying agent and a gelling/thickening agent are required for its formation.

The emulsifying agent and the gelling/thickening agent can be either the same or different, and this has a significant impact on the properties of the emulsion gel. The filler particles of the emulsion gel, being the oil droplets, affect the rheological properties of the emulsion gel in several ways: the size and concentration of the filler particles; the mechanical properties of the filler particles; the nature of the filler particle–gel matrix interaction (Tolstoguzov & Braudo, 1983; van Vliet, 1988).

Based on the nature of the filler–matrix interaction, the filler particles can be classified as either active or inactive depending on their contribution to the rheology of the network (Ring & Stainsby, 1985). Active fillers are bound to the gel matrix (strong interaction), and thus contribute to gel strength; inactive fillers are not bound to the gel matrix (weak or no interaction), and thus act as defects to the network and lead to a monotonic decrease in gel strength (Chen et al., 2000; Jampen et al., 2001). In addition, phase separation of the oil droplets has been observed in emulsion gels with inactive filler particles, which was caused by depletion flocculation (Chen et al., 2000; Kerstens et al., 2006). This would lead to an inhomogeneous distribution of the oil droplets in the gel matrix, which could be another contributing factor to the decrease in gel strength.

The oil droplet release from emulsion gels containing either active or inactive filler particles and their sensory perception have been studied by Sala, van de Velde, Cohen Stuart, and van Aken (2007b). They found that the gels with inactive filler particles had higher sensory scores such as creaminess, possibly due to higher oil droplet release from these gels upon shearing. However, they also concluded that oil droplet release was not the main mechanism for creaminess perception in emulsion-filled gels as an increase in creaminess perception was also observed in emulsion gels containing active filler particles with increasing oil content. Moreover, the method they used to measure oil

droplet release under shear was to squeeze the gels in a syringe. In our study, we aimed to establish the inter-relationship between the structure and rheology of the gel, the in-mouth breakdown behaviour (i.e. oil droplet release during mastication; masticated gel bolus particle size) and the sensory perception of emulsion gels containing either active or inactive filler particles.

In Chapter 4, we investigated the effects of oil droplet size and NaCl concentration on the oral processing behaviour and mouth burn perception of whey protein emulsion gels containing capsaicinoids. The gels were all characterized as emulsion gels with active filler particles because the protein-coated oil droplets had strong interactions with the protein gel matrix. Results showed that for emulsion gels with active filler particles, the oil droplet release during mastication was very low and did not affect the gel breakdown behaviour and sensory perception. The masticated bolus particle size was negatively correlated with Young's modulus while the perceived mouth burn perception was negatively correlated with masticated bolus particle size.

In this study, we aimed to investigate the effect of active versus inactive filler particles on the rheological behaviour, oral processing behaviour and sensory perception of emulsion gels containing capsaicinoids. The CAP-loaded whey protein emulsion gels (i.e. whey proteins as both emulsifier and gelling agent, oil droplet size $d_{4,3}$ of ~ 1, 0.5 or 0.2 μm, 100 mM NaCl) were characterized as gels with active filler particles, the CAP-loaded Tween 80 emulsion gels (i.e. Tween 80 as emulsifier, whey proteins as gelling agent, oil droplet size $d_{4,3}$ of ~ 1, 0.5 or 0.2 μm, 100 mM NaCl) were characterized as gels with inactive filler particles. Both gels systems were used in this study. The hypothesis is that inactive filler particles in emulsion gels containing capsaicinoids could cause a drastic decrease in gel strength, which could lead to much less chewing effort

and a smaller bolus particle size; also, the gels with inactive filler particles would release more oil droplets during mastication, all of which would contribute to a greater mouth burn perception, as compared to emulsion gels containing active filler particles.

This study would provide knowledge on designing food structure to increase the incorporation of bioactive compounds in foods, especially those with undesirable flavours; while maintaining acceptable sensory perception.

5.3 Results and discussion

5.3.1 Large deformation properties

Table 5-1 presents the large deformation properties of the CAP-loaded whey protein emulsion gels and the CAP-loaded Tween 80 emulsion gels at 100 mM NaCl. For both gel systems, with decreasing oil droplet size, Young's modulus, fracture stress, fracture modulus and fracture energy gradually increased. At a given oil droplet size, the CAP-loaded whey protein emulsion gels always had higher Young's modulus, fracture stress, fracture modulus and fracture energy ($P < 0.05$) than the CAP-loaded Tween 80 emulsion gels. The mechanisms are well-established in literature (Langley & Green, 1989; McClements et al., 1993; Sala et al., 2009).

For CAP-loaded whey protein emulsion gels, there was no significant difference in fracture strain between gels with different oil droplet sizes. van Vliet and Walstra (1995) established the starting point for the theory on fracture mechanics that all the materials are inhomogeneous; all materials contain tiny cracks or weak spots (defects). Upon compression, stress is concentrated around the defects and eventually lead to fracture. In emulsion-filled gel systems, the defects could be the filler particles, i.e. the oil droplets, or the intrinsic defects presented in the gel matrix. The change in oil droplet size did not

lead to change in fracture strain for CAP-loaded whey protein emulsion gels is probably because that the length of the intrinsic defects presented in whey protein isolate gel (without the oil droplets) is larger than the oil droplet size used in this study. Therefore, during compression, fracture initiated from the intrinsic defects in whey protein gel matrix, rather than the oil droplets. Sala, Van Aken, Stuart, and Van De Velde (2007a) have reported similar findings in cold-set whey protein gel systems. Kim, Renkema, and Van Vliet (2001) have also reported that the oil droplet size did not affect fracture strain significantly in soybean protein isolate emulsion-filled gels, and they deduced that the intrinsic defect length of the soybean protein isolate gel is larger than 10 μm (which was the largest oil droplet size observed in their systems), which explained why when oil droplet size was smaller than 10 μm, it did not affect fracture strain significantly.

As explained above, if the intrinsic defect length in whey protein gel matrix is larger than the oil droplet size, then the use of either whey proteins or Tween 80 as emulsifier would not cause a significant difference in fracture strain. However, at a given oil droplet size, the CAP-loaded whey protein emulsion gels always had higher fracture strain than the CAP-loaded Tween 80 emulsion gels ($P < 0.05$; Table 5-1). This observation is most probably caused by flocculation / aggregation of oil droplets in CAP-loaded Tween 80 emulsion gels (Fig. 5-2 A: CAP-loaded Tween 80 emulsion gels and Fig. 5-2 B: CAP-loaded whey protein emulsion gels). The possible explanation is that the size of the oil droplet flocs presented in the CAP-loaded Tween 80 emulsion gels have exceeded the size of the intrinsic defects presented in whey protein gel matrix, and therefore existed as weaker points, causing fracture at a lower strain.

Table 5-1 Large deformation properties of CAP-loaded whey protein emulsion gels [W_1-100 ($d_{4,3}$ ~ 1 µm), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm), $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm)] and CAP-loaded Tween 80 emulsion gels [T_1-100 ($d_{4,3}$ ~ 1 µm), $T_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm), $T_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm)]. All emulsion gels contain 100 mM NaCl. Results are shown as mean ± standard deviation of N = 6 independent experiments.

Emulsion gel type	Young's modulus (kPa)	Fracture stress (kPa)	Fracture strain	Fracture modulus (kPa)	Fracture energy (mJ)
W_1-100	$106.8 \pm 1.9^{a,x}$	$88.0 \pm 2.9^{a,x}$	$0.87 \pm 0.04^{a,x}$	$101.6 \pm 6.2^{a,x}$	$417.7 \pm 20.2^{a,x}$
$W_{0.5}$-100	$137.3 \pm 5.9^{b,x}$	$121.7 \pm 10.0^{b,x}$	$0.90 \pm 0.04^{a,x}$	$135.9 \pm 8.7^{b,x}$	$617.2 \pm 60.9^{b,x}$
$W_{0.2}$-100	$174.4 \pm 3.3^{c,x}$	$152.3 \pm 3.8^{c,x}$	$0.94 \pm 0.05^{a,x}$	$161.4 \pm 9.3^{c,x}$	$854.5 \pm 55.5^{c,x}$
T_1-100	$44.4 \pm 1.7^{a,y}$	$29.1 \pm 0.6^{a,y}$	$0.70 \pm 0.03^{a,y}$	$41.9 \pm 2.7^{a,y}$	$101.6 \pm 6.9^{a,y}$
$T_{0.5}$-100	$51.6 \pm 2.0^{b,y}$	$33.7 \pm 1.4^{b,y}$	$0.69 \pm 0.02^{a,y}$	$49.0 \pm 1.9^{b,y}$	$118.6 \pm 8.1^{b,y}$
$T_{0.2}$-100	$58.4 \pm 2.1^{c,y}$	$37.4 \pm 2.3^{c,y}$	$0.70 \pm 0.04^{a,y}$	$53.3 \pm 5.1^{b,y}$	$142.2 \pm 9.8^{c,y}$

$^{a–c}$Values with different letters within the same emulsifier group differ significantly ($P < 0.05$).

^{x,y}Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

5.3.2 SLCM images of CAP-loaded emulsions and emulsion gels

Figure 5-1 shows CLSM images of the CAP-loaded Tween 80 emulsions before and after mixing with protein solution, and the CAP-loaded whey protein emulsions. The oil droplets in the CAP-loaded Tween 80 stock emulsions (30 wt% oil phase) were homogeneously distributed and no flocculation was observed (Fig. 5-1 A). After mixing with whey protein solution, the oil droplets were found to be flocculated (Fig. 5-1 B). This flocculation was probably depletion flocculation, caused by the excess whey protein in the aqueous phase (Kerstens et al., 2006). Figure 5-2 shows CLSM images of the CAP-loaded Tween 80 emulsion gels at 100 mM NaCl and the CAP-loaded whey protein emulsion gels at 100 mM NaCl. After heat-set gelation, all the CAP-loaded Tween 80 emulsion gels at 100 mM NaCl showed a discontinuous protein network (green colour) with pores (black colour); the oil droplets (red colour) still appeared to be in a flocculated state and were unevenly distributed in the protein network (Fig. 5-2 A). The CAP-loaded whey protein emulsions appeared to be slightly flocculated (Fig. 5-1 C), because of the excess free protein in the aqueous phase; however, the flocs appeared to be much smaller than that of the CAP-loaded Tween 80 emulsions. This is in agreement with Kerstens et al. (2006), who stated that, with the presence of excess free protein in the aqueous phase, β-lactoglobulin-stabilized emulsions were slightly flocculated; however, the flocs was smaller than for the emulsions with both Tween 20 and excess free protein. After heat-set gelation, all the CAP-loaded whey protein emulsion gels showed discontinuous protein networks (green) with pores (black); the oil droplets appeared to be relatively evenly distributed in the protein matrix (Fig. 5-2 B).

When the CLSM images of the CAP-loaded Tween 80 emulsion gels and the CAP-loaded whey protein emulsion gels were compared (Figs. 5-2 A and B), the pores in the

protein network of the CAP-loaded Tween 80 emulsion gels appeared to be larger; this could have been caused by the presence of large flocs of oil droplets. These large pores acted as defects in the gel system and significantly decreased the gel strength. In addition, as Tween 80 is a non-ionic small-molecule surfactant, there were no interactions between the CAP-loaded Tween-80-coated emulsion droplets and the protein matrix. In contrast, for the CAP-loaded whey protein emulsion gels, the whey-protein-coated emulsion droplets would participate in gel formation upon heating, and therefore would contribute to the gel strength. From their rheological properties, gel microstructures, and the nature of the interactions between the emulsion droplets and the protein matrix, the CAP-loaded Tween 80 emulsion gels were characterized as gels with inactive filler particles and the CAP-loaded whey protein emulsion gels were characterized as gels with active filler particles (Dickinson, 2012).

The CAP-loaded emulsion gels had essentially the same oil droplet size distribution as the CAP-loaded emulsions (data not shown), confirming that there was no coalescence of the oil droplets during the preparation of the emulsion gels.

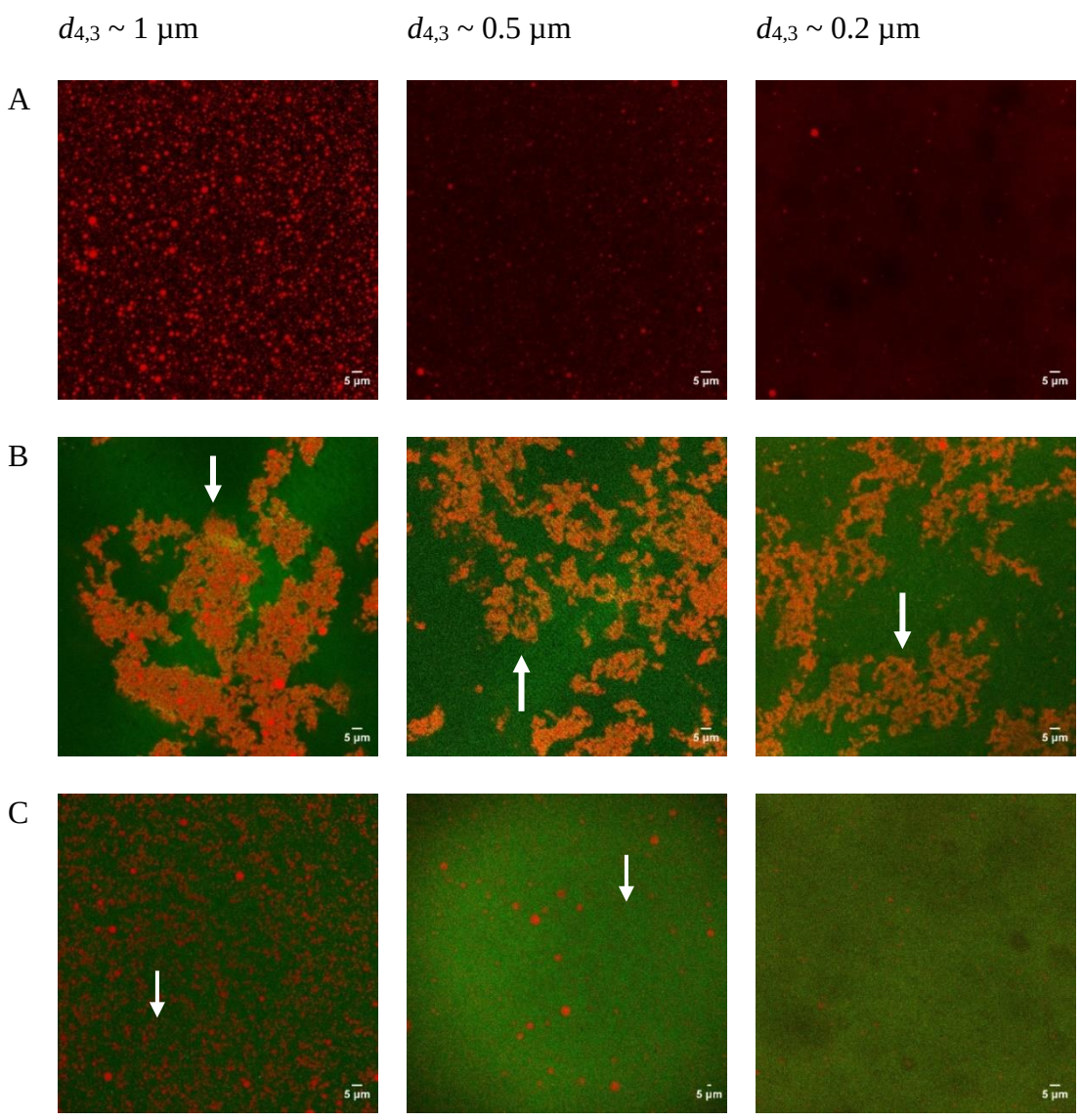


Figure 5-1 CLSM images of (A) CAP-loaded Tween 80 stock emulsions, (B) CAP-loaded Tween 80 emulsions after mixing with WPI stock solution and (C) CAP-loaded whey protein emulsions, with $d_{4,3}$ ~ 1, 0.5 and 0.2 µm. Arrows in Fig. 5-1 B and Fig. 5-1 C indicate examples of the flocs of the oil droplets. No indication for WPI emulsions with $d_{4,3}$ ~ 0.2 µm because the oil droplets were too small and therefore difficult to identify the flocs of the oil droplets.

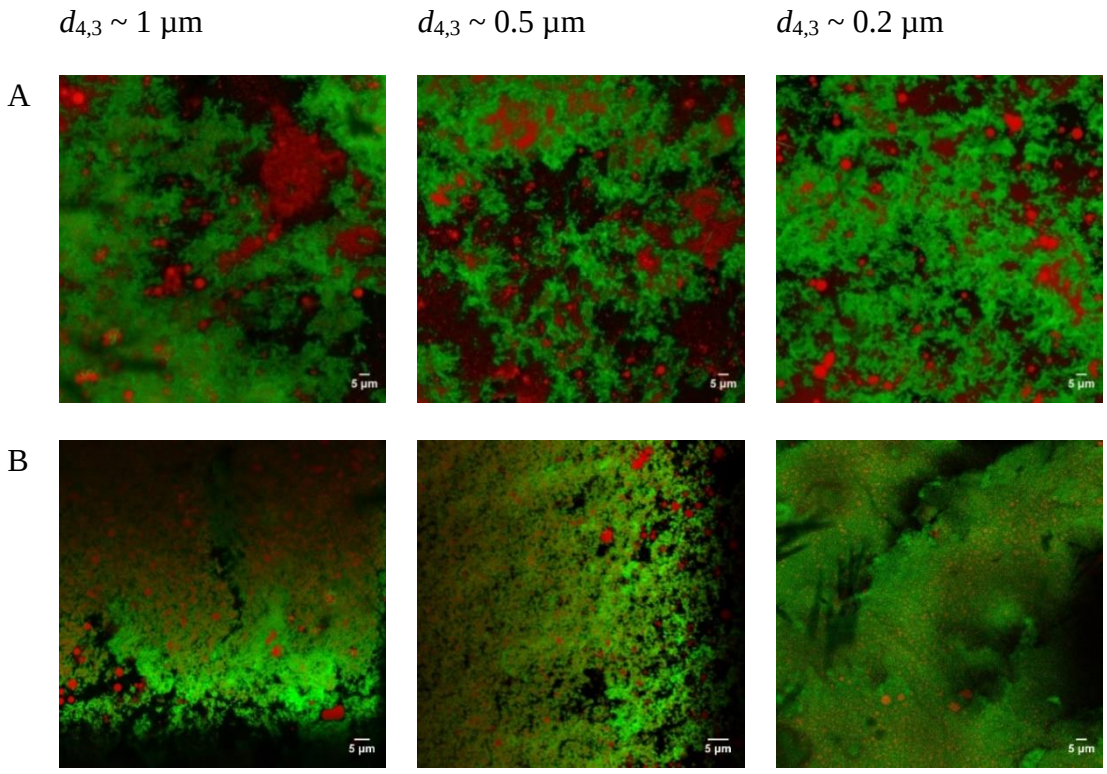


Figure 5-2 CLSM images of (A) CAP-loaded Tween 80 emulsion gels and (B) CAP-loaded whey protein emulsion gels, with $d_{4,3}$ ~ 1, 0.5 and 0.2 µm at 100 mM NaCl.

5.3.3 Oral processing behaviour

5.3.3.1 Mastication parameters

The mastication parameters of the CAP-loaded emulsion gels are presented in Table 5-2. The CAP-loaded whey protein emulsion gels had significantly greater numbers of chews and longer chewing durations than the CAP-loaded Tween 80 emulsion gels, probably because the whey protein emulsion gels had higher fracture stress. This is in agreement with previous studies that showed the number of chews is positively correlated with fracture stress (Engelen et al., 2005; Guo et al., 2013; Gwartney et al., 2004; Kohyama et al., 2017; Wilson & Brown, 1997).

For the CAP-loaded Tween 80 emulsion gels, there was no significant difference in the mastication parameters between samples with different oil droplet sizes. With decreasing oil droplet size from T_1-100 to $T_{0.2}$-100, fracture stress gradually increased ($P < 0.05$; Table 5-1); however, it did not significantly affect the number of chews or the chewing duration. This indicates that mastication is a complicated process and is not solely dependent on fracture stress. For instance, the presence of extra liquids would facilitate bolus formation and therefore would reduce the number of chewing cycles (van der Bilt et al., 2007; van Eck, et al., 2019b).

Table 5-2 Mastication parameters of CAP-loaded whey protein emulsion gels [W_1-100 ($d_{4,3}$ ~ 1 μm), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 μm), $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 μm)] and CAP-loaded Tween 80 emulsion gels [T_1-100 ($d_{4,3}$ ~ 1 μm), $T_{0.5}$-100 ($d_{4,3}$ ~ 0.5 μm), $T_{0.2}$-100 ($d_{4,3}$ ~ 0.2 μm)]. All emulsion gels contain 100 mM NaCl. Results of number of chews, chewing duration and chewing frequency are shown as mean ± standard error obtained from 18 human participants. Results of d_{frag} and oil droplet release are shown as mean ± standard deviation of N = 3 replicates for d_{frag}; N = 2 replicates for oil droplet release after mastication.

Emulsion gel type	Number of chews	Chewing duration (s)	Chewing frequency (s^{-1})	d_{frag} (mm)	Oil droplet release during mastication
W_1-100	$32.8 \pm 3.7^{a,x}$	$19.2 \pm 2.1^{a,x}$	$1.69 \pm 0.05^{a,x}$	$0.60 \pm 0.03^{a,x}$	$3.1 \pm 0.05\%^{a,x}$
$W_{0.5}$-100	$36.8 \pm 4.8^{ab,x}$	$22.2 \pm 2.1^{ab,x}$	$1.63 \pm 0.05^{a,x}$	$0.80 \pm 0.11^{a,x}$	$0.26 \pm 0.26\%^{b,x}$
$W_{0.2}$-100	$43.4 \pm 3.5^{b,x}$	$26.6 \pm 2.1^{b,x}$	$1.65 \pm 0.05^{a,x}$	$0.69 \pm 0.11^{a,x}$	$0.12 \pm 0.04\%^{b,x}$
T_1-100	$16.9 \pm 2.8^{a,y}$	$11.9 \pm 1.6^{a,y}$	$1.38 \pm 0.07^{a,y}$	$0.15 \pm 0.02^{a,y}$	$61.7 \pm 3.4\%^{a,y}$
$T_{0.5}$-100	$17.3 \pm 2.7^{a,y}$	$11.6 \pm 1.5^{a,y}$	$1.47 \pm 0.07^{a,x}$	$0.12 \pm 0.01^{a,y}$	$49.1 \pm 0.3\%^{a,y}$
$T_{0.2}$-100	$22.2 \pm 2.7^{a,y}$	$14.0 \pm 1.5^{a,y}$	$1.57 \pm 0.07^{a,x}$	$0.14 \pm 0.05^{a,y}$	$55.4 \pm 3.8\%^{a,y}$

^{a,b}Values with different letters within the same emulsifier group differ significantly ($P < 0.05$).

^{x,y}Values with different letters within the same oil droplet size group differ significantly ($P < 0.05$).

5.3.3.2 Oil droplet release during mastication

The CAP-loaded Tween 80 emulsion gels had significantly greater release of oil droplets from the protein matrix during mastication than the CAP-loaded whey protein emulsion gels, as shown in Figure 5-3. These results can be attributed to the differences in their gel structures (Fig. 5-2). The CAP-loaded Tween-80-coated emulsion droplets had no interactions with the whey protein matrix, and the emulsion droplets were flocculated and disrupted the protein matrix. During mastication, the mechanical compression and shearing would further disrupt the gel structure and lead to a reduction in the bolus particle size. As the Tween-80-coated emulsion droplets were not bound with the protein matrix, they were less likely to be protected by it under mechanical forces and were more likely to be liberated from the gel matrix under stress. In contrast, the CAP-loaded whey-protein-coated emulsion droplets had strong interactions with the protein matrix, through hydrophobic interaction and disulphide binding. During mastication, the CAP-loaded whey-protein-coated emulsion droplets would be protected by the protein coating and would be retained in the fragmented gel particles.

As the released oil droplets facilitated the lubrication of food particles and bolus formation by providing liquid (van Eck et al., 2019b). Faster bolus formation also contributed to the significantly fewer chewing cycles and the shorter chewing durations of the CAP-loaded Tween 80 emulsion gels.

The masticated gel boluses had essentially the same oil droplet size distributions as the parent emulsions (data not shown), confirming that there was no coalescence of the oil droplets during mastication.

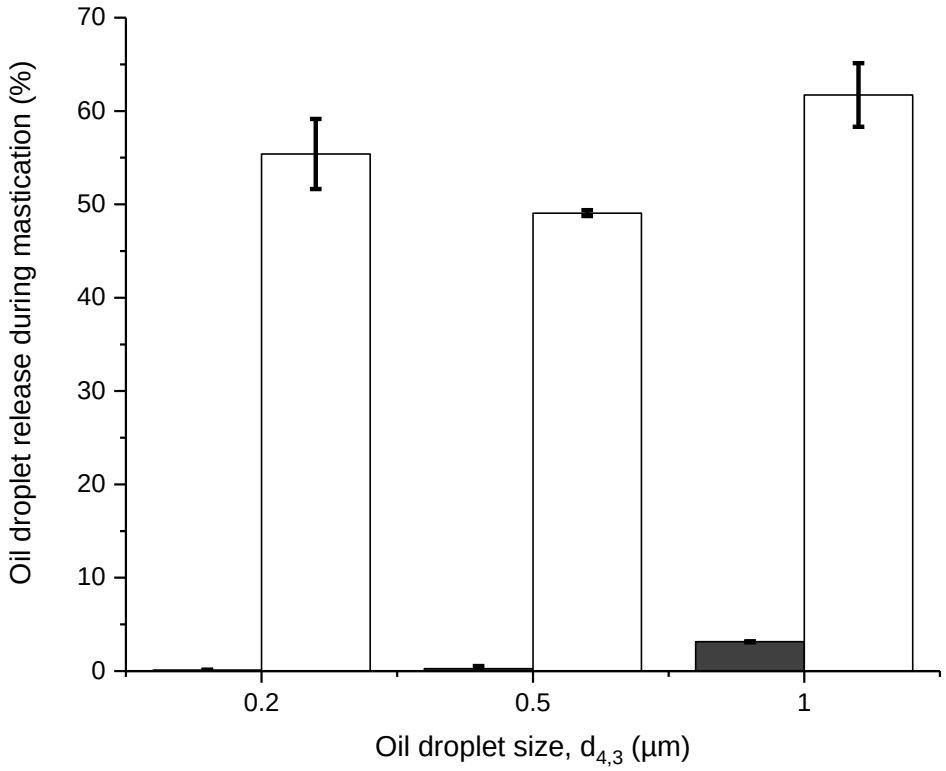


Figure 5-3 Oil droplet release during mastication of CAP-loaded whey protein emulsion gels (filled columns) and CAP-loaded Tween 80 emulsion gels (open columns). Error bars represent standard deviation of N = 2 replicates.

5.3.3.3 Degree of fragmentation of masticated gel bolus

The cumulative dry mass percentages of masticated gel particles passing through sieves of a given size (0.038, 0.425, 0.850, 1.40, 2.00 and 3.15 mm) are plotted in Figure 5-4; the geometric mean diameters of the masticated gel boluses, d_{frag}, were calculated using Equation 3-5 and are presented in Table 5-2. A higher d_{frag} indicates a lower degree of fragmentation. The differences in oil droplet size did not affect the degree of gel fragmentation significantly; however, the CAP-loaded Tween 80 emulsion gels were significantly more fragmented than the CAP-loaded whey protein emulsion gels.

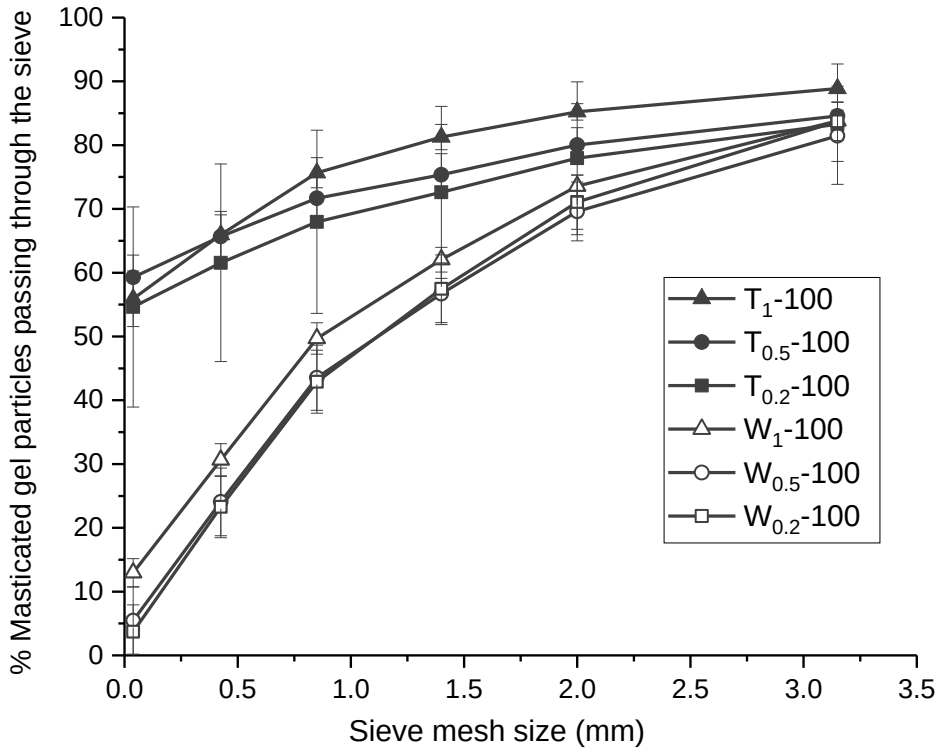


Figure 5-4 Cumulative dry mass percentage of masticated gel particles passing through sieves of given sizes (0.038, 0.425, 0.850, 1.40, 2.00 and 3.15 mm). Error bars: standard deviations of N = 3 replicates.

In Chapter 4, we investigated the effect of gel structure on the rheological properties and *in vivo* oral processing behaviour of CAP-loaded whey protein emulsion gels and established a power-law relationship between the gel hardness (represented by Young's modulus) and the masticated bolus particle size (represented by d_{frag}): $Y = 25.38X^{-0.29}$, with R^2 = 0.69. However, the results for the CAP-loaded Tween 80 emulsion gels did not fit this curve well. This indicates that, in the present study, gel hardness may not have been the dominating factor determining the bolus particle size.

Firstly, in our previous study in Chapter 4, the whey protein emulsion gels had minor amounts of oil droplet release during mastication (values < 9%), whereas, in the present study, the Tween 80 emulsion gels had significant amounts of oil droplet release (values > 49%). The released oil droplets could easily pass through the sieve of size 0.038 mm and

significantly decreased the bolus particle size. Also, as the Tween-80-coated emulsion droplets were flocculated and not bound with the protein matrix, they acted as 'slippery defects' in the gel matrix and made fragmentation easier during mechanical compression and shearing.

Secondly, the CAP-loaded Tween 80 emulsion gels had significantly lower fracture stress and fracture strain than the CAP-loaded whey protein emulsion gels, indicating that they were weaker in gel strength and more brittle (Barrangou, Drake, Daubert, & Foegeding, 2006). Devezeaux de Lavergne, Strijbosch, van den Broek, van de Velde, and Stieger (2016) investigated the impact of different fracture stresses and fracture strains on the oral breakdown of emulsion-filled gels. They found that gels with low fracture stress and low fracture strain and gels with high fracture stress and low fracture strain had the lowest masticated bolus particle size, followed by gels with high fracture stress and high fracture strain; gels with low fracture stress and high fracture strain were found to be least fragmented.

In our study, the CAP-loaded Tween 80 emulsion gels at 100 mM NaCl could be characterized as gels with low fracture stress and low fracture strain and had the lowest bolus particle size. From our previous study, CAP-loaded whey protein gels at 100 mM NaCl can be characterized as gels with high fracture stress and high fracture strain and had intermediate bolus particle size; CAP-loaded whey protein gels at 10 mM NaCl could be characterized as gels with low fracture stress and high fracture strain and had larger bolus particle size. The results obtained in our study were in agreement with the findings of Devezeaux de Lavergne et al. (2016). Fracture strain has a significant impact on bolus particle size: lower fracture strain leads to the formation of many small and round particles because the gels can be easily fragmented at small deformation; gels with high

fracture strain produce bigger particles and, because of their high deformability, these bigger particles are safe for swallowing as such (Devezeaux de Lavergne et al., 2016; Luo et al., 2019).

5.3.4 Sensory perception

5.3.4.1 Mouth feel firmness

Figure 5-5 A presents the mouth-feel firmness ratings of the CAP-loaded whey protein emulsion gels and the CAP-loaded Tween 80 emulsion gels. For the CAP-loaded whey protein emulsion gels, the gel hardness ($P < 0.05$; Table 5-1) and the perceived mouth-feel firmness gradually increased with decreasing oil droplet size. For the CAP-loaded Tween 80 emulsion gels, the gel hardness gradually increased ($P < 0.05$; Table 5-1) but the mouth-feel firmness ratings were not significantly affected with decreasing oil droplet size. This can probably be attributed to the large amounts of oil droplet release during mastication. The released oil droplets lubricated the bolus particles formed and provided liquid, which may have mitigated the differences in their perceived firmness. Similarly, van Eck, Fogliano, Galindo-Cuspinera, Scholten, and Stieger (2019a) investigated the perceived softness of breads with different mechanical strengths (high, medium and low) without and with mayonnaise. They found that, when the breads were consumed without mayonnaise, they would be perceived as having high, medium and low softness respectively. When they were consumed with mayonnaise, the differences in mechanical strength between the different breads were mitigated and they were all perceived as having low softness. However, this mitigation is only to a certain extent; when the differences in the initial food hardness are large, consumer sensitivity to discriminate between foods would not be affected by the presence of other food items (i.e. extra liquid etc.).

At a given oil droplet size, the CAP-loaded whey protein emulsion gels always had higher mouth-feel firmness ratings than the CAP-loaded Tween 80 emulsion gels. This is in agreement with Liu et al. (2015), who found that mouth-feel attributes (i.e. MF-firm, MF-elastic etc.) were positively related to the physical properties.

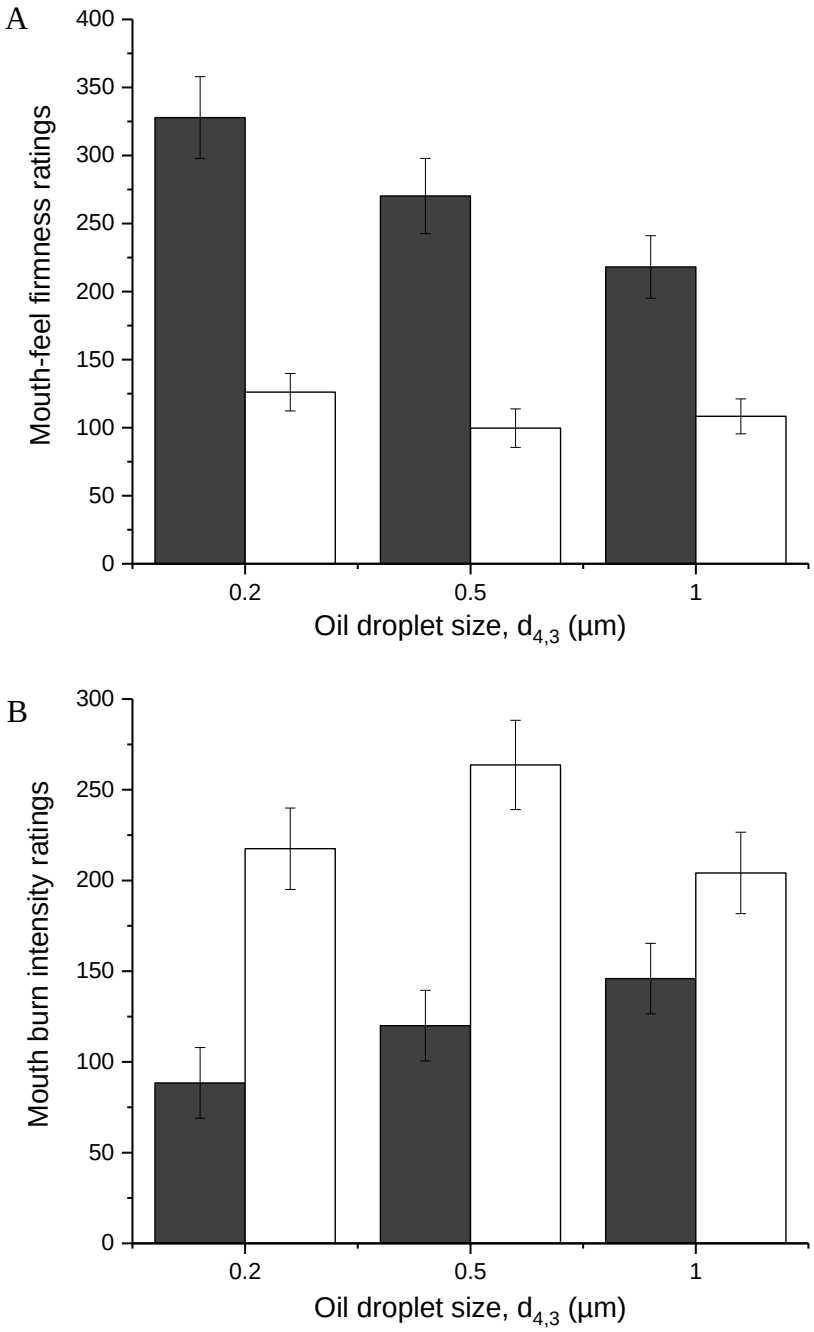


Figure 5-5 (A) Mouth-feel firmness and (B) mouth burn intensity ratings of CAP-loaded whey protein emulsion gels (filled columns) and CAP-loaded Tween 80 emulsion gels (open columns). Error bars represent standard error obtained from 18 human participants.

5.3.4.2 Mouth burn intensity

Figure 5-5 B presents the mouth burn intensity ratings of the CAP-loaded whey protein emulsion gels and the CAP-loaded Tween 80 emulsion gels. For the CAP-loaded whey protein emulsion gels, the mouth burn perception gradually decreased with decreasing oil droplet size, because of the increased gel mechanical strength. This can be explained by two possible mechanisms, which were described in detail in Chapter 4. Firstly, the higher is the gel mechanical strength, the lower is the diffusion rate of the CAP molecules through the gel matrix, and therefore, lower mouth burn perception [it should be noted that capsaicinoids have water solubility of 60 mg/L, as reported by Turgut et al., (2004)]. Secondly, the higher is the gel mechanical strength, the higher is the perceived mouth-feel firmness. From a neurological point of view, according to the cross-modal integration theory (Driver & Spence, 2000), an increase in one attribute may lead to enhancement or suppression in another attribute. As sensory pathways widely overlap from the very periphery, the gustatory nerves may respond to both taste and temperature or tactile stimulations and the same stimulus may elicit both gustatory and somatosensory responses (Cerf-Ducastel et al., 2001). In our study, an increase or decrease in mouth-feel firmness led to a decrease or increase in mouth burn perception of the CAP-loaded emulsion gels, or vice versa.

There was no significant difference in mouth burn perception for CAP-loaded Tween 80 emulsion gels with different oil droplet sizes. This may also be ascribed to the large amounts of oil droplet release during mastication. As CAP molecules were dissolved in the oil droplets, the CAP-loaded Tween 80 emulsion gels with different oil droplet sizes had similar oil droplet release (Table 5-2); therefore, there was no significant difference in their mouth burn perception.

At a given oil droplet size, the CAP-loaded Tween 80 emulsion gels always had significantly higher mouth burn perception than the CAP-loaded whey protein emulsion gels ($P < 0.05$). The CAP-loaded Tween 80 emulsion gels had lower gel hardness and greater amounts of oil droplet release during mastication, both of which contributed to their higher mouth burn perception.

5.3.5 Correlation between degree of fragmentation of the masticated gel bolus and mouth burn perception

In Chapter 4, we investigated the effect of gel structure on the rheological, *in vivo* oral processing and sensory properties of whey protein emulsion gels containing CAPs and established a power-law relationship between mouth burn perception and masticated bolus particle size (d_{frag}): $Y = 119.5X^{-0.25}$, with $R^2 = 0.76$. We re-plotted the graph with the results obtained from the CAP-loaded Tween 80 emulsion gels and found that they fitted well into the curve (Fig. 5-6). With more data points, the updated power-law relationship was: $Y = 123.9X^{-0.27}$, with $R^2 = 0.81$. This confirmed that an increase in surface exposure during mastication would generally lead to an increase in mouth burn perception.

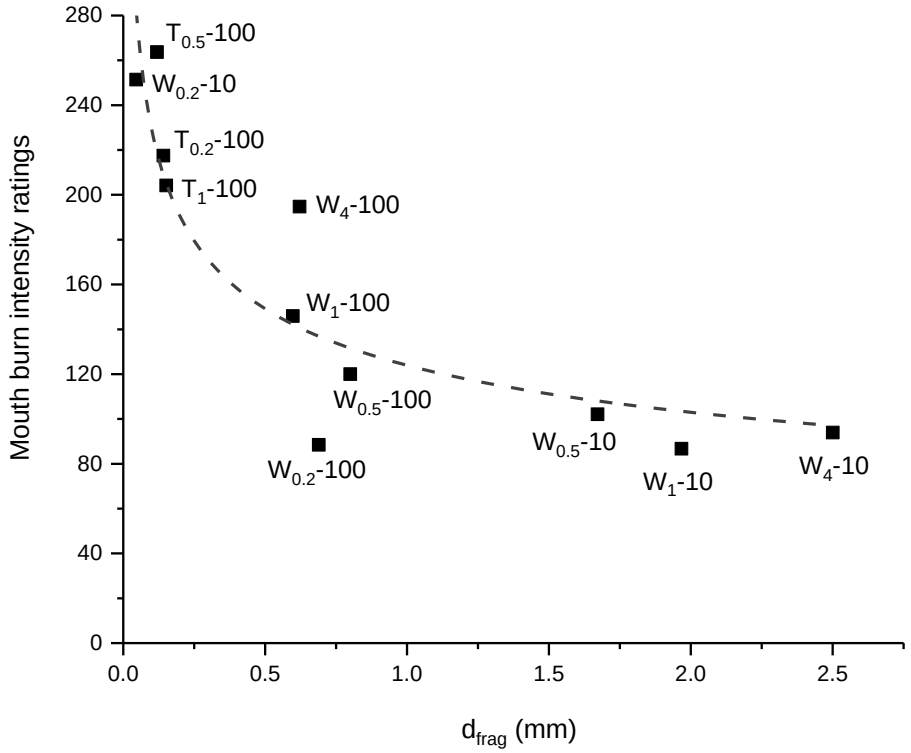


Figure 5-6 Correlation between d_{frag} of masticated gel boluses and mouth burn perception of CAP-loaded whey protein emulsion gels W_4-10 ($d_{4,3}$ ~ 4 μm with 10 mM NaCl), W_1-10 ($d_{4,3}$ ~ 1 μm with 10 mM NaCl), $W_{0.5}$-10 ($d_{4,3}$ ~ 0.5 μm with 10 mM NaCl), $W_{0.2}$-10 ($d_{4,3}$ ~ 0.2 μm with 10 mM NaCl), W_4-100 ($d_{4,3}$ ~ 4 μm with 100 mM NaCl), W_1-100 ($d_{4,3}$ ~ 1 μm with 100 mM NaCl), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 μm with 100 mM NaCl) and $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 μm with 100 mM NaCl), and CAP-loaded Tween 80 emulsion gels T_1-100 ($d_{4,3}$ ~ 1 μm with 100 mM NaCl), $T_{0.5}$-100 ($d_{4,3}$ ~ 0.5 μm with 100 mM NaCl) and $T_{0.2}$-100 ($d_{4,3}$ ~ 0.2 μm with 100 mM NaCl).

5.3.6 Correlation between oil droplet release during mastication and mouth burn perception

Figure 5-7 presents the correlation between oil droplet release during mastication and mouth burn perception. The CAP-loaded Tween 80 emulsion gels had higher mouth burn perception than the CAP-loaded whey protein emulsion gels, which could partly be attributed to their significantly greater amounts of oil droplet release during mastication. As CAP molecules are lipophilic and are dissolved mostly in the oil phase, when large amounts of oil droplets are liberated from the gel matrix, the contact area of CAPs with

the tongue will increase and will promote the mouth burn perception (Sala, de Wijk, van de Velde, & van Aken, 2008; Sala et al., 2007b). Moreover, the oil droplets in the CAP-loaded Tween 80 emulsion gels were flocculated (Fig. 1), whereas those in the CAP-loaded whey protein emulsion gels were more homogeneously distributed. The inhomogeneous distribution of the oil droplets could also have enhanced the mouth burn perception. This is in agreement with Fuhrmann, Kalisvaart, Sala, Scholten, and Stieger (2019) and Mosca, Rocha, Sala, van de Velde, and Stieger (2012), who studied the effect of fat distribution on the sensory properties of emulsions and gelled foods. A possible mechanism is that an inhomogeneous distribution of the oil droplets can alter the rheological and tribological properties of the food being consumed, and therefore can alter the sensory perception and enhance fat-related sensory attributes (Fuhrmann et al., 2019).

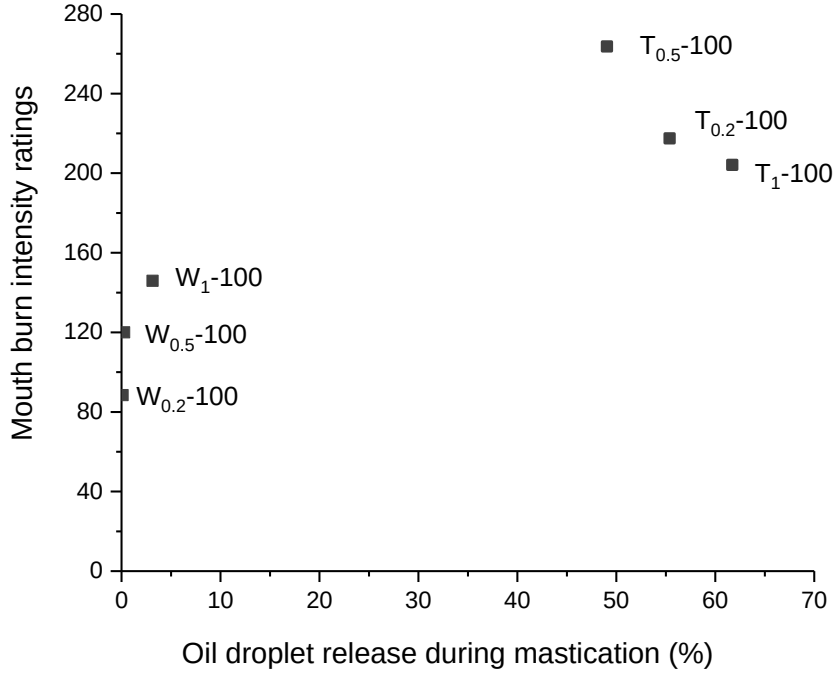


Figure 5-7 Correlation between oil droplet release during mastication and mouth burn perception of CAP-loaded whey protein emulsion gels W_1-100 ($d_{4,3}$ ~ 1 µm), $W_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm) and $W_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm), and CAP-loaded Tween 80 emulsion gels T_1-100 ($d_{4,3}$ ~ 1 µm $T_{0.5}$-100 ($d_{4,3}$ ~ 0.5 µm) and $T_{0.2}$-100 ($d_{4,3}$ ~ 0.2 µm). All emulsion gels contain 100 mM NaCl.

5.4 Conclusion

This study explored the effects of active versus inactive filler particles on the structure, rheological properties, *in vivo* oral breakdown behaviour and sensory perception of emulsion gels containing CAPs. As a result, emulsion gels containing inactive filler particles were more easily broken down into small fragments in the mouth at small deformation with less chewing effort (i.e. lower fracture energy, fewer chewing cycles and shorter chewing duration) and released large amounts of oil droplets during mastication. A power-law relationship between the masticated bolus particle size and the mouth burn intensity was established: $Y = 123.9X^{-0.27}$, with R^2 = 0.81 (Y represents mouth burn intensity ratings, X represents bolus particle size). A higher degree of fragmentation led to greater surface exposure (i.e. new surface area) during mastication and therefore to higher mouth burn perception. The presence of inactive filler particles in the emulsion gels caused an inhomogeneous distribution of fat, which also enhanced mouth burn perception. This work has provided useful information; by manipulating the droplet-matrix interaction, not only the structure and rheological properties, but also the oral processing behaviour of the food and the release behaviour of the lipophilic CAPs are altered, through multiple mechanisms. This outcome has potential for designing foods with different purposes, such as increasing the incorporation of bioactive compounds in foods, especially those with undesirable flavours while maintaining acceptable sensory perception.

Chapter 6: Effect of gel structure on the *in vitro* gastrointestinal digestion behaviour of whey protein emulsion gels and bioaccessibility of capsaicinoids

6.1 Abstract

This chapter investigated the effect of gel structure on the *in vitro* gastric digestion behaviour of whey protein emulsion gels containing CAP using a human gastric simulator, and the effect of the characteristics of gastric digesta on the intestinal digestion and bioaccessibility of CAP. The hard gel (i.e. CAP-loaded whey protein emulsion gel with oil droplet size $d_{4,3}$ ~ 0.5 μm, containing 200 mM NaCl) had a more compact particulate gel structure, which may have hindered the cleavage sites of pepsin, leading to slower disintegration of gel particles and slower hydrolysis of whey proteins during gastric digestion. The oil droplets started to coalesce after 60 min of gastric digestion in the soft gel (i.e. CAP-loaded whey protein emulsion gel with oil droplet size $d_{4,3}$ ~ 0.5 μm, containing 10 mM NaCl), while no oil droplet coalescence was observed for the hard gel throughout the gastric digestion. Gastric digesta from hard gel was disintegrated slower than the soft gel during intestinal digestion because of its structure, except for the gastric digesta emptied at 240 min. Oil droplet coalescence was observed in gastric digesta emptied at 120 min from the soft gel, leading to reduced surface area for the lipolytic enzymes to react during intestinal digestion, and therefore had lower extent of lipid digestion. A power-law fitting was established between the bioaccessibility of CAP (Y) and the extent of lipid digestion (X): $Y = 49.2 \times (X - 305.3)^{0.104}$, with $R^2 = 0.84$. A higher extent of lipid digestion would lead to greater release of CAP from the food matrix; also, more lipolytic products would be produced and participate in micelle

formation that help solubilize the released CAP, and therefore resulting in higher bioaccessibility of CAP.

6.2 Introduction

Emulsion-based systems for delivery of lipophilic bioactive compounds, such as capsaicin, β-carotene and curcumin by incorporating the bioactive compounds in the emulsion droplets have been widely studied for the purposes of increased solubility, reduced irritation, increased stability and bioaccessibility (Ahmed et al., 2012; Humberstone & Charman, 1997; Lin et al., 2018; Lu et al., 2016; Salvia-Trujillo et al., 2013). For instance, Lu et al. (2016) investigated the use of nanoemulsion for delivery of capsaicin and reported that the bioaccessibility of capsaicin increased from about 10% in unformulated form to about 80% in capsaicin-loaded nanoemulsion after *in vitro* lipid digestion. However, the digestion condition used in their study was very simple and did not represent gastrointestinal conditions.

The behaviour of liquid emulsions during *in vitro* gastrointestinal digestion has been widely investigated in recent decades, one of the common behaviours is flocculation / coalescence of oil droplets in the GI tract (Shani-Levi, Levi-Tal, & Lesmes, 2013; Singh & Ye, 2013; Wang, Lin, Ye, Han, & Singh, 2019). Different from the liquids, the solid/semi-solid foods undergo more complicated processes during digestion, since they require mastication during oral processing, trituration and disintegration of solid particles in the stomach to form particles small enough to pass through the pyloric sphincter. Therefore, the solid/semi-solid foods usually take longer time to digest and empty from the stomach; the digestion behaviour would be quite different and would affect the release behaviour of the nutrients and their bioaccessibility (Dias et al., 2019).

Emulsion gel has been widely used as a model system for solid/semi-solid foods, to investigate the structure, rheology, breakdown behaviour during digestion (Chen & Dickinson, 1998a, 1998b, 1999; Guo et al., 2014a, 2014b, 2016; Gwartney et al., 2004; Ye & Taylor, 2009). The structure and rheological properties of the emulsion gel can be tailored so that its disintegration and digestion behaviour would be altered (Guo et al., 2014a, 2014b, 2016). Macierzanka et al. (2012) studied the effect of protein structure on the kinetics of simulated gastrointstinal digestion of bovine β-lactoglobulin by varying the pH or heating conditions under which the protein gel was formed, and reported that the gel formed near the isoelectric point was more resistant against protein hydrolysis during simulated gastric digestion. Guo et al. (2014b) also reported that whey protein emulsion gel containing 200 mM NaCl was disintegrated much slower during *in vitro* gastric digestion than whey protein emulsion gel containing 10 mM NaCl. However, there is limited information on using emulsion gel-based systems for delivery of bioactive compounds and how the gel structure and digestion behaviour would affect their bioaccessibility.

Gastric emptying into the small intestine is a dynamic and continuous process, controlled by the pyloric sphincter. The food chyme leaving the stomach at different digestion times may have distinct characteristics, which may have an impact on the bioaccessibility of the nutrients. However, little information is available on how the characteristics of gastric chyme would affect its behaviour during intestinal digestion and the bioaccessibility of nutrients. Therefore, in the present work, we collected gastric digesta from three different digestion times to represent gastric digestion at the beginning, intermediate and final phase, to investigate how the characteristics of gastric digesta would affect the intestinal digestion and bioaccessibility.

In the present work, we used whey protein emulsion gel as a model for solid/semi-solid delivery systems, where the capsaicinoids (CAP) were dissolved in the emulsion droplets. The gel structure was modified by changing the NaCl concentration of the emulsion gel to study the effect of gel structure on the disintegration and digestion behaviour of whey protein emulsion gels containing CAP, and understand how it affects the bioaccessibility of CAP after *in vitro* gastrointestinal digestion.

The CAP-loaded whey protein emulsion gel $W_{0.5}$_10 (oil droplet size $d_{4,3}$ ~ 0.5 µm with 10 mM NaCl; characterized as soft gel) and the CAP-loaded whey protein emulsion gel $W_{0.5}$_200 ($d_{4,3}$ ~ 0.5 µm with 200 mM NaCl; characterized as hard gel) were used in this study. *In vitro* dynamic gastric digestion was carried out for 240 min using the Human Gastric Simulator (HGS), and *in vitro* intestinal digestion was carried out for 120 min using pH-stat.

6.3 Results and discussion

6.3.1 pH profiles during *in vitro* gastric digestion in the HGS

During 240 min of *in vitro* gastric digestion in the human gastric simulator (HGS), with the constant secretion of simulated gastric fluid (SGF, pH: 1.5), the pH of the emptied gastric digesta gradually decreased from 5.82 ± 0.39 at 0 min to 2.57 ± 0.16 at 240 min for CAP-loaded whey protein emulsion gel $W_{0.5}$_10 (oil droplet size $d_{4,3}$ ~ 0.5 µm with 10 mM NaCl; characterized as soft gel) and from 5.94 ± 0.20 at 0 min to 2.41 ± 0.24 at 240 min for CAP-loaded whey protein emulsion gel $W_{0.5}$_200 ($d_{4,3}$ ~ 0.5 µm with 200 mM NaCl; characterized as hard gel), as shown in Figure 6-1. The pH profiles were in agreement with an *in vivo* study done by Kalantzi et al. (2006), where they fed the human participants with 500 ml of Ensure Plus® (a complete nutrient drink with protein

content of 62 mg/ml, fat content of 49.2 mg/ml and carbohydrates content of 202 mg/ml) and measured the pH of the gastric content every 30 min for 3.5 h. An average pH of 2.7 was reported after 210 min of ingestion, which is comparable to the results obtained from our study (pH ~ 2.80 ± 0.16 for the soft gel and pH ~ 2.64 ± 0.24 for the hard gel at 210 min of digestion).

The trends of the pH profiles during gastric digestion were similar for both gels, indicating that the structure of the protein matrix did not have significant effect on the buffering capacity of the emulsion gel. Similar results were reported by Guo et al. (2014b) where they compared the pH changes during *in vitro* gastric digestion of soft gel (whey protein emulsion gel containing 10 mM NaCl) and hard gel (whey protein emulsion gel containing 200 mM NaCl).

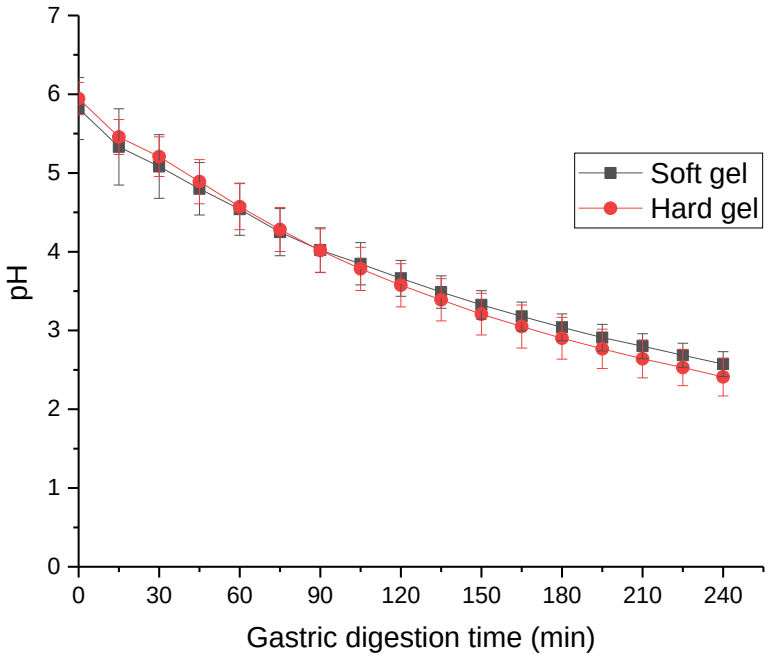


Figure 6-1 Changes in pH during *in vitro* gastric digestion in the HGS of CAP-loaded whey protein emulsion gels: soft gel ($d_{4,3}$ ~ 0.5 µm, 10 mM NaCl) and hard gel ($d_{4,3}$ ~ 0.5 µm, 200 mM NaCl). Error bars represent standard deviations obtained from N = 3 independent experiments.

6.3.2 Physiochemical characteristics of emptied gastric digesta

6.3.2.1 Solid content of emptied gastric digesta

Figure 6-2 presents the solid content of emptied gastric digesta as a function of digestion time. The results for 0 min and 15 min were missing because the gastric emptying of solid foods only started after 30 min of ingestion (Siegel et al., 1988; Urbain et al., 1989). During the first 90 min of digestion, the solid content of emptied gastric digesta from the hard gel was higher than the soft gel ($P < 0.05$), suggesting that the hard gel was emptied out faster in the beginning of digestion. This could be attributed to the fact that the hard gel had a significantly smaller initial bolus particle size entering the stomach, according to the results obtained from previous *in vivo* oral processing study (Table 4-2, Section 4.3.2.2 in Chapter 4: masticated bolus particle size d_{frag} of 0.8 mm for hard gel and 1.7 mm for soft gel). During 90 to 180 min of digestion, no significant difference was found on the solid content of emptied gastric digesta between soft gel and hard gel; the solid content of emptied gastric digesta gradually decreased for both gels, due to the dilution of gastric content by constant gastric secretion and emptying. During 180 to 240 min of digestion, the solid content of emptied gastric digesta gradually increased for the soft gel while it remained relatively constant for the hard gel. This means that towards the end of digestion, the soft gel was disintegrated faster than the hard gel. The results were in agreement with the report from Guo et al. (2014b).

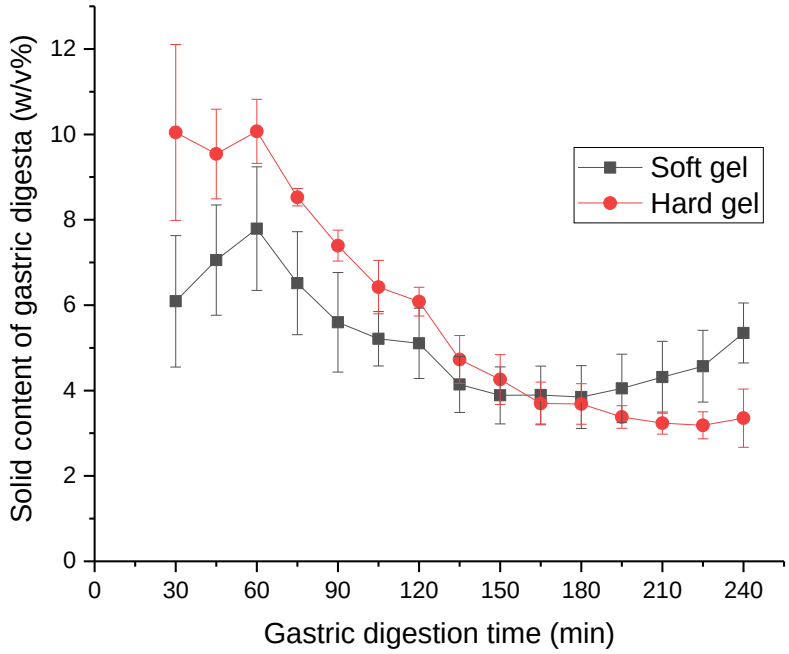


Figure 6-2 Solid content of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

6.3.2.2 Particle size distribution of emptied gastric digesta

The changes in weight-to-volume diameter ($d_{4,3}$, μm) of emptied gastric digesta as a function of digestion time are shown in Figure 6-3. Overall, the average particle size of the emptied gastric digesta gradually decreased with increasing digestion time for both gels, indicating the disintegration of gel particles during gastric digestion. At 30 min (i.e. the time point for the first gastric emptying), the emptied gastric digesta from the hard gel had significantly bigger particle size than that of the soft gel. The gastric sieving, mimicked by using the mesh bag, only allowed particles smaller than 1 mm to pass through and be emptied out. During oral processing, the soft gel was mainly fragmented into bigger particles (Chapter 4, Fig. 4-3), about 75% of the masticated gel bolus from the soft gel were larger than 1.0 mm, which could not be emptied out at the beginning of gastric digestion. On the other hand, the hard gel was fragmented into smaller particles

during mastication, about 50% of the masticated gel bolus from the hard gel were smaller than 1.0 mm and could be emptied out from the beginning of gastric digestion.

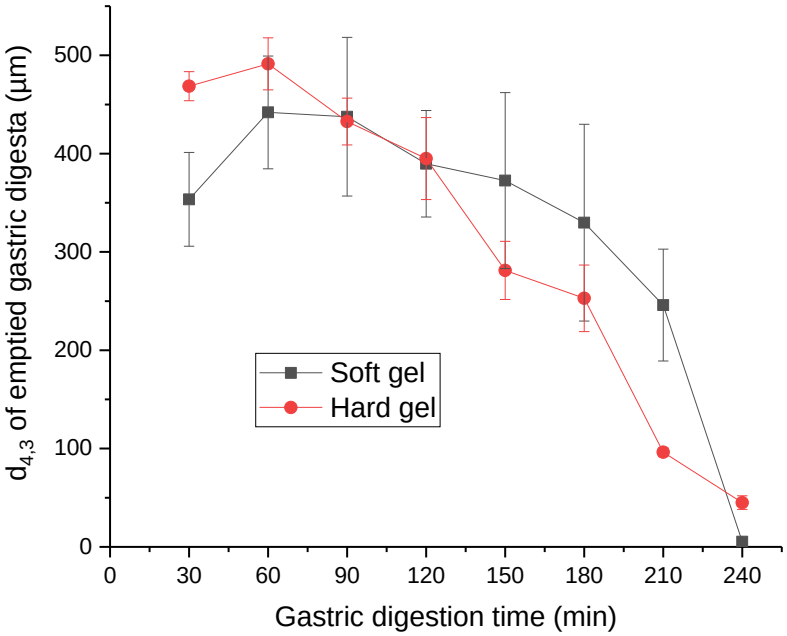


Figure 6-3 $d_{4,3}$ of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

Table 6-1 shows the results of the percentage of dry matter of gel fragments retained on the sieve of a given size obtained from our previous *in vivo* oral processing study. The hard gel produced significantly more gel particles in the size range of 0.85 to 1.4 mm, 0.425 to 0.85 mm and 0.038 to 0.425 mm than the soft gel after mastication. Therefore, during the initial gastric emptying (30 min), more larger particles can be emptied out from the hard gel.

Table 6-1 Percentage of dry matter of gel fragments retained on the sieve of a given size after *in vivo* oral processing. Results are shown as mean ± standard deviation of N = 3 replicates.

Sieve mesh size (mm)	Percentage of dry matter of gel fragments retained on the sieve (%)	
	Soft gel	Hard gel*
0.038	$9.7^{a} \pm 2.3$	$19.7^{b} \pm 1.1$
0.425	$10.7^{a} \pm 3.5$	$20.6^{b} \pm 0.9$
0.85	$8.8^{a} \pm 3.4$	$14.0^{b} \pm 0.6$
1.4	$11.0^{a} \pm 0.2$	$13.7^{b} \pm 0.8$
2.0	$17.5^{a} \pm 1.5$	$12.5^{b} \pm 1.3$
3.15	$42.3^{a} \pm 10.5$	$19.5^{b} \pm 3.4$

[a,b]Values with different superscripts differ significantly ($P < 0.05$).

*The results presented for the hard gel was the CAP-loaded whey protein emulsion gel with $d_{4,3}$ of ~ 0.5 μm and 100 mM NaCl, obtained from our previous *in vivo* oral processing study (Chapter 4). Guo et al. (2013) reported that the whey protein emulsion gel ($d_{4,3}$ of ~ 0.5 μm) containing 100 mM or 200 mM NaCl had similar degree of fragmentation during *in vivo* oral processing. Therefore, we deduced that the results of the CAP-loaded whey protein emulsion gel containing 100 mM NaCl can represent the CAP-loaded whey protein emulsion gel containing 200 mM NaCl.

During the first 90 min of digestion, the soft gel showed an increasing trend in $d_{4,3}$ of emptied gastric digesta, while the hard gel showed a slight decreasing trend. A possible reason for this could be the higher swelling of the soft gel. Guo (2015) reported that the soft gel (whey protein emulsion gel containing 10 mM NaCl) had significantly higher swelling ratio in SGF (pH 1.5) than the hard gel (whey protein emulsion gel containing 200 mM NaCl). The swelling ratio gradually increased with increasing incubation time in the SGF for both gels, however, the soft gel had about 10% swelling after 4 h of incubation while the hard gel had about 2%. Another possible reason could be that the

gel particles bigger than 1 mm from the soft gel gradually disintegrated and were emptied out.

During 90 to 240 min of digestion, the $d_{4,3}$ of emptied gastric digesta gradually decreased for both gels; the decrease was especially noticeable after 180 min, when the gastric pH dropped to below 3.0 and the gel particles were rapidly disintegrated.

The particle size distributions of the emptied gastric digesta are presented in Figure 6-4. The emptied gastric digesta showed a bimodal or a trimodal pattern, depending on the digestion time. With increasing digestion time, the peak near 1000 μm gradually decreased for both gels, indicating the disintegration of gel particles during gastric digestion. At 150 min of digestion, a peak near 0.7 μm appeared from the emptied gastric digesta from the soft gel. This peak (i.e. the peak between 0.1 to 1 μm) gradually increased in volume from 150 min to 240 min of digestion. This peak overlapped with the peak of the CAP-loaded whey protein emulsion, suggesting that it may be the free oil droplets released from the gel matrix during gastric digestion. For the hard gel, the peak between 0.1 to 1 μm only showed up after 210 min of digestion, with a much smaller volume, suggesting that the hard gel was more resistant to the disintegration and digestion in the stomach and that the oil droplets were mainly protected by the protein matrix of the hard gel and remained in the gel particles.

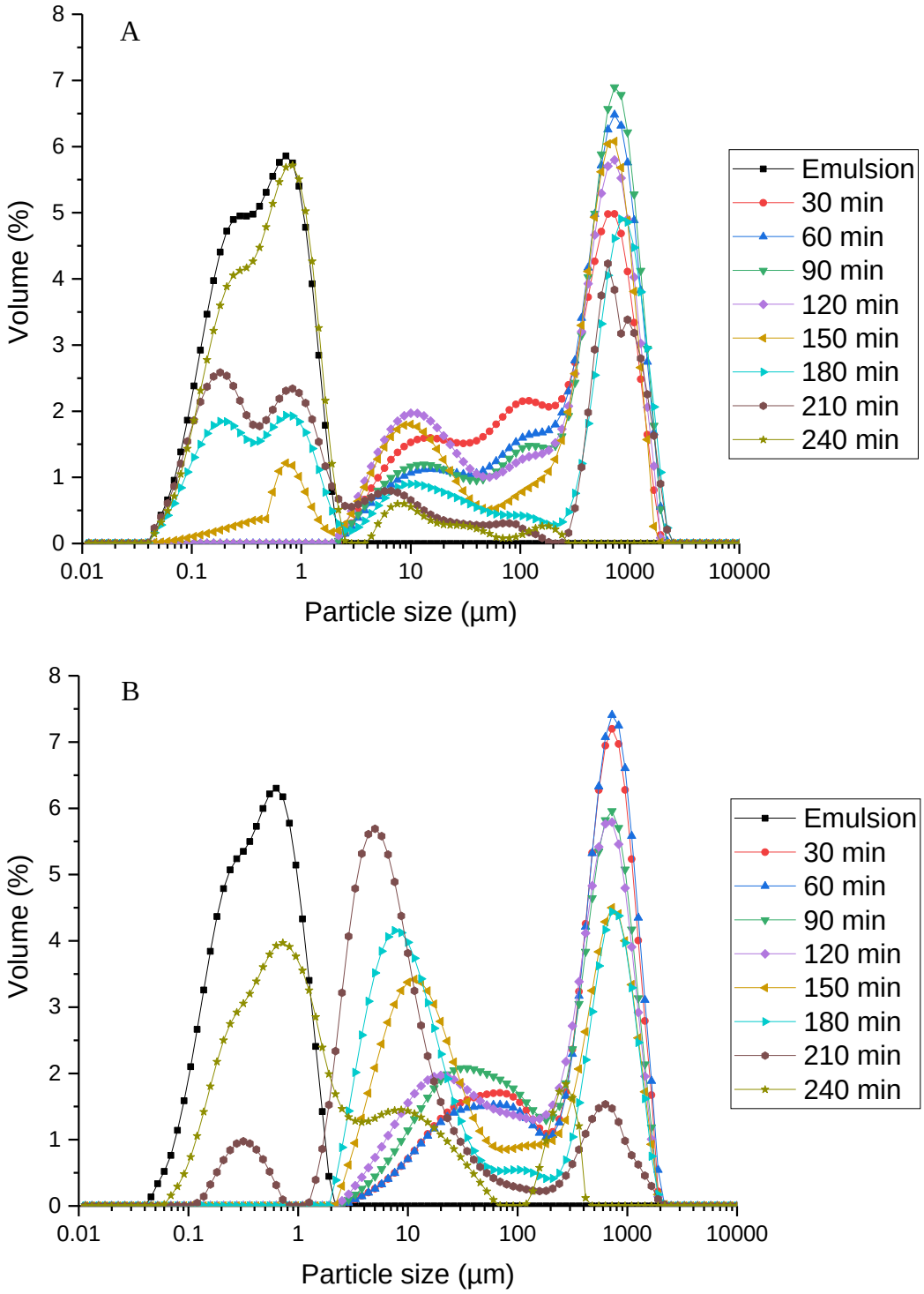


Figure 6-4 Particle size distributions of the CAP-loaded whey protein emulsion and the emptied gastric digesta of (A) soft gel (CAP-loaded whey protein emulsion gel containing 10 mM NaCl, oil droplet size $d_{4,3}$ of 0.5 μm) and (B) hard gel (CAP-loaded whey protein emulsion gel containing 200 mM, oil droplet size $d_{4,3}$ of 0.5 μm), as a function of digestion time.

6.3.2.3 Stability of oil droplets of emptied gastric digesta

The $d_{4,3}$ of the oil droplets of emptied gastric digesta as a function of digestion time is presented in Figure 6-5. The oil droplet size of emptied gastric digesta from the hard gel increased slightly after 210 min of digestion, indicating minor coalescence of the oil droplets at the end of digestion. For the soft gel, the oil droplet size increased between 60 and 150 min of digestion, indicating possible oil droplet coalescence; it showed a decreasing trend between 150 min and 240 min of digestion.

Figure 6-6 presents the particle size distributions of the oil droplets of emptied gastric digesta as a function of digestion time. For the hard gel, the oil droplet size distributions of the emptied gastric digesta were similar and no new peaks were formed, suggesting that there was no oil droplet coalescence during digestion in the stomach. For the soft gel, a peak near 8 μm started to appear at 60 min of digestion; the volume of this peak increased from 60 to 150 min and decreased from 150 to 210 min; this peak disappeared at 240 min of digestion. These observations indicate that the hard gel was more resistant to protein hydrolysis by pepsin.

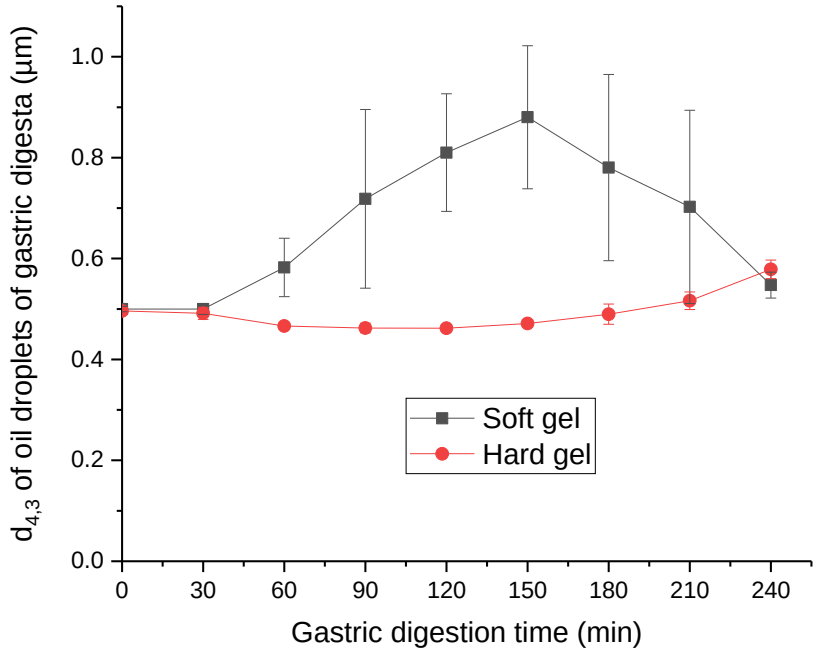


Figure 6-5 $d_{4,3}$ of oil droplets of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

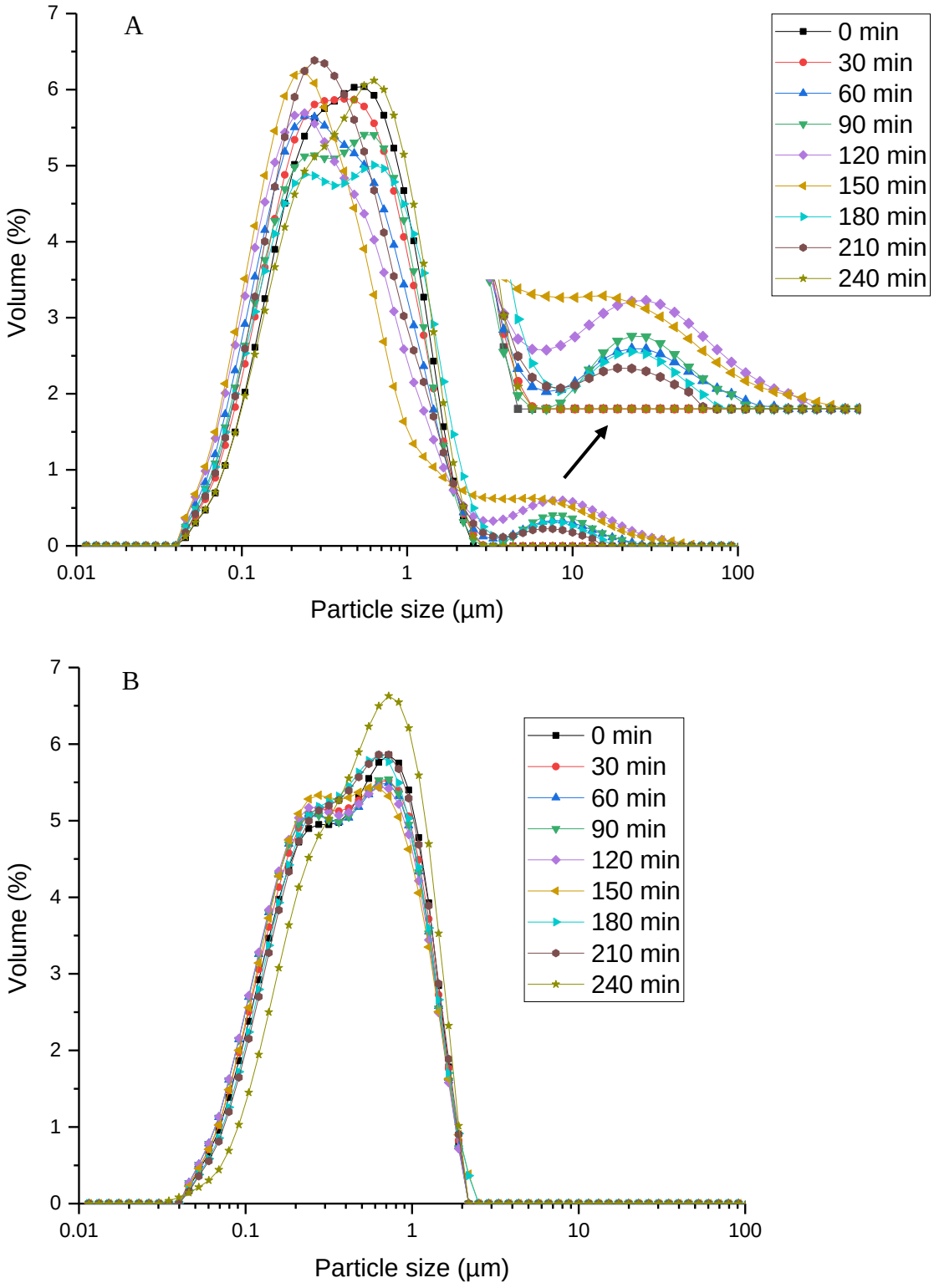


Figure 6-6 Particle size distributions of the oil droplets of emptied gastric digesta from: (A) soft gel (CAP-loaded whey protein emulsion gel containing 10 mM NaCl, oil droplet size $d_{4,3}$ of 0.5 μm) and (B) hard gel (CAP-loaded whey protein emulsion gel containing 200 mM, oil droplet size $d_{4,3}$ of 0.5 μm), as a function of digestion time.

6.3.2.4 Microstructure of emptied gastric digesta

The CLSM images of the emptied gastric digesta showed the gradual disintegration of gel particles for both gels with increasing digestion time (Fig. 6-7). The disintegration of gel particles was not obviously observed in the first 120 min of digestion for the hard gel, which is consistent with the results presented in Figure 6-3. For the soft gel, at 90 min of digestion, many large gel particles (~ 500 μm) in the digesta disappeared and disintegrated into smaller particles. At 240 min of digestion, gel particles of ~ 200 μm in size were still observed in the digesta from the hard gel, while the soft gel seemed to be mainly broken down into particles smaller than 100 μm; this is consistent with the particle size distribution results shown in Figure 6-4. The CLSM images indicate that the soft gel was disintegrated much faster than the hard gel during gastric digestion.

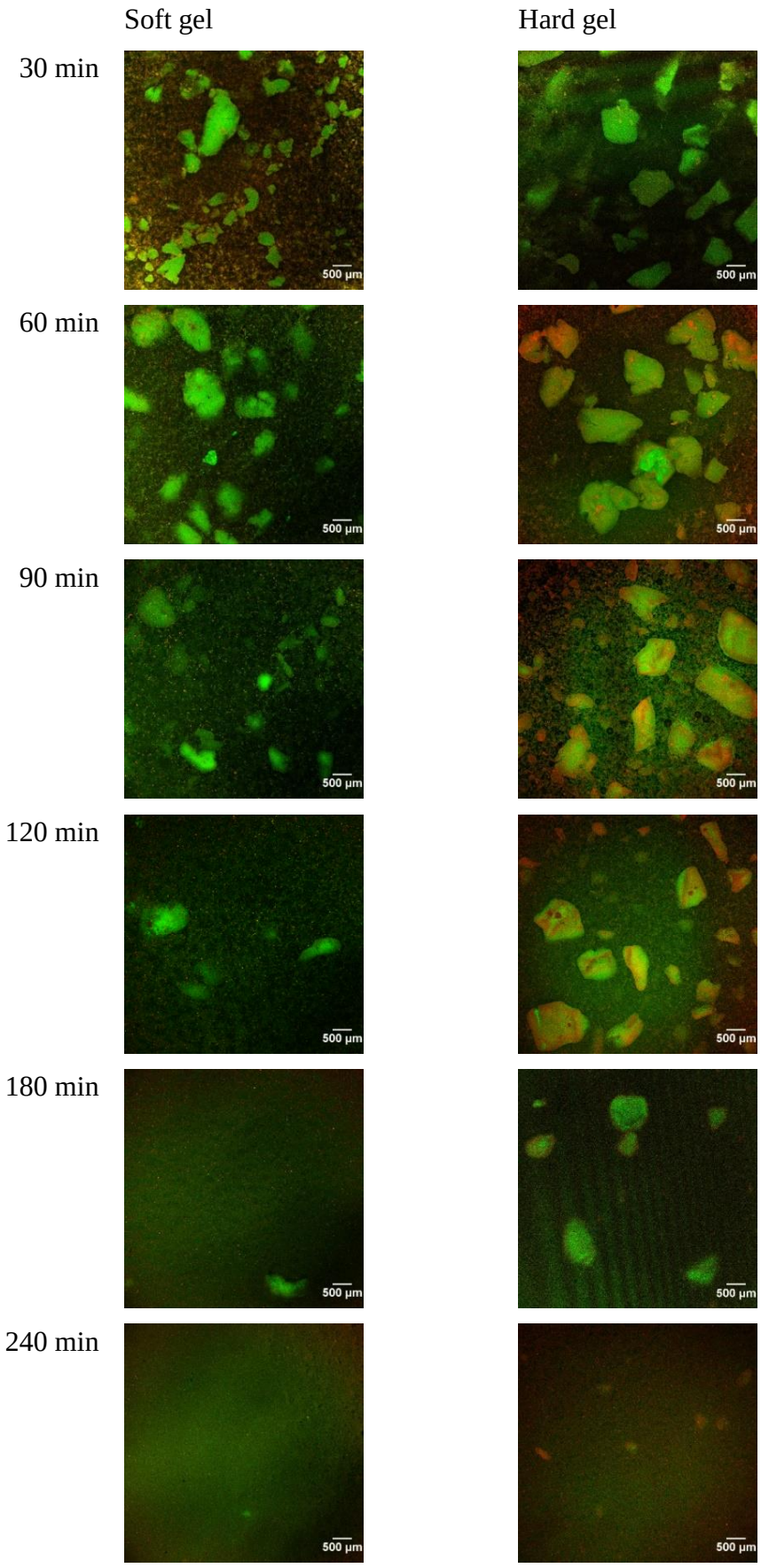


Figure 6-7 CLSM images of gastric digesta as a function of digestion time.

6.3.2.5 SDS-PAGE patterns of emptied gastric digesta

Figure 6-8 shows the Tricine SDS-PAGE patterns under reducing conditions of the emptied gastric digesta as a function of digestion time. Whey protein isolate (WPI) was used as a marker to indicate the locations of bovine serum albumin (BSA, ~ 69 kDa), β-lactoglobulin (β-lg, ~ 18.4 kDa) and α-lactalbumin (α-la, ~ 14.2 kDa). The combined intensity of all the bands represents the protein concentration in the sample, which showed a decreasing trend with increasing digestion time, indicating the gradual dilution of samples during gastric digestion. The trend is consistent with the trend of solid content of the emptied gastric digesta (Fig. 6-2).

The bands at 0 min from both gels mainly consisted of BSA, β-lg and α-la. Peptide bands appeared after 30 min of digestion for both gels, indicating protein hydrolysis by pepsin. However, the intensities of the peptide bands from the hard gel at 30 to 210 min of digestion were much lower than the soft gel, while the intensities of the BSA, β-lg and α-la bands were more intense, even though the solid content of the digesta from the hard gel at 30, 60 and 90 min of digestion was significantly lower than the soft gel but was similar at 120, 150, 180 and 210 min of digestion. This indicates that the whey proteins in the hard gel were hydrolysed slower than the soft gel. The result is in agreement with the reports from Guo et al. (2014b) and Liang, Leung Sok Line, Remondetto, and Subirade (2010), where they stated that the β-lg was degraded faster when the β-lg stabilized emulsion gel had a filamentous structure (soft gel) than a particulate structure (hard gel). At 240 min of digestion, there were no intact whey proteins seen in both gels.

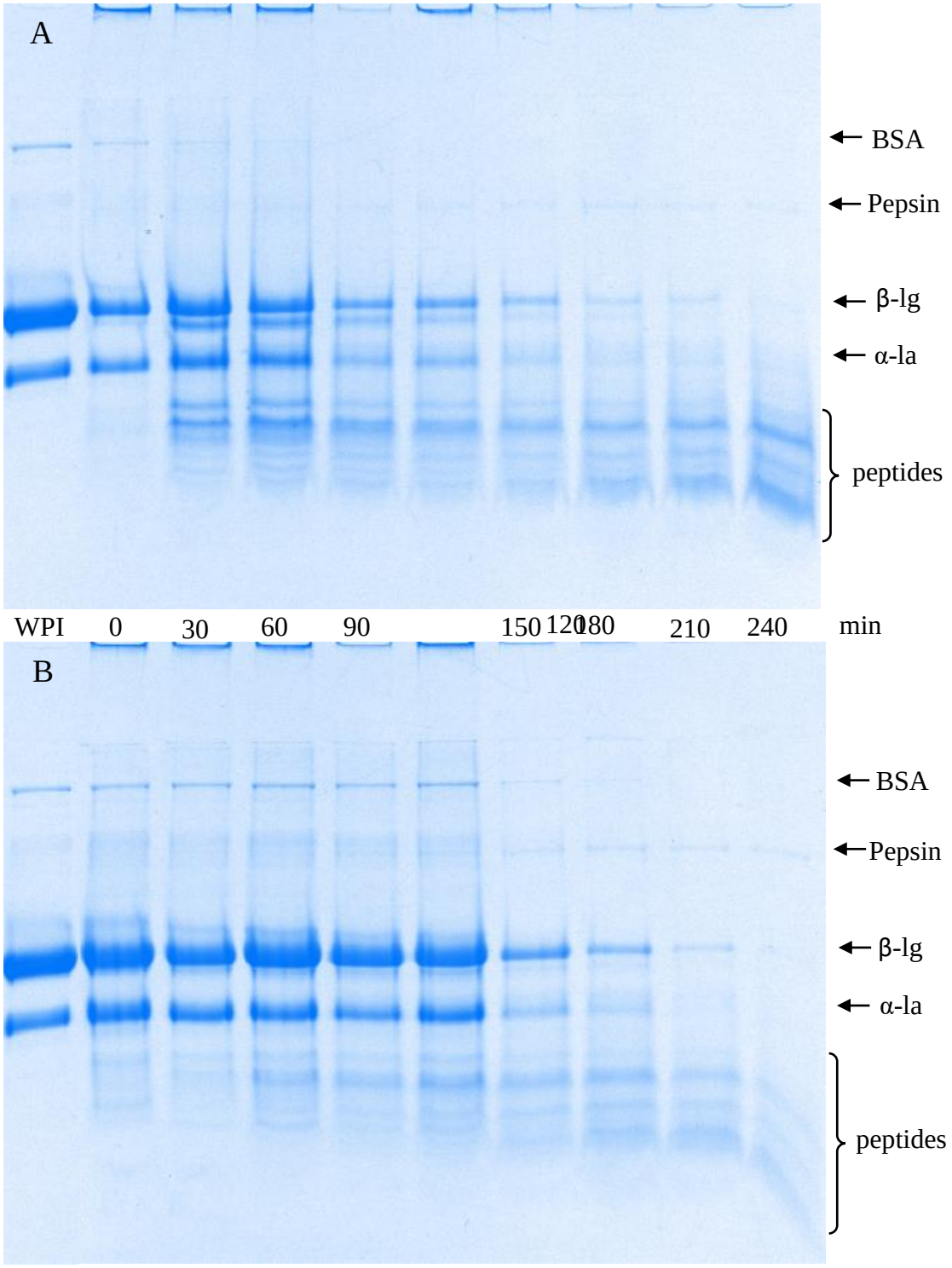


Figure 6-8 Tricine SDS-PAGE patterns under reducing conditions of emptied gastric digesta as a function of digestion time: (A) soft gel; (B) hard gel.

6.3.2.6 Fat content of emptied gastric digesta

The fat content of the emptied gastric digesta at 60, 120 and 240 min of digestion is shown in Figure 6-9. With increasing digestion time, the fat content of emptied gastric digesta decreased initially and then increased for the soft gel and gradually decreased for the hard gel. The trend was consistent with the trend of solid content of emptied gastric digesta shown in Figure 6-2.

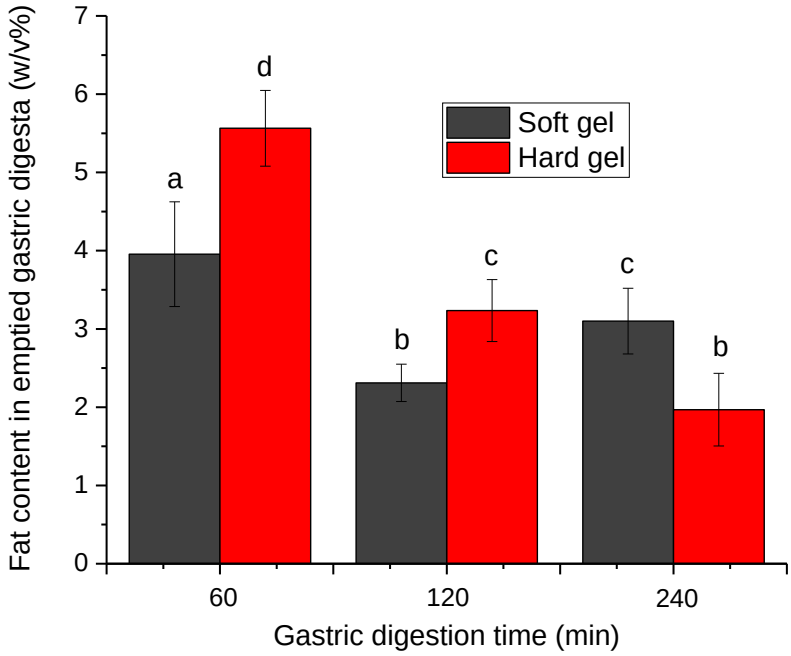


Figure 6-9 Fat content of the gastric digesta at 60, 120 and 240 min of digestion. Error bars represent standard deviations obtained from N = 3 independent experiments, each conducted in duplicate. Different lowercase letters indicate significant differences between samples ($P < 0.05$).

6.3.3 Gel retention during *in vitro* gastric digestion

Figure 6-10 presents the gel retention in the HGS during 240 min of gastric digestion. Gastric emptying only started after 30 min because of the existence of the lag phase during digestion of solid foods (Siegel et al., 1988). The hard gel was emptied out faster than the soft gel, probably due to the significantly smaller masticated bolus particle size

of the hard gel. The result is in agreement with the report from Guo et al. (2015), where they investigated the effect of gel structure (i.e. soft gel versus hard gel) on the gastric emptying rate of whey protein emulsion gel.

The curves were fitted to a modified power exponential function as described in Equation 6-1:

$$y(t) = 1 - (1 - e^{-kt})^{\beta} \qquad \textbf{Equation 6-1}$$

where, $y(t)$ was the fractional gel retention at time t, k was the gastric emptying rate (min-1), β was the extrapolated y-intercept from the terminal portion of the curve (Siegel et al., 1988). The parameters of the fitted curves are presented in Table 6-2. The hard gel had higher gastric emptying rate (i.e. k) than the soft gel, and it required less time to empty out 50% of the solids ($P < 0.05$). This is probably also attributed to the significantly smaller masticated bolus particle size of the hard gel. Koike et al. (2013) investigated the effect of malocclusion (leading to insufficient chewing) on gastric emptying rate and found that malocclusion was correlated with decreased gastric emptying rate. Pera et al. (2002) also reported that an improvement in mastication efficiency would lead to a significant increase in gastric emptying rate and a significant decrease in T_{half}. Ample mastication would greatly reduce bolus particle size, and therefore shorten the time required by the stomach to further triturate the particles small enough to pass through the pylorus. However, in their study, they used the same food with different mastication efficiency; the effect of food rheological properties was not considered. In the current study, the differences in gel matrix would also have affected gastric emptying. The fact that the soft gel was disintegrated faster than the hard gel (Fig. 6-3, Fig. 6-4, Fig. 6-7) would have promoted the emptying of the soft gel. Nevertheless,

the effect of masticated bolus particle size seemed to have a greater impact on gastric emptying.

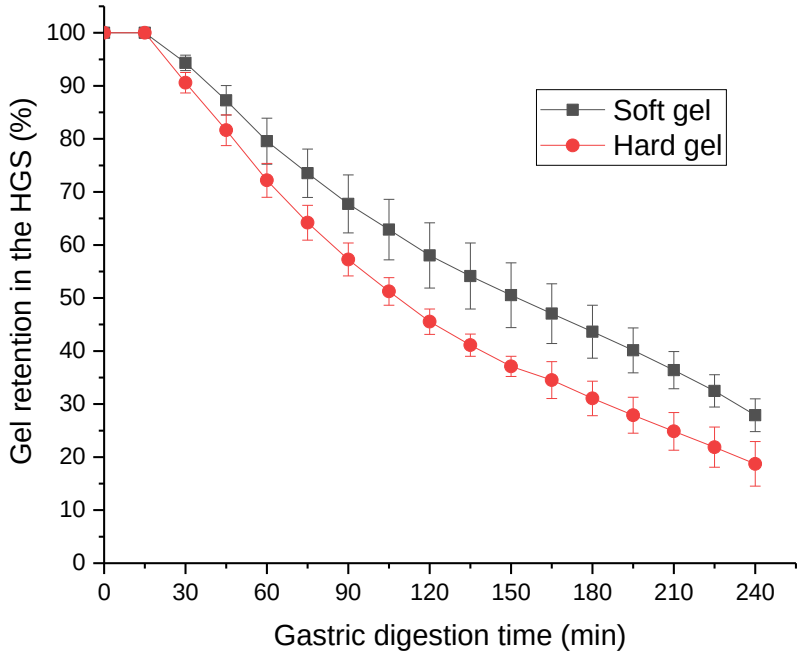


Figure 6-10 Gel retention in the HGS during gastric digestion. Error bars represent standard deviations obtained from N = 3 independent experiments.

Table 6-2 Parameters of the fitted power exponential function for gastric emptying. Results are shown as mean ± standard deviation of N = 3 independent experiments.

Parameters	Soft gel	Hard gel
$\boldsymbol{k}$ (min^{-1})	$0.00632^{a} \pm 0.000788$	$0.00840^{b} \pm 0.000817$
β	$1.366^{a} \pm 0.156$	$1.412^{a} \pm 0.110$
R^2	0.992 ± 0.006	0.994 ± 0.003
T_{half}^{*} (min)	$146.2^{a} \pm 12.2$	$113.1^{b} \pm 8.6$
T_{lag}^{**} (min)	$47.8^{a} \pm 12.4$	$40.6^{a} \pm 8.2$

*T_{half} is defined as the time needed to empty out half of the solids.

**T_{lag} was calculated as $ln\beta/k$ (Siegel et al., 1988).

[a,b]Values with different superscripts differ significantly ($P < 0.05$).

6.3.4 Discussion on the effect of emulsion gel structure on *in vitro* gastric digestion

The structure of a heat-set protein-based emulsion gel depends on the properties of the protein matrix (e.g. protein concentration, ionic strength), the properties of the emulsion droplets (e.g. the size distribution, concentration and mechanical properties of the emulsion droplets) and the emulsion droplets-protein matrix interaction (e.g. type, number and magnitude of interaction) (Tolstoguzov & Braudo, 1983; van Vliet, 1988). During heat-set whey protein gel formation at neutral pH, whey proteins would firstly unfold and aggregate into primary aggregates (tens of nanometers in size) mainly through hydrophobic interaction and disulphide binding (Ikeda, 2003; Ikeda & Morris, 2002). At low ionic strength (e.g. 10 mM NaCl), the intermolecular electrostatic repulsion was high, the primary aggregates would associate in a 'head-to-tail' manner and form a fine-stranded gel. At high ionic strength (e.g. 200 mM NaCl), the electrostatic repulsion is screened down, the primary aggregates would associate randomly and eventually form a particulate gel (Ako, Nicolai, Durand, & Brotons, 2009b; Ikeda & Morris, 2002).

In the formation of a heat-set protein-based emulsion gel, the protein-stabilized emulsion droplets would also participate in the network formation (Dickinson, 2012). Similarly, at low salt concentration, the emulsion droplets and the protein aggregates in the solution would associate in a 'head-to-tail' manner because of the relatively high electrostatic repulsion and form a so-called fine-stranded gel. At high salt concentration, the emulsion droplets and the protein aggregates were randomly aggregated in the formation of a compact particulate gel, and the emulsion droplets were more evenly distributed in the emulsion gel. Also, because of the low repulsion at high salt concentration, more cross-links between whey proteins can be formed through

hydrophobic interaction and disulphide bonding (Macierzanka et al., 2012; Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001; Verheul & Roefs, 1998). As a result, a hard gel was formed at high salt concentration and a soft gel at low salt concentration. There would be more interactions between the adsorbed proteins at interface and the proteins in the gelling solution in the hard gel.

Because of the more compact particulate gel structure and more cross-linking between whey proteins in the hard gel, some cleavage sites may be changed and become more difficult for pepsin to approach during gastric digestion, leading to slower hydrolysis of whey proteins and slower disintegration of gel particles in the hard gel. Therefore, even though the whey proteins in both gels were starting to be hydrolysed by pepsin at approximately the same time (Figure 6-8, peptide formation started from 30 min of digestion for both gels), the rate of proteolysis was slower in the hard gel and further hydrolysis of the large peptides formed may be different. Creusot, Gruppen, van Koningsveld, de Kruif, and Voragen (2006) stated that peptide-peptide and protein-peptide interactions were observed in mixtures of WPI and WPI hydrolysates and that the peptide-peptide interaction were most likely to be dominated by hydrophobic interaction while the protein-peptide interaction relied on the balance between electrostatic repulsion and hydrophobic attraction. This means that the peptides formed in the hard gel would still be associated through hydrophobic interactions and disulphide bonding while in the soft gel, the interactions would be much weaker because of the high electrostatic repulsion. Consequently, the soft gel were broken down faster, with the liberation of oil droplets from the protein matrix during digestion.

Another factor contributing to the slower proteolysis and disintegration of the hard gel could be the slower diffusion rate of pepsin in the gel particles of the hard gel because

of its higher gel hardness, which was extensively explained in Chapter 4 (Section 4.3.7). According to the Stokes-Einstein equation (Equation 6-2), higher gel hardness leads to lower diffusion rate, and therefore slower digestion.

$$D = \frac{k_B T}{6\pi\eta r} \qquad \textbf{Equation 6-2}$$

where D is the diffusion coefficient or 'diffusivity', k_B is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity (measured by small strain oscillatory rheology, positively correlated with gel hardness) and r is the radius of the spherical particle.

There were no intact whey proteins remaining after 240 min of digestion (Fig. 6-8), suggesting that even though proteolysis were slowed down in the hard gel, the cleavage sites may be more difficult to access by pepsin but not hindered.

6.3.5 *In vitro* small intestinal digestion and bioaccessibility of CAP

6.3.5.1 Physicochemical properties of the emptied gastric digesta

The pH, solid content, particle size, oil droplet size and fat content of the gastric digesta are summarized in Table 6-3. Gastric digesta emptied at 60, 120 and 240 min of digestion were used for *in vitro* intestinal digestion because these time points can represent gastric digestion at three different phases: the initial, intermediate and final phase. The gastric digesta collected at these time points had different compositions and properties, which would help us understand how physicochemical properties of gastric digesta would affect intestinal digestion.

Table 6-3 pH, solid content, particle size, oil droplet size and fat content of gastric digesta emptied at 60, 120 and 240 min of digestion from the soft gel (CAP-loaded whey protein emulsion gel with oil droplet size $d_{4,3}$ ~ 0.5 μm and 10 mM NaCl) and the hard gel (CAP-loaded whey protein emulsion gel with oil droplet size $d_{4,3}$ ~ 0.5 μm and 200 mM NaCl). Results are shown as mean ± standard deviation of N = 3 independent experiments.

Physicochemical properties	Soft gel			Hard gel		
	60 min	120 min	240 min	60 min	120 min	240 min
pH	$4.5^{a,x} \pm 0.3$	$3.7^{b,x} \pm 0.2$	$2.6^{c,x} \pm 0.2$	$4.6^{a,x} \pm 0.3$	$3.6^{b,x} \pm 0.3$	$2.4^{c,x} \pm 0.2$
Solid content (w/v%)	$7.8^{a,x} \pm 1.4$	$5.1^{b,x} \pm 0.8$	$5.3^{b,x} \pm 0.7$	$10.1^{a,y} \pm 0.8$	$6.1^{b,x} \pm 0.3$	$3.4^{c,y} \pm 0.7$
Particle size ($d_{4,3}$, μm)	$441.9^{a,x} \pm 57.4$	$389.7^{a,x} \pm 54.1$	$5.2^{b,x} \pm 1.9$	$491.3^{a,x} \pm 26.4$	$395.0^{b,x} \pm 41.8$	$45.0^{c,y} \pm 6.9$
Oil droplet size ($d_{4,3}$, μm)	$0.58^{a,x} \pm 0.13$	$0.81^{b,x} \pm 0.26$	$0.55^{a,x} \pm 0.06$	$0.47^{a,y} \pm 0.01$	$0.46^{a,y} \pm 0.01$	$0.58^{b,x} \pm 0.03$
Fat content (w/v%)	$4.0^{a,x} \pm 0.7$	$2.3^{b,x} \pm 0.2$	$3.1^{c,x} \pm 0.4$	$5.6^{a,y} \pm 0.5$	$3.2^{b,y} \pm 0.4$	$2.0^{c,y} \pm 0.5$

$^{a\text{-}c}$Values with different letters within the same gel type differ significantly ($P < 0.05$).

^{x,y}Values with different letters for the gastric digesta collected at the same digestion time differ significantly ($P < 0.05$).

6.3.5.2 Breakdown of gel particles during intestinal digestion

Figure 6-11 presents the changes in particle size distributions during *in vitro* intestinal digestion of gastric digesta from soft gel and hard gel emptied at 60, 120 and 240 min of gastric digestion. The gastric digesta emptied at 60 min from both gels had similar particle size distribution showing a bimodal pattern with a narrow peak near 1000 μm and another broader peak in the range of 2 to 100 μm. There was no peak for both gels in the range of 0.1 to 1 μm, indicating no oil droplet release from protein matrix (Fig. 6-11 A1 and A2). At 10 min of intestinal digestion, the digesta from hard gel still showed a bimodal distribution where the peak near 10 μm became narrower and increased in volume while the peak near 1000 μm decreased slightly in volume, indicating the disintegration of gel particles. There were no free oil droplets released from protein matrix at this stage. The digesta from soft gel showed a trimodal distribution with a new peak appearing in the range of 0.04 to 4 μm, indicating the liberation of free oil droplets from protein matrix at 10 min of intestinal digestion; the peaks in the range of 2 to 100 μm and near 1000 μm both decreased in volume, indicating the disintegration of gel particles. At 30 min of intestinal digestion, the digesta from hard gel showed a trimodal distribution with a new peak appearing in the range of 0.05 to 2 μm, suggesting oil droplet release from protein matrix; the peak near 10 μm increased in volume and the peak near 1000 μm decreased in volume. The peak in the range of 0.04 to 4 μm from the digesta from soft gel continued to increase in volume while the peak in the range of 4 to 300 μm decreased in volume, indicating further breakdown of gel particles and more oil droplet release at 30 min of intestinal digestion.

At 60, 90 and 120 min of intestinal digestion, the particle size distributions of the digesta from soft gel were similar with a major peak in the range of 0.04 to 4 μm and

another small peak with a tail in the range of 4 to 300 μm. The peak in the range of 0.04 to 4 μm slowly transformed into a bimodal pattern with a peak near 0.1 μm and another peak near 1 μm. The peak near 0.1 μm might be the mixed micelles and vesicles formed during intestinal digestion or the digested oil droplets, the peak near 1 μm might be the undigested oil droplets. The peak with a tail in the range of 4 to 300 μm might be the coalesced / flocculated oil droplets. At 60, 90 and 120 min of intestinal digestion, the digesta from hard gel also showed a bimodal distribution with a major peak in the range of 0.04 to 4 μm and a second peak in the range of 4 to 100 μm. The peak in the range of 4 to 100 μm gradually decreased in volume from 60 to 120 min of intestinal digestion; the peak in the range of 0.04 to 4 μm gradually increased in volume and showed a bimodal distribution at 120 min of digestion. Although, the peak near 0.1 μm (possibly representing the mixed micelles and vesicles or the digested oil droplets) was much smaller than that from the soft gel. This qualitatively indicates that the soft gel was digested to a higher extent.

The changes in particle size distributions during intestinal digestion of the gastric digesta emptied at 120 min are shown in Fig. 6-11 B1 (soft gel) and B2 (hard gel). The particle size distributions of the digesta from both gels before intestinal digestion (i.e. 0 min) were similar with a peak near 1000 μm and another peak in the range of 2 to 200 μm; there was no peak in the range of 0.1 to 1 μm, indicating no oil droplet release from protein matrix. At 10 min of intestinal digestion, for digesta from both gels, a new peak in the range of 0.03 to 2 μm appeared, indicating the release of oil droplets from protein matrix; the peak near 1000 μm was similar to that of the digesta at 0 min. The peak in the range of 2 to 200 μm decreased in the digesta from soft gel and increased in the digesta from hard gel. At 30 min of intestinal digestion, the peak near 1000 μm disappeared in the digesta from soft gel, indicating complete breakdown of large gel

particles from the soft gel, while the digesta from hard gel still had this peak. In general, the peaks gradually shifted to the left for both gels, indicating particle size reduction during intestinal digestion. At 120 min of intestinal digestion, there were a major peak near 0.1 μm for both gels, indicating the digested oil droplets or the formation of mixed micelles and vesicles, and some small peaks in the larger size ranges, which could be the undigested and/or coalesced / flocculated oil droplets.

The changes in particle size distributions of gastric digesta emptied at 240 min are shown in Fig. 6-11 C1 (soft gel) and C2 (hard gel). At 0 min, the digesta from soft gel showed bimodal distribution with one peak near 10 μm representing small gel particles and another peak near 0.5 μm representing free oil droplets released from protein matrix; the digesta from hard gel showed trimodal distribution with a peak near 300 μm and a peak near 10 μm representing gel particles and a third peak near 0.7 μm representing free oil droplets released from protein matrix. Starting from 30 min of intestinal digestion, the digesta from both gels showed bimodal distribution in the range of 0.04 to 4 μm with a peak near 0.1 μm and another peak near 1 μm. The peak near 0.1 μm gradually increased in volume and reached maximum at the end of intestinal digestion, while the peak near 1 μm gradually decreased in volume. This indicates the gradual digestion of oil droplets and the formation of mixed micelles and vesicles during intestinal digestion.

In summary, the results indicate that the digesta from hard gel emptied at 60 and 120 min were more resistant to disintegration and digestion in the small intestine, and this could be attributed to the higher solid content of the digesta from hard gel emptied at 60 and 120 min (Table 6-3); the effect of protein matrix is another contributing factor, as explained previously in section 6.3.4.

In general, gastric digesta emptied at 240 min were disintegrated the fastest in the small intestine, followed by gastric digesta emptied at 120 min and gastric digesta emptied at 60 min. This is probably because of the lower solid content and smaller gel particle size of the gastric digesta emptied at 240 min.

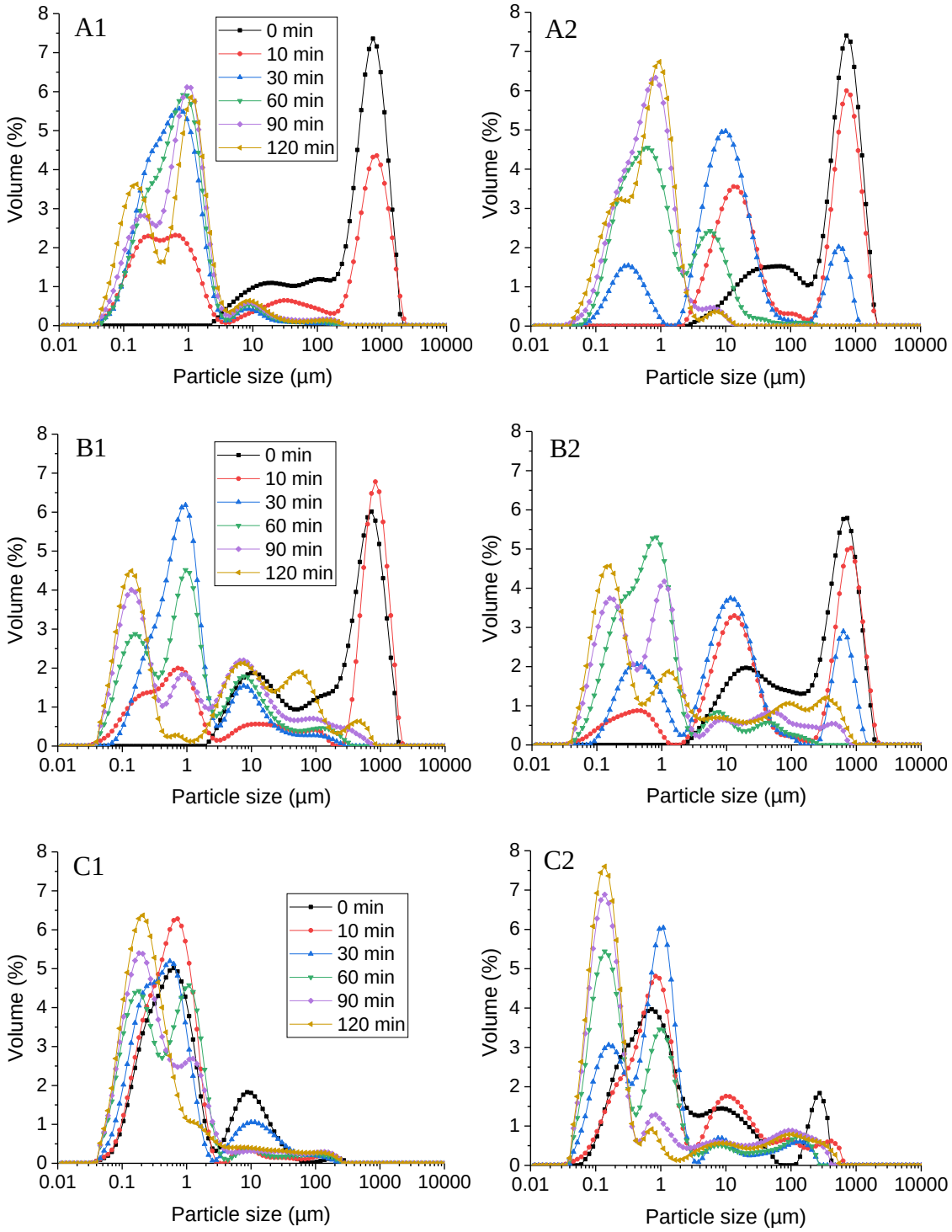


Figure 6-11 Changes in particle size distributions of emptied gastric digesta during intestinal digestion (0, 10, 30, 60, 90 and 120 min): (A1) gastric digesta from soft gel emptied at 60 min; (A2) gastric digesta from hard gel emptied at 60 min; (B1) gastric digesta from soft gel emptied at 120 min; (B2) gastric digesta from hard gel emptied at 120 min; (C1) gastric digesta from soft gel emptied at 240 min; (C2) gastric digesta from hard gel emptied at 240 min.

6.3.5.3 Initial lipolysis rate

The initial lipolysis rate (i.e. the free fatty acid released per ml of digestion mixture per minute during the initial 2 min of reaction) calculated from Figure 6-12 is presented in Table 6-4. For gastric digesta emptied at 60 min, the soft gel had higher lipolysis rate than the hard gel ($P < 0.05$); no significant difference was found for gastric digesta emptied at 120 and 240 min between the soft gel and the hard gel. Based on the Michaelis-Menten equation, the rate of enzymatic reaction is positively correlated with the substrate concentration until the reaction rate reaches the maximum value and becomes plateau (i.e. the enzyme becomes saturated). However, the limitation of the Michaelis-Menten model is that it assumes the diffusion to be free. For gastric digesta emptied at 60 min, the hard gel had significantly higher fat content than the soft gel (Fig. 6-9 and Table 6-3), however, the initial lipolysis rate was significantly lower. This suggests that the gel structure had a stronger effect on lipolysis rate than the concentration of substrate (i.e. fat). The oil droplets in the gel particles were covered by protein coating in both gels, so the diffusion of enzymes through the solid gel particles is not free diffusion. Also, according to the Stokes-Einstein equation (Equation 6-2), the diffusion rate would be lower in hard gel than in soft gel.

For gastric digesta emptied at 120 min, the hard gel had significantly higher fat content than the soft gel (Fig. 6-9 and Table 6-3), but no significant difference was found in initial lipolysis rate between them. This could also be attributed to the effect of the gel structure of the soft gel.

For gastric digesta emptied at 240 min, the soft gel had higher initial lipolysis rate than the hard gel. There could be several contributing factors: gastric digesta from the

soft gel had significantly higher fat content, smaller gel particle size, and more oil droplet release from protein matrix.

For both gels, gastric digesta emptied at 240 min had higher lipolysis rate than gastric digesta emptied at 60 min. At 240 min of gastric digestion, the solid content was much lower, the gel particles were broken down into smaller sizes, and there were free oil droplets released from protein matrix. These would make the substrate easier for the enzyme to access. Also, the proteins in the gastric digesta at 240 min were more readily hydrolysed than the proteins in the gastric digesta at 60 min, which may have also contributed to the higher lipolysis rate of gastric digesta at 240 min.

Table 6-4 Initial lipolysis rate ($\mu mol \cdot ml^{-1} \cdot min^{-1}$) of emptied gastric digesta during intestinal digestion. Results are shown as mean ± standard deviation of N= 3 independent experiments.

	Soft gel			Hard gel		
	60 min	120 min	240 min	60 min	120 min	240 min
Initial lipolysis rate	$1.05^{a,x}$ ± 0.21	$1.03^{a,x}$ ± 0.38	$1.48^{a,x}$ ± 0.37	$0.46^{a,y}$ ± 0.06	$0.64^{ab,x}$ ± 0.26	$1.07^{b,x}$ ± 0.21

^{a,b}Values with different letters within the same gel type differ significantly ($P < 0.05$).
^{x,y}Values with different letters for the gastric digesta collected at the same digestion time differ significantly ($P < 0.05$).

6.3.5.4 Free fatty acid release profiles

The free fatty acid (FFA) release profiles per ml of digestion mixture of gastric digesta emptied at 60, 120 and 240 min during 120 min of intestinal digestion are shown in Figure 6-12. For gastric digesta at 60 min, the fatty acid release reached plateau at about 50 min of intestinal digestion for the soft gel, while it did not reach plateau at the end of intestinal digestion for the hard gel. This indicates that the oil droplets from soft

gel were hydrolysed faster than the hard gel, and this could also be attributed to the effect of gel structure. The oil droplets were liberated from protein matrix faster in the soft gel than in the hard gel and the solid gel particles of the soft gel were quickly broken down into free oil droplets (indicated by particle size distribution presented in Fig. 6-11 A1 and A2), which made it easier for the pancreatic lipase and co-lipase to access to the interface of the oil droplets. Similar results were observed for gastric digesta at 120 min. For gastric digesta at 240 min, it took 30 min for the soft gel to reach plateau while it took 10 min for the hard gel. This could be attributed to the lower fat content of the gastric digesta from hard gel.

For the gastric digesta emptied at 60 min, the lipolysis rate (i.e. the slope of the curve) of the soft gel gradually decreased in the first 50 min of digestion because of the gradual lipolysis of the oil droplets (i.e. decreased substrate concentration leading to decreased rate of reaction), and the rate became zero after 50 min of intestinal digestion. For the hard gel, the lipolysis rate slightly decreased in the first 30 min and then was kept relatively constant until the end of digestion. This means that in the first 30 min, the concentration of the accessible substrate gradually decreased, and after that, the ratio of accessible substrate to enzyme was relatively constant, indicating that the hard gel was broken down at a slower rate during intestinal digestion, leading to gradual release of accessible oil droplets. Similar results were observed for gastric digesta emptied at 120 min.

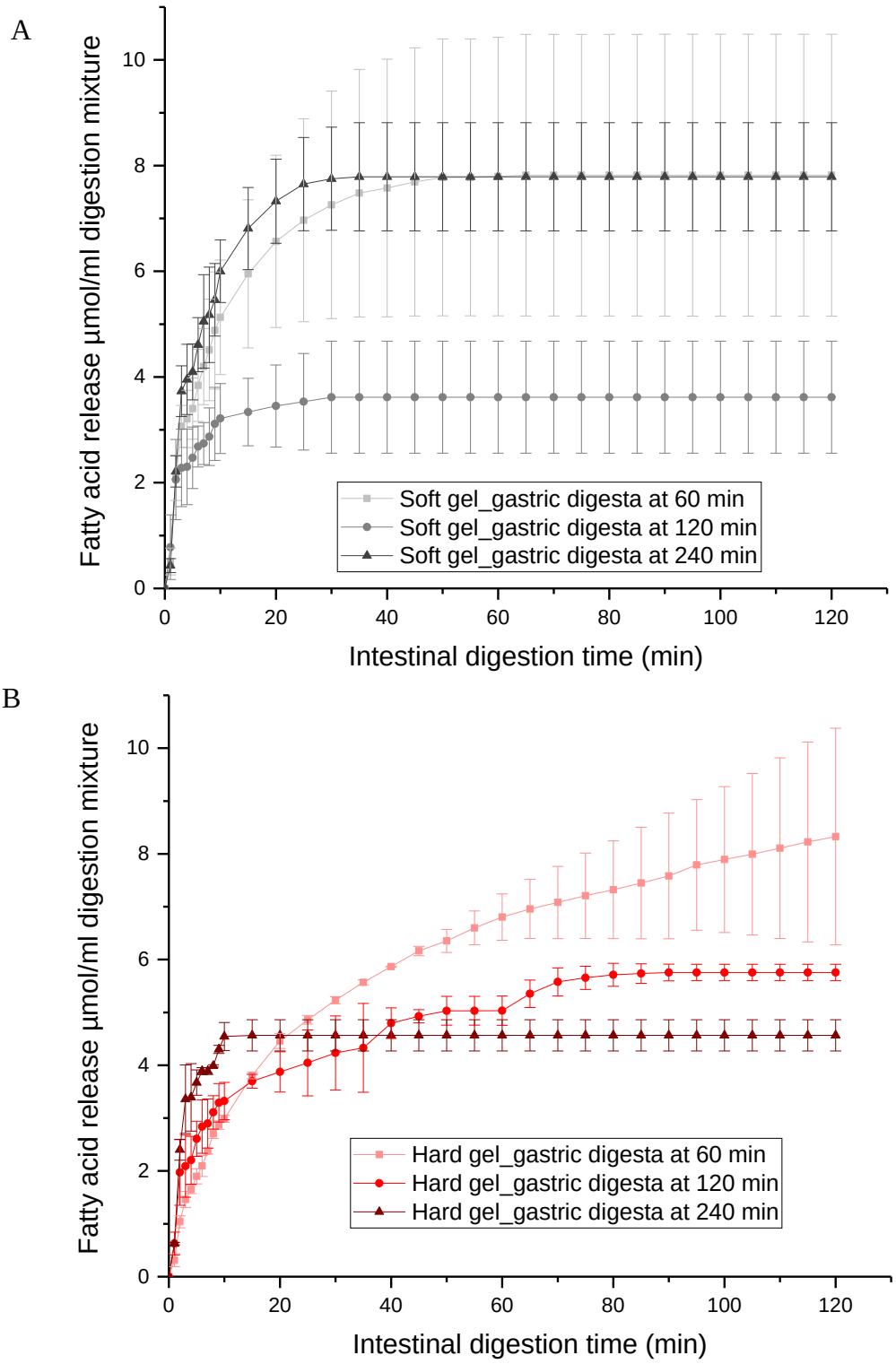


Figure 6-12 Free fatty acid release profile per ml of digestion mixture of gastric digesta emptied at 60, 120 and 240 min during intestinal digestion. (A) soft gel; (B) hard gel. Error bars represent standard deviations obtained from N = 3 independent experiments.

Figure 6-13 shows the free fatty acid release profiles per g of fat, which indicates the extent of lipolysis during intestinal digestion. For both gels, the gastric digesta emptied at 240 min had higher extent of lipid digestion than gastric digesta emptied at 60 and 120 min ($P < 0.05$); no significant difference was found between gastric digesta emptied at 60 and 120 min. For gastric digesta emptied at 60 min, the soft gel had higher extent of lipid digestion than the hard gel ($P < 0.05$); there was no significant difference for the gastric digesta emptied at 120 and 240 min between soft gel and hard gel.

The extent of lipid digestion is affected by multiple factors, such as the accessibility of lipolytic enzymes to the substrate, the concentration of the substrate, the concentration of the enzyme and the bile salts, the effect of calcium, the characteristics of the lipids (e.g. type of lipid, droplet size, emulsifier type), etc (Li, Hu, & McClements, 2011; Li & McClements, 2010; Malaki Nik, Wright, & Corredig, 2011; Maldonado-Valderrama et al., 2011; Salvia-Trujillo et al., 2013). It is expected that less fat content would generally lead to a higher extent of lipid digestion (Li et al., 2011), because of a higher ratio of calcium / bile salts to the substrate / FFA. The role of bile salts in lipid digestion is that they could replace the proteins / peptides at the oil-water interface, so that the co-lipase and lipase could anchor onto the interface and hydrolyse the lipids; bile salts also replace the lipolytic products (i.e. FFA, monoglycerides and diglycerides) from the interface and help solubilize them by forming mixed micelles (Maldonado-Valderrama et al., 2011). With a higher calcium /bile salts-to-substrate ratio, the lipolytic products would be more effectively precipitated by calcium and displaced by bile salts from the oil-water interface. This prevents the accumulation of lipolytic products at the interface, which would free the interface for the enzymes to further hydrolyse the lipid. The effect of lower fat content on the extent of lipid digestion was seen: 1) for the hard gel, the gastric digesta emptied at 240 min had significantly higher extent of lipid digestion than the gastric digesta

emptied at 60 and 120 min; 2) for the gastric digesta emptied at 60 min, the soft gel had higher extent of lipid digestion than the hard gel; 3) for the gastric digesta emptied at 240 min, the hard gel had higher extent of lipid digestion than the soft gel.

However, for the gastric digesta emptied at 120 min from the soft gel, even though it had lower fat content than the gastric digesta emptied at 60 and 240 min (Fig. 6-9 and Table 6-3), the extent of lipid digestion was lower. At 120 min, it is likely that oil droplet coalescence has occurred (Fig. 6-6), which increased the oil droplet size, as compared to gastric digesta emptied at 60 and 240 min (Table 6-3). An increase in oil droplet size would lead to a decrease in interface area exposed to lipolytic enzymes, and therefore leading to a decrease in the rate of lipid digestion (Li et al., 2011; Salvia-Trujillo et al., 2013).

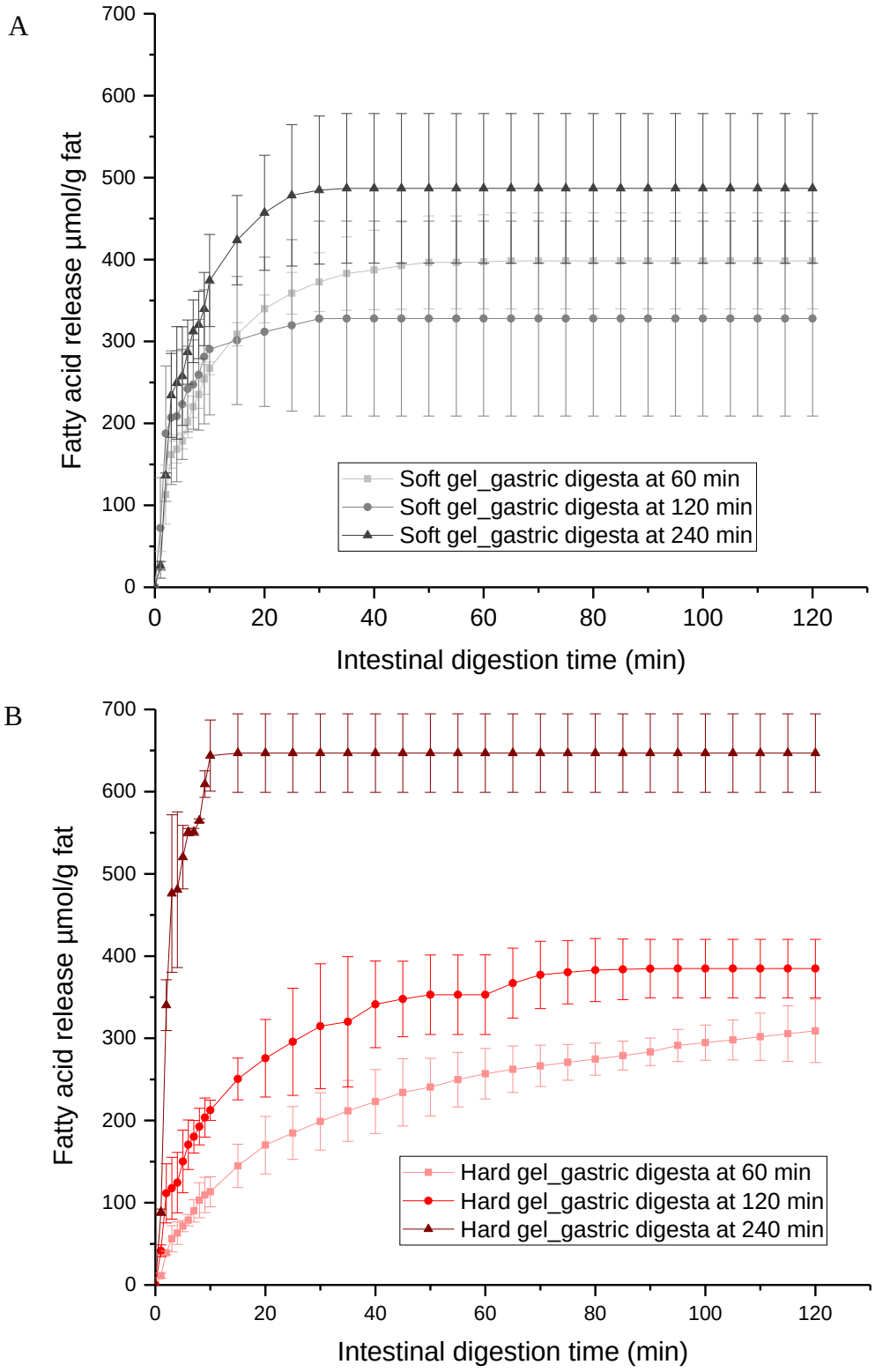


Figure 6-13 Free fatty acid release profile per g of fat of gastric digesta emptied at 60, 120 and 240 min during 120 min of intestinal digestion. (A) soft gel; (B) hard gel. Error bars represent standard deviations obtained from N = 3 independent experiments.

6.3.5.5 Bioaccessibility of CAP

Figure 6-14 presents the bioaccessibility of CAP after *in vitro* gastrointestinal digestion. For both gels, gastric digesta emptied at 240 min had significantly higher bioaccessibility of CAP than gastric digesta emptied at 60 and 120 min. For gastric digesta emptied at 60 min, the soft gel had higher bioaccessibility of CAP than the hard gel ($P < 0.05$); for gastric digesta emptied at 120 min, the hard gel had higher bioaccessibility of CAP than the soft gel ($P < 0.05$); no significant difference was found for gastric digesta emptied at 240 min between the soft gel and the hard gel. A power-law fitting was done between the bioaccessibility of CAP (Y) and the extent of lipid digestion (X; represented by the final FFA release per g of fat): $Y = 49.2 \times (X - 305.3)^{0.104}$, with $R^2 = 0.84$ (Fig. 6-15). In general, the bioaccessibility of CAP was positively correlated with the extent of lipid digestion.

The absorption of dietary CAP includes the following steps: the release of CAP from the food matrix during digestion in the GI tract, the solubilization of CAP in the aqueous phase through the formation of micelles and vesicles, the transportation of CAP to the epithelium and the absorption of CAP by the epithelial cells. In *in vitro* studies, only the first two steps were considered, which represent the bioaccessibility of CAP. A higher extent of lipid digestion would lead to more release of CAP from the food matrix, also, more lipolytic products would also participate in micelle formation and help solubilize the released CAP, and therefore led to higher bioaccessibility. The results were in agreement with the report from Lu et al. (2016), where they investigated the effect of different oil type (MCT, corn oil or canola oil) and found that the use of MCT as the lipid carrier for capsaicin in nanoemulsion had significantly higher extent of lipolysis during *in vitro* digestion and this led to higher bioaccessibility of capsaicin. In a study

investigating the bioaccessibility of β-carotene in emulsions and nanoemulsions, Salvia-Trujillo et al. (2013) also reported that the bioaccessibility of β-carotene and the final FFA released to be positively linearly correlated.

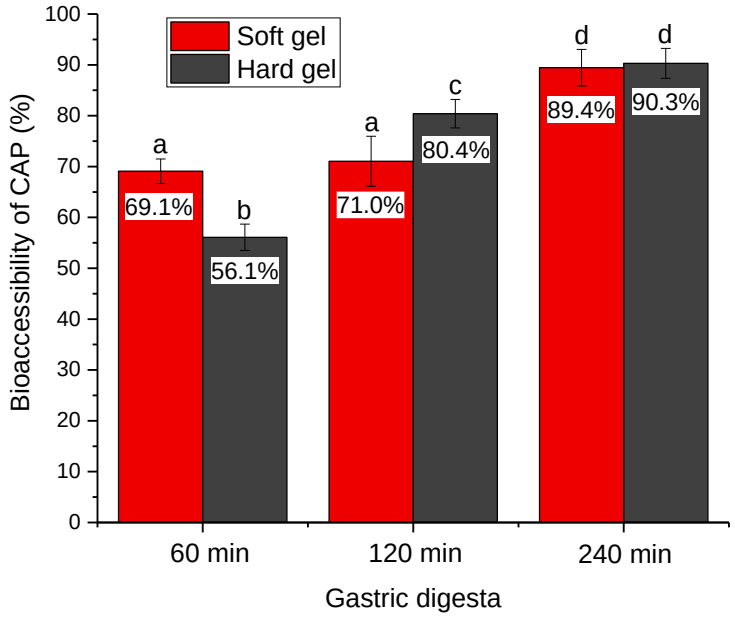


Figure 6-14 Bioaccessibility of CAP after *in vitro* gastrointestinal digestion. Error bars represent standard deviations obtained from N = 3 independent experiments, each conducted in duplicate.

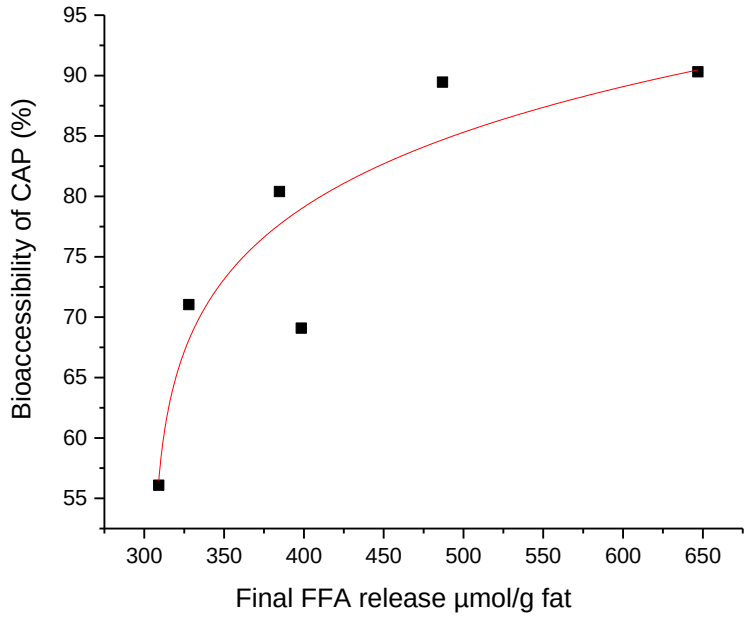


Figure 6-15 Correlation between the bioaccessibility of CAP and the final FFA release per g of fat.

6.4 Conclusions

In this chapter, we investigated the effect of gel structure on the *in vitro* gastric digestion behaviour of whey protein emulsion gels containing CAP using a human gastric simulator, and the effect of the physicochemical characteristics and colloidal structure of gastric digesta on its *in vitro* intestinal digestion behaviour and bioaccessibility of CAP. The soft gel was mostly disintegrated into free oil droplets at the end of gastric digestion while the hard gel had partial release of oil droplets from the protein matrix. Because the gel particles were disintegrated faster and there was less cross-linking between whey proteins in the soft gel to protect the oil droplets from flocculation / coalescence, the oil droplets started to coalesce after 60 min of gastric digestion. No oil droplet coalescence was observed for the hard gel during gastric digestion.

During intestinal digestion, the hard gel was also disintegrated slower than the soft gel during intestinal digestion because of its gel structure, except for the gastric digesta emptied at 240 min, where the hard gel had higher extent of lipid digestion probably due to its lower fat content. Lower fat content would generally lead to higher extent of lipid digestion, and it is mainly because of higher effective concentration of calcium and bile salts. The calcium and bile salts are more effective in precipitating the lipolytic products and removing them from the oil-water interface and free the interface for further lipolysis. Oil droplet coalescence was observed in gastric digesta emptied at 120 min from the soft gel, leading to reduced surface area for the lipolytic enzymes to react during intestinal digestion, and therefore had lower extent of lipid digestion.

A power-law fitting was done between the bioaccessibility of CAP (Y) and the extent of lipid digestion (X; represented by the final FFA release per g of fat): $Y = 49.2 \times (X - 305.3)^{0.104}$, with $R^2 = 0.84$. A higher extent of lipid digestion would lead

to more release of CAP from the food matrix, also, more lipolytic products would be produced and participate in micelle formation that help solubilize the released CAP, and therefore led to higher bioaccessibility of CAP. This work has provided useful insights in food structural design for delivery of lipophilic bioactive compounds; by manipulating the gel structure, the digestion behaviour in the GI tract can be altered, which would affect the bioaccessibility of the incorporated bioactive compounds. Also, this work has provided information on how the characteristics of gastric digesta would affect the intestinal digestion and bioaccessibility of incorporated bioactive compounds, by using digesta emptied at the beginning, intermediate and end of gastric digestion. Gastric emptying into the small intestine is a dynamic process, therefore, the digesta at different times would be different and also have an effect on the behaviour during intestinal digestion. This factor should be taken into consideration to better assess the bioaccessibility of nutrients in future *in vitro* studies.

Chapter 7: Digestion behaviour of capsaicinoids-loaded emulsion gels and bioaccessibility of capsaicinoids: Effect of emulsifier type

7.1 Abstract

In this study, the effect of emulsifier type, i.e. whey proteins versus Tween 80 on the digestion behaviour of emulsion gels containing capsaicinoids (CAP) were examined. Results indicate that CAP-loaded Tween 80 emulsion gel was emptied out significantly faster during gastric digestion than CAP-loaded whey protein emulsion gel, mainly due to its smaller masticated bolus particle size entering the stomach. The Tween-80-coated oil droplets seemed to be in a flocculated state in the emulsion gel, disrupting the protein network structure; they had no interactions with the protein matrix and started to be released from the protein matrix during gastric digestion. The whey-protein-coated oil droplets had strong interactions with the protein matrix, and the thick protein layer formed around the oil droplets protected their liberation during gastric digestion.

During intestinal digestion, CAP-loaded Tween 80 emulsion gel showed lower extent of lipolysis than CAP-loaded whey protein emulsion gel, probably because Tween 80 had certain resistance against displacement by bile salts at the interface, and / or Tween 80 formed interfacial complexes with bile salts / lipolytic enzymes. Due to the softer structure of CAP-loaded Tween 80 emulsion gel, the gel particles were broken down much faster and the oil droplets were liberated from the protein matrix much faster than CAP-loaded whey protein emulsion gel during intestinal digestion; this promoted the release of CAP molecules from the gel. Meanwhile, the Tween 80 molecules displaced

from the interface would participate in the formation of mixed micelles and help solubilize the released CAP molecules, leading to improved bioaccessibility of CAP. Information obtained from this work could be useful in designing functional foods for delivery of lipophilic bioactive compounds.

7.2 Introduction

Liquid emulsion-based systems have been widely studied for their uses in delivering lipophilic bioactive compounds, where the bioactive compounds are dissolved in the emulsion droplets to improve their stability (Ahmed et al., 2012; Humberstone & Charman, 1997; Lin et al., 2018; Lu et al., 2016; Salvia-Trujillo et al., 2013). However, limited information is available on the use of emulsion gel as the delivery system. The solid/semi-solid emulsion gel possesses very different digestion behaviour from the liquid emulsion systems, since it requires mastication in the mouth and further disintegration in the subsequent GI tract. The solid/semi-solid systems normally require more time to digest; the release behaviour of the nutrients and their bioaccessibility are also different between liquid versus solid systems (Dias et al., 2019).

An emulsion gel is a complex colloidal system that exists as both an emulsion and a gel (Dickinson, 2012). Its formation requires both an emulsifying agent and a gelling agent. The selection of the emulsifying agent and the gelling agent has a considerable impact on the structure and rheological properties of the emulsion gel. For instance, the effect of using whey proteins versus Tween 20 as emulsifier on the structure and rheological properties of heat-set whey protein emulsion gel have been investigated by Chen et al. (2000). The whey-protein-coated oil droplets have strong interactions with the surrounding whey protein matrix, and thus contribute to gel strength; the Tween-20-coated oil droplets are not bound to protein matrix (weak or no interaction), and thus act

as defects to the network and lead to a monotonic decrease in gel strength. The emulsion gel using proteins or non-ionic small molecule surfactant as emulsifier possesses distinct gel structure and rheological properties; however, their breakdown and digestion behaviour in mouth and subsequent GI tract have not been studied.

The effects of using different types of emulsifiers on the *in vitro* digestion behaviour of liquid oil-in-water emulsions have been widely studied in recent decades. van Aken, Bomhof, Zoet, Verbeek, and Oosterveld (2011) studied the use of different emulsifiers on the *in vitro* gastric digestion behaviour of emulsions and reported that the whey protein stablized emulsion with additional Tween 80 was more resistant against flocculation, but had more oil droplet coalesence during gastric digestion, as compared to emulsion stabilized with whey proteins alone. Li and McClements (2011) investigated the effect of additional non-ionic, anionic and cationic low-molecular weight surfactants on the rate and extent of lipolysis of emulsion stabilized with β-lactoglobulin under simulated gastrointestinal conditions and found that there was a lag phase during intestinal digestion with the presence of surfactants; when surfactants were added to a sufficient concentration, the extent of lipolysis was significantly reduced. The effect of surfactant type on the bioaccessiblity of β-carotene after *in vitro* digestion of emulsion was studied by Mun, Kim, Shin, and McClements (2015), and the extent of lipolysis was similar between emulsions stabilized with whey proteins or Tween 20; however, emulsion stabilized with Tween 20 had significantly higher bioaccessibility of β-carotene.

In Chapter 5, the effect of using whey proteins versus Tween 80 on the *in vivo* oral processing behaviour and sensory perception of emulsion gels containing CAP was investigated. It was found that CAP-loaded Tween 80 emulsion gel was significantly more fragmented and released large amounts of oil droplets in the mouth, leading to

higher mouth burn perception. Further to that, in this chapter, we aim to investigate the simulated gastrointestinal digestion behaviour of emulsion gels using either whey proteins or Tween 80 as emulsifier and the impact on the bioaccessibility of CAP.

The CAP-loaded whey protein emulsion gel $W_{0.5}$-100 (i.e. whey proteins as both emulsifier and gelling agent, oil droplet size $d_{4,3}$ of ~ 0.5 µm, 100 mM NaCl; characterized as gel with active filler particles) and the CAP-loaded Tween 80 emulsion gel $T_{0.5}$-100 (i.e. Tween 80 as emulsifier, whey proteins as gelling agent, oil droplet size $d_{4,3}$ of 0.5 µm, 100 mM NaCl; characterized as gel with inactive filler particles) were used in this study. The hypothesis was that the CAP-loaded Tween 80 emulsion gel would break down much faster and empty out much faster during gastric digestion, because of its loose gel structure. *In vitro* dynamic gastric digestion was carried out for 240 min using the Human Gastric Simulator (HGS), and *in vitro* intestinal digestion was carried out for 120 min using pH-stat.

7.3 Results and discussion

7.3.1 pH profiles during *in vitro* gastric digestion in the HGS

Figure 7-1 presents the pH profiles during 240 min of *in vitro* gastric digestion in the Human Gastric Simulator (HGS) of CAP-loaded whey protein emulsion gel ($W_{0.5}$-100: whey proteins as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 µm, 100 mM NaCl) and CAP-loaded Tween 80 emulsion gel ($T_{0.5}$-100: Tween 80 as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 µm, 100 mM NaCl). The pH of emptied gastric digesta gradually decreased from 5.88 ± 0.27 at 0 min to 2.40 ± 0.23 at 240 min for CAP-loaded whey protein emulsion gel and from 5.70 ± 0.12 at 0 min to 2.27 ± 0.06 at 240 min for CAP-loaded Tween 80 emulsion gel. The pH profiles during gastric digestion of these two gels were similar,

indicating that their buffering capacity was not significantly affected by the type of emulsifier used.

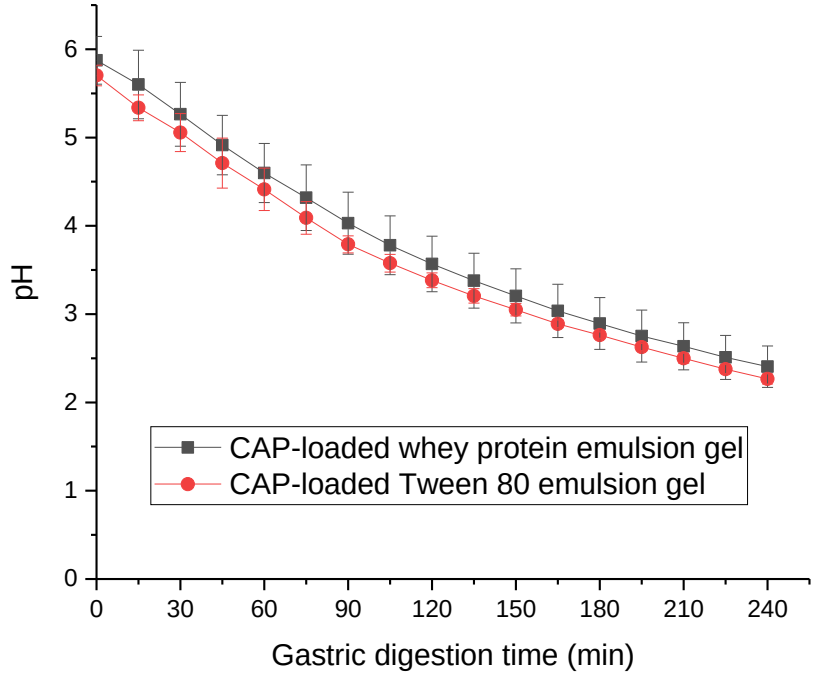


Figure 7-1 Changes in pH during *in vitro* gastric digestion in the HGS of CAP-loaded whey protein emulsion gel (whey proteins as emulsifier, $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl) and CAP-loaded Tween 80 emulsion gel (Tween 80 as emulsifier, $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl). Error bars represent standard deviations obtained from N = 3 independent experiments.

7.3.2 Physiochemical characteristics of emptied gastric digesta

7.3.2.1 Solid content of emptied gastric digesta

The solid content of emptied gastric digesta as a function of digestion time is presented in Figure 7-2. Overall, with increasing digestion time, the solid content of gastric digesta gradually decreased for both gels, because of constant gastric secretion and emptying. Gastric emptying only occurred after 30 min because of the lag phase during digestion of solid/semi-solid foods (Siegel et al., 1988). For CAP-loaded whey protein emulsion gel, there was no significant difference in the solid content of gastric

digesta emptied at 30 to 60 min or at 180 to 240 min; the solid content of gastric digesta gradually decreased from 60 to 180 min. For CAP-loaded Tween 80 emulsion gel, no significant difference was found in the solid content of gastric digesta emptied at 30 to 60 min; the solid content of the gastric digesta gradually decreased from 60 to 105 min, increased at 120 min and then gradually decreased from 120 to 240 min.

Gastric digesta emptied at 30 to 165 min from CAP-loaded Tween 80 emulsion gel had significantly higher solid content than those from CAP-loaded whey protein emulsion gel; no significant difference was found between two gels for gastric digesta emptied at 180 to 240 min. The results suggested that the CAP-loaded Tween 80 emulsion gel was emptied out faster than CAP-loaded whey protein emulsion gel. This was probably due to the smaller gel particles after mastication of CAP-loaded Tween 80 emulsion gel entering the stomach (Table 5-2 in Section 5.3.3.3, Chapter 5: the masticated bolus particle size d_{frag} of CAP-loaded whey protein emulsion gel and CAP-loaded Tween 80 emulsion gel were 0.80 ± 0.11 and 0.12 ± 0.01 mm respectively). Gastric sieving only allows particles smaller than 1 mm to pass through; CAP-loaded Tween 80 emulsion gel had about 73% of the solids smaller than 1 mm while CAP-loaded whey protein emulsion gel had about 47% after oral processing (estimated based on *in vivo* data presented in Figure 5-4 from Chapter 5). Greater proportion of smaller gel particles from the CAP-loaded Tween 80 emulsion gel facilitated its emptying from the stomach. Similar results were reported by Koike et al. (2013) and Pera et al. (2002) that insufficient mastication (leading to bigger masticated bolus particle size) would result in a reduced rate of gastric emptying.

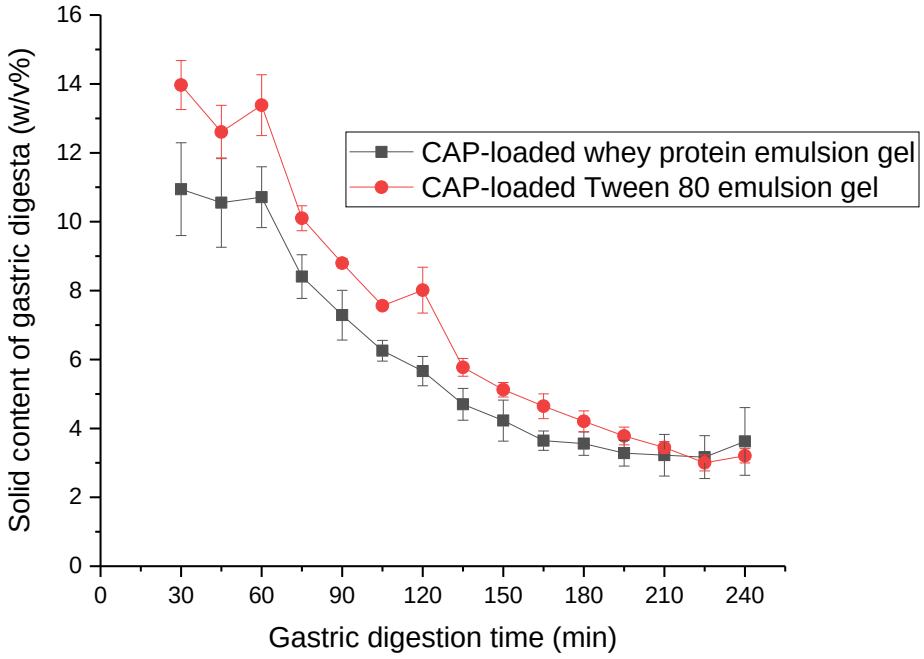


Figure 7-2 Solid content of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

7.3.2.2 Particle size distribution of emptied gastric digesta

With increasing digestion time, the average particle size ($d_{4,3}$) of the emptied gastric digesta gradually decreased for both gels (Fig. 7-3), showing the gradual breakdown of gel particles during gastric digestion. During 30 to 180 min of gastric digestion, emptied digesta from CAP-loaded whey protein emulsion gel had larger particle size than digesta from CAP-loaded Tween 80 emulsion gel; this was attributed to the smaller masticated bolus particle size and the release of oil droplets from protein matrix during gastric digestion (Fig. 7-4) of CAP-loaded Tween 80 emulsion gel. No significant difference was found in particle size of gastric digesta emptied at 210 and 240 min between two gels. At 210 min of digestion, the pH had decreased to ~ 2.5 (Fig. 7-1) for both gels. Pepsin has higher activity at this pH value and, therefore, is more effective in cleaving peptide bonds and facilitate gel disintegration. This effect was more evident in CAP-

loaded whey protein emulsion gel, which may explain a large decrease in particle size of gastric digesta emptied from 180 to 240 min.

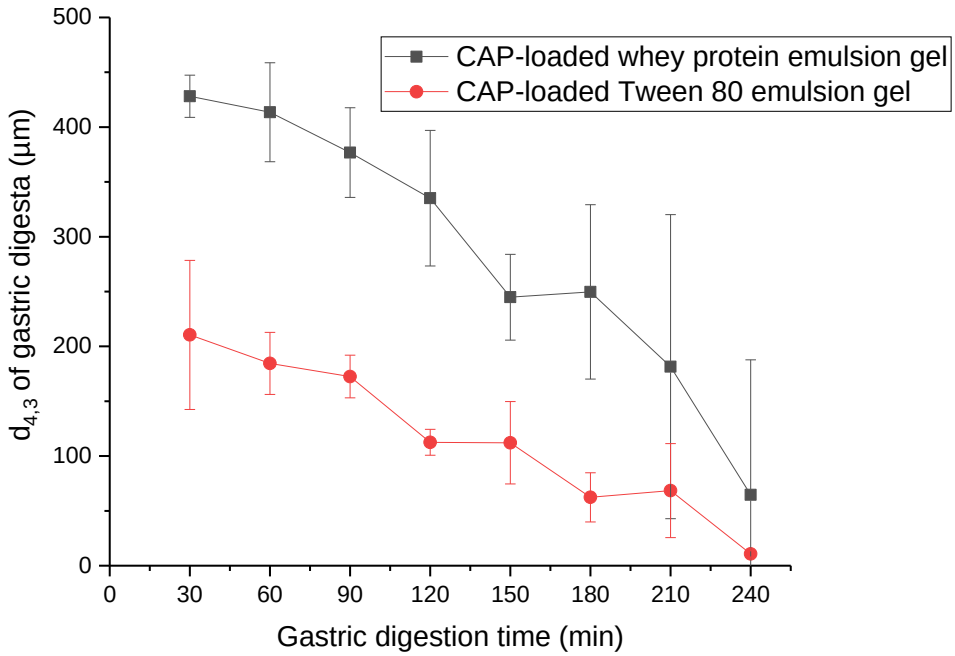


Figure 7-3 Average particle size ($d_{4,3}$, μm) of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

Figure 7-4 presents the particle size distributions of CAP-loaded liquid emulsions and the emptied gastric digesta as a function of digestion time. For CAP-loaded whey protein emulsion gel (Fig. 7-4 A), the gastric digesta emptied at 30, 60, 90 and 120 min had similar particle size distributions that resemble a trimodal pattern with a high and narrow peak near 1000 μm, a small and broader peak near 10 μm and another smaller peak near 100 μm. With increasing digestion time from 30 to 120 min, the peak near 1000 μm showed a slight decrease while the peak near 10 μm showed a slight increase in volume; the peaks near 100 μm were similar. This indicates that the disintegration of gel particles during the first two hours of digestion were not significant. At 150 and 180 min of digestion, the particle size distributions of the gastric digesta also showed trimodal

distribution with three peaks near 10, 100 and 1000 μm; the peak near 1000 μm decreased significantly while the peak near 10 μm increased significantly and the peak near 100 μm decreased slightly in volume, as compared to the particle size distributions of gastric digesta emptied at 30 to 120 min. This indicates the breakdown of large gel particles and the production of small particles during 120 to 180 min of digestion. At 210 min of digestion, two new peaks appeared in the range of 0.04 to 2 μm, which could be the oil droplets liberated from protein matrix; the peak near 10 and 100 μm decreased significantly in size while the peak near 1000 μm stayed similar, as compared to the digesta emptied at 150 and 180 min. This indicates further breakdown of gel particles. At 240 min of digestion, the peak near 1000 μm disappeared while the peak near 10 μm increased in volume, as compared to gastric digesta emptied at 210 min, suggesting the total disintegration of large gel particles; the two peaks in the range of 0.04 to 2 μm also increased in size, suggesting more oil droplet release from protein matrix.

The results from CAP-loaded whey protein emulsion gel suggested that gel disintegration was not significant during 0 to 120 min of digestion. During 0 to 120 min of digestion, the pH of gastric digesta decreased from 5.88 ± 0.27 to 3.57 ± 0.31; the pH decreased to 4.03 ± 0.35 at 90 min of digestion. Clearly after 120 min of digestion, pepsin became more active due to low pH in hydrolysing proteins and facilitating the breakdown of gel particles by disrupting the protein network structure.

The diffusion of pepsin through gel particles was another factor affecting gel disintegration during gastric digestion. During the first two hours of digestion, the particle size of the gastric digesta was relatively large, as compared to the gastric digesta collected after 120 min of digestion. The diffusion of pepsin through smaller gel particles was more efficient, which would also facilitate gel disintegration.

Moreover, with constant secretion of SGF (simulated gastric fluid), the concentration of pepsin in the stomach gradually increased. Meanwhile, the solid content of gastric digesta gradually decreased (Fig. 7-2). The gradual increase of the enzyme-to-substrate ratio is another contributing factor that the disintegration of gel particles was more evident during the later stage of gastric digestion.

For CAP-loaded Tween 80 emulsion gel (Fig. 7-4 B), the gastric digesta emptied at 30, 60 and 90 min of digestion had similar particle size distributions of a trimodal pattern with three peaks near 1, 6 and 200 μm. The small peak near 1 μm could be the small amounts of released oil droplets, due to the loose connection between Tween-80-coated oil droplets and the protein matrix. At 120 min of digestion, the peaks near 1 and 200 μm shifted slightly to the left, indicating the breakdown of gel particles; the peak near 1 μm increased in volume, indicating further release of oil droplets from protein matrix. At 150, 180 and 210 min of digestion, the peak near 100 μm gradually decreased in volume while the peak in the range of 2 to 30 μm increased in volume; the peak near 1 μm became broader with no increase in height. This indicates the gradual breakdown of large gel particles and more release of oil droplets from protein matrix. At 240 min of digestion, the gastric digesta had a small peak near 200 μm and two major peaks near 0.6 and 4 μm; the peaks near 0.6 and 4 μm both increased in volume as compared to gastric digesta collected from previous digestion times. The peak near 0.6 μm was likely to be the released oil droplets and the peak near 200 μm was likely to be the solid gel particles; the peak near 4 μm may consisted of small solid gel particles and / or the flocculated / coalesced oil droplets.

Similar to CAP-loaded whey protein emulsion gel, gel disintegration was not significant during 0 to 90 min of digestion of CAP-loaded Tween 80 emulsion gel, when

the pH was 5.70 ± 0.12 at 0 min and 3.79 ± 0.10 at 90 min. After 90 min, pepsin activity gradually increased to its optimum and promoted gel disintegration.

CAP-loaded whey protein emulsion gel had minor oil droplet release during the last 30 min of gastric digestion, mainly due to the rapid gel disintegration and protein hydrolysis. However, most of the oil droplets remained inside the solid gel particles, because the protein / peptide-coated oil droplets have strong interactions with surrounding protein matrix through disulphide binding and hydrophobic interaction. As previously explained in Chapter 6, emulsion gels formed at relatively high NaCl concentrations have compact particulate structure; the thick protein layer around the oil droplets could prevent them from liberation during gastric digestion. On the other hand, CAP-loaded Tween 80 emulsion gel had oil droplet release at the beginning of gastric digestion which became more evident at the end. During oral processing, there was already substantial release of oil droplets because of the mechanical compression, shearing and cutting (Fig. 5-3 from Section 5.3.3.2, Chapter 5).

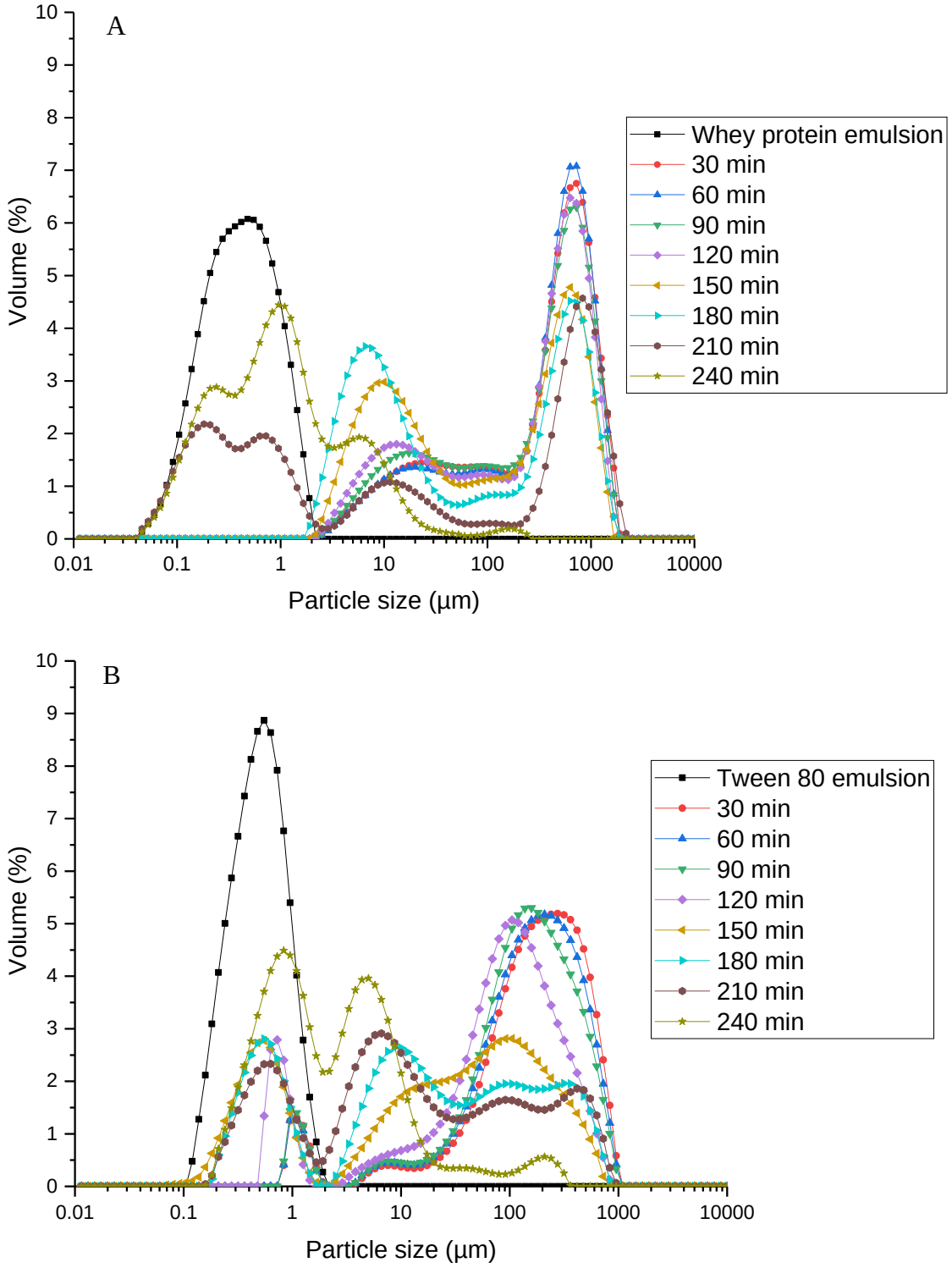


Figure 7-4 Particle size distributions of (A) CAP-loaded whey protein emulsion ($d_{4,3}$ ~ 0.5 μm) and emptied gastric digesta from CAP-loaded whey protein emulsion gel and (B) CAP-loaded Tween 80 emulsion ($d_{4,3}$ ~ 0.5 μm) and emptied gastric digesta from CAP-loaded Tween 80 emulsion gel, as a function of digestion time.

7.3.2.3 Particle size of oil droplets in the emptied gastric digesta

Figures 7-5 and 7-6 present the average particle size ($d_{4,3}$) and the particle size distribution of the oil droplets of emptied gastric digesta as a function of digestion time, respectively. The oil droplet size of gastric digesta from CAP-loaded whey protein emulsion gel increased slightly at the end of digestion. From 0 to 210 min of digestion, the particle size distribution of the oil droplets of gastric digesta from CAP-loaded whey protein emulsion gel showed a bimodal pattern with two peaks near 0.2 and 1 μm; the particle size distribution did not change significantly. At 240 min of digestion, the peak near 0.2 μm decreased slightly and the peak near 1 μm increased slightly in volume, which may represent some oil droplet coalescence at the end of digestion (Fig. 7-6 A).

For CAP-loaded Tween 80 emulsion gel, the oil droplet size did not change significantly during the first 180 min and increased significantly at 210 and 240 min of digestion. From 0 to 180 min of digestion, the particle size distributions of oil droplets of gastric digesta were similar and displayed a single peak near 0.5 μm. At 210 and 240 min of digestion, the peak near 0.5 μm decreased in volume and a new peak appeared in the range of 3 to 11 μm, representing the coalesced oil droplets formed at the end of digestion. Oil droplet coalescence was much more evident in CAP-loaded Tween 80 emulsion gel. This indicates that the whey-protein-coated oil droplets were more stable against coalescence than the Tween-80-coated oil droplets during gastric digestion.

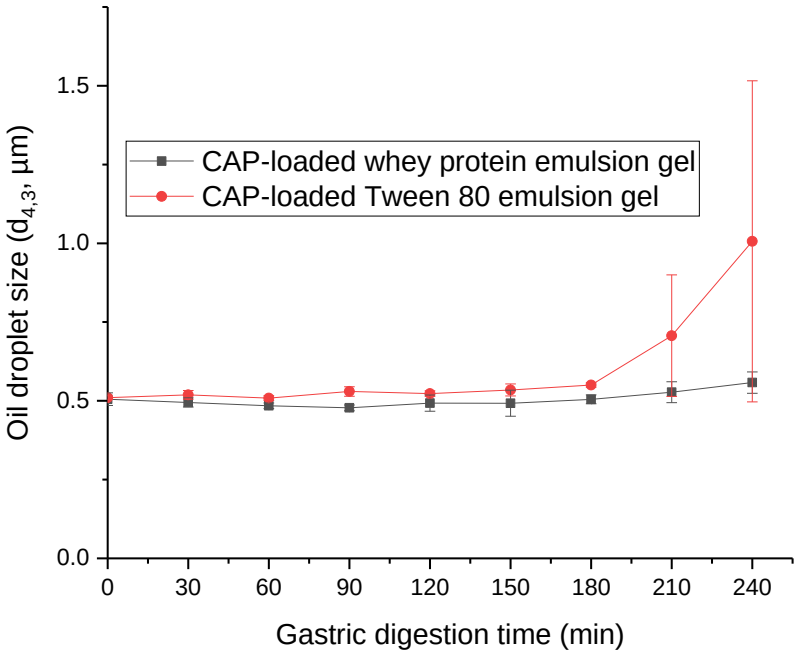


Figure 7-5 $d_{4,3}$ of oil droplets of emptied gastric digesta as a function of digestion time. Error bars represent standard deviations obtained from N = 3 independent experiments.

The degree of lipolysis by gastric lipase had an influence on oil droplet coalescence (gastric lipase: amano lipase A from *Aspergillus niger* at 50 U/ml digestion mixture). For CAP-loaded whey protein emulsion gel, the whey-protein-coated oil droplets were well protected by the surrounding thick protein layer, which would prevent the oil droplets from coalescence. The extent of lipolysis during gastric digestion would also be much lower in CAP-loaded whey protein emulsion gel, because the majority of the oil droplets were kept in solid gel particles. However, towards the end of digestion, there could be minor coalescence resulting from a combined effect of protein hydrolysis (leading to smaller gel particle size and the release of oil droplets from protein matrix) and the displacement of proteins / peptides at the interface by the lipolytic products (van Aken et al., 2011).

For CAP-loaded Tween 80 emulsion gel, oil droplet release was observed from the beginning of gastric digestion, due to low degree of interaction between Tween-80-coated oil droplets and protein matrix. The released oil droplets were more susceptible to being digested by gastric lipase, resulting in earlier and higher extent of lipolysis. The possible mechanism of oil droplet coalescence in CAP-loaded Tween 80 emulsion gel during gastric digestion could be the displacement of Tween 80 from the oil-water interface by the lypolytic products produced. The lypolytic products (i.e. free fatty acids, monoglycerides and diglycerides) are non-ionic at gastric pH, and they are known to be not good emulsifiers for oil-in-water emulsions (van Aken et al., 2011). Another possible mechanism is that Tween 80 could act as a substrate for lipases and was hydrolysed during gastric digestion (Plou et al., 1998; van Aken et al., 2011). Nevertheless, the oil droplet coalescence happened much more slowly and was much less apparent for CAP-loaded whey protein emulsion gel, as compared to CAP-loaded Tween 80 emulsion gel. Similar observations were reported by van Aken et al. (2011), where they studied the *in vitro* gastric behaviour of four liquid systems including a whey protein-stablized emulsion, a whey protein-stabilized emulsion with added sodium casinate, a Tween 80 stabilized emulsion, and pasteurized and homogenized full fat milk. They observed that the Tween 80 stabilized emulsion and full fat milk showed apparent oil droplet coalascence during gastric digestion, and that Tween 80 stabilizied emulsion had higher extent of lipolysis than whey protein stabilized emulsion during gastric digestion. The whey protein-stabilized emulsion showed some coalescence at the end of digestion, which proceeded more slowly or subsequent to flocculation. The additional sodium casinate in whey protein-stabilzed emulsion could stabilize the emulsion against coalescence.

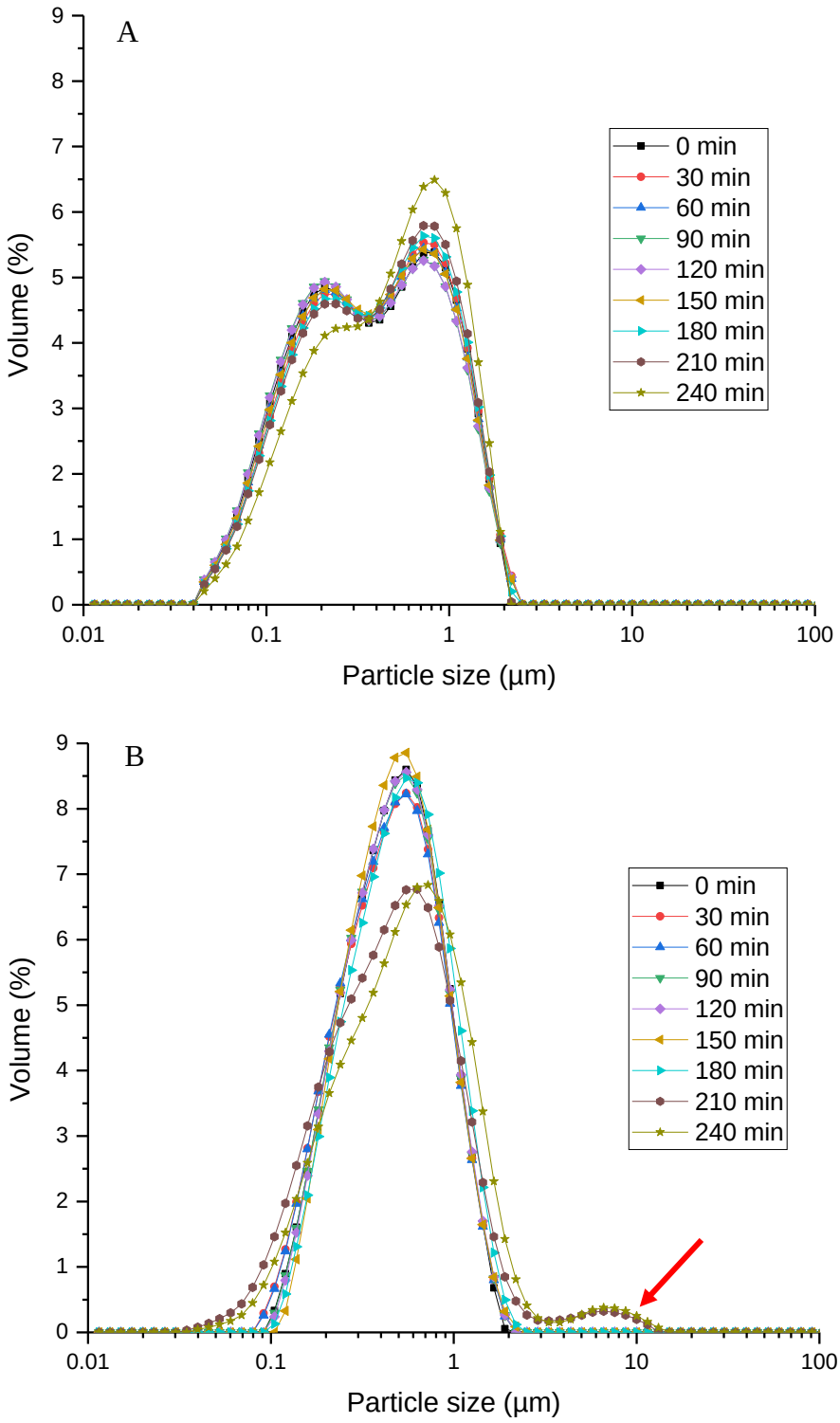


Figure 7-6 Particle size distributions of the oil droplets of emptied gastric digesta from (A) CAP-loaded whey protein emulsion gel and (B) CAP-loaded Tween 80 emulsion gel, as a function of digestion time. The red arrow indicates the coalesced oil droplets in gastric digesta emptied at 210 and 240 min from CAP-loaded Tween 80 emulsion gel.

7.3.2.4 Microstructure of emptied gastric digesta

The confocal laser scanning microscopy (CLSM) images of gastric digesta emptied at different digestion times are presented in Figure 7-7. For both gels, with increasing digestion time, the size of the gel particles gradually decreased, indicating gel disintegration during gastric digestion. For both gels, no significant difference was observed in the CLSM images of gastric digesta emptied at 30, 60 or 90 min. At 240 min of digestion, gel particles of ~ 500 μm in size were still observed in the digesta from CAP-loaded whey protein emulsion gel, while CAP-loaded Tween 80 emulsion gel were mainly broken down into smaller particles, qualitatively indicating that CAP-loaded Tween 80 emulsion gel was more easily broken down during gastric digestion, as compared to CAP-loaded whey protein emulsion gel.

Oil droplet release was observed in the gastric digesta emptied at 30, 60, 90, 120 and 150 min from CAP-loaded Tween 80 emulsion gel. In CLSM images of gastric digesta emptied at 180, 210 and 240 min from CAP-loaded Tween 80 emulsion gel, the gel particles were further broken down into smaller pieces, with more oil droplet release. In CLSM images of gastric digesta emptied before 240 min from CAP-loaded whey protein emulsion gel, no obvious oil droplet release was observed, indicating that most of the oil droplets were retained in the solid gel particles during gastric digestion.

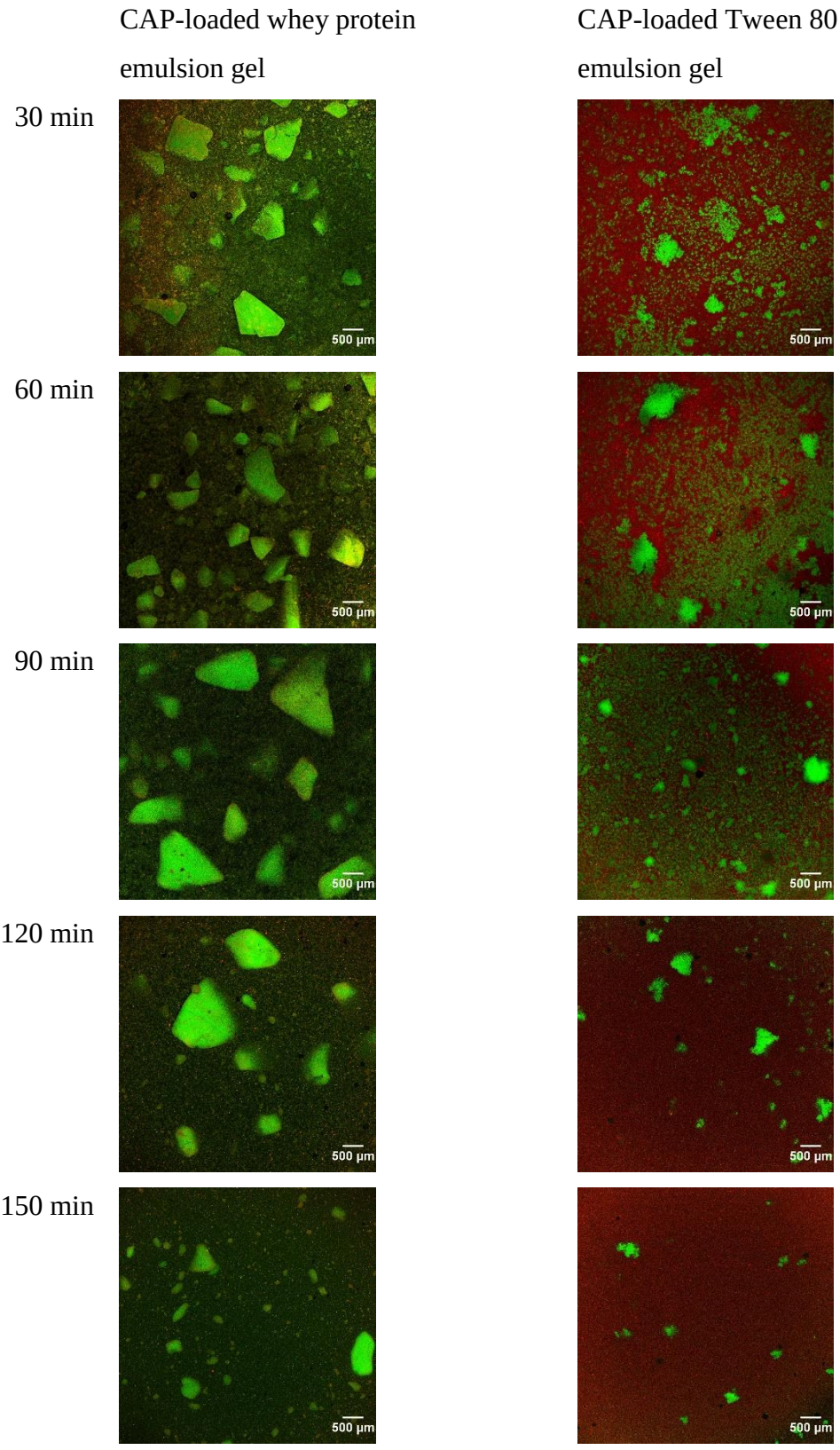


Figure 7-7 Representative CLSM images of emptied gastric digesta as a function of digestion time (continued)

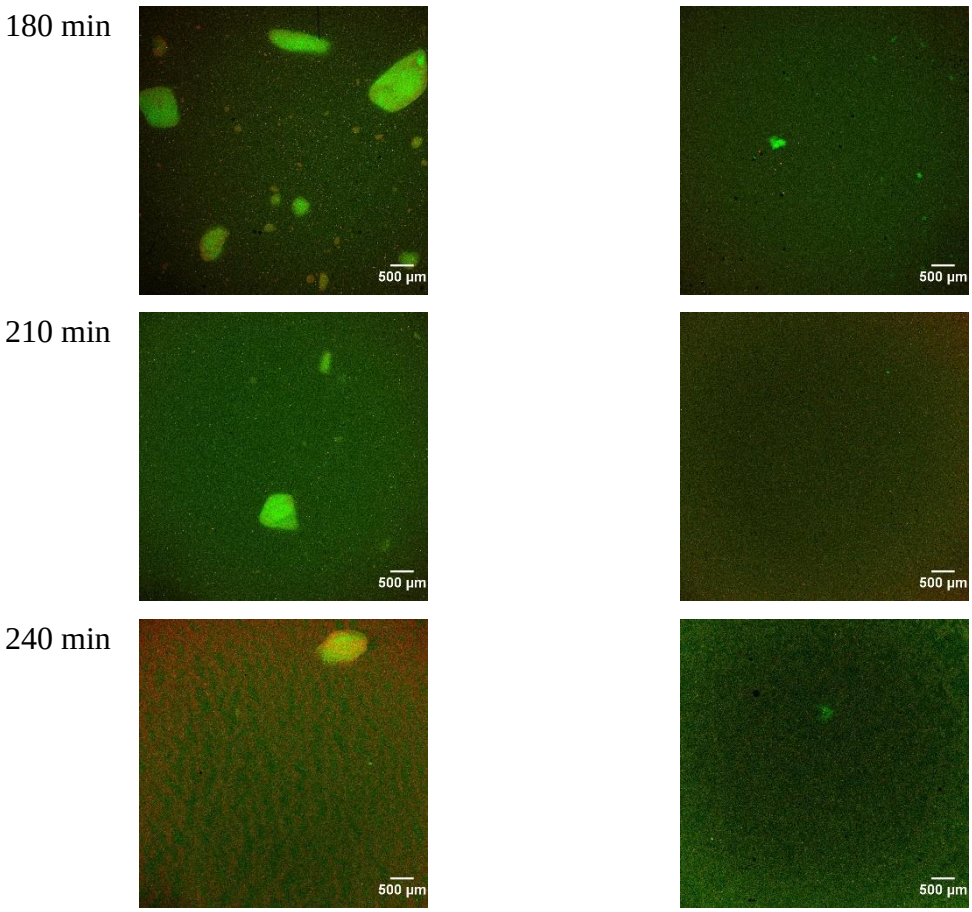


Figure 7-7 Representative CLSM images of emptied gastric digesta as a function of digestion time. Green colour represents proteins; red colour represents oil; black colour represents water or air.

7.3.2.5 SDS-PAGE patterns of emptied gastric digesta

The Tricine SDS-PAGE patterns under reducing conditions of the emptied gastric digesta as a function of digestion time are presented in Figure 7-8. WPI was used as a marker to indicate the locations of bovine serum albumin (BSA, ~ 69 kDa), β-lactoglobulin (β-lg, ~ 18.4 kDa) and α-lactalbumin (α-la, ~ 14.2 kDa). For both gels, with increasing digestion time, the band intensity of β-lg and α-la gradually decreased, which was caused by protein hydrolysis by pepsin and gradual dilution of gastric content during gastric digestion. The bands from gastric digesta from CAP-loaded Tween 80 emulsion gel were more intense than those from CAP-loaded whey protein emulsion gel emptied at the same digestion time, indicating higher protein concentration in the gastric digesta from CAP-loaded Tween 80 emulsion gel.

At 0 min, the bands from both gels were mainly composed of BSA, β-lg and α-la. Starting from 30 min of digestion, peptide bands appeared in the gastric digesta from both gels, indicating the occurrence of protein hydrolysis at pH value above 5. At 240 min of digestion, no intact whey proteins were found in the gastric digesta from both gels. Overall, the SDS-PAGE patterns were similar between two gels, indicating the proteins were hydrolysed at similar rate.

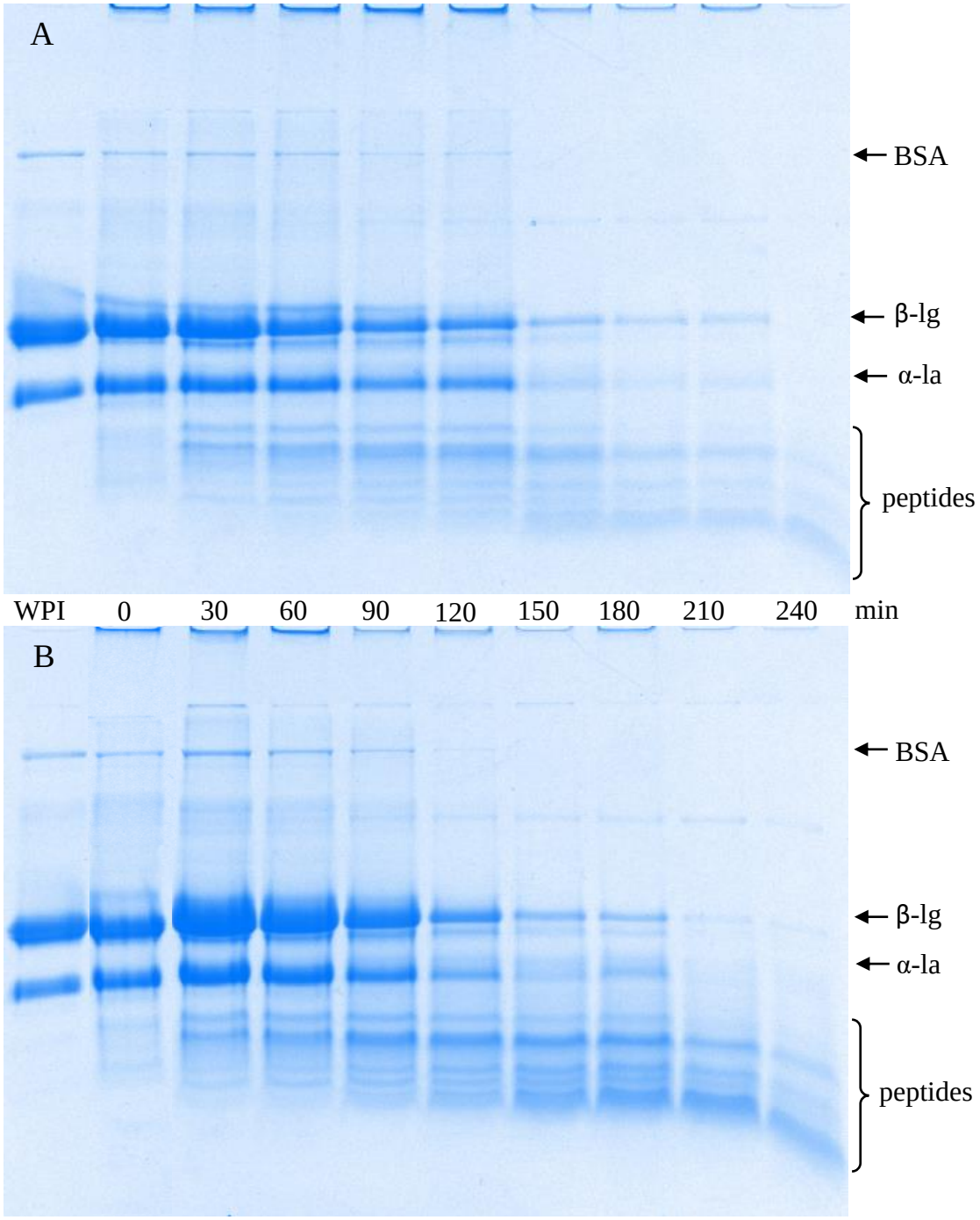


Figure 7-8 Tricine SDS-PAGE patterns under reducing conditions of emptied gastric digesta from: (A) CAP-loaded whey protein emulsion gel; (B) CAP-loaded Tween 80 emulsion gel, as a function of digestion time.

7.3.2.6 Oil contents of emptied gastric digesta

For both gels, with increasing digestion time, the oil content of gastric digesta gradually decreased (Fig. 7-9). For gastric digesta emptied at 60 and 120 min, CAP-loaded Tween 80 emulsion gel had significantly higher oil content than CAP-loaded whey protein emulsion gel; no significant difference was found in gastric digesta emptied at 240 min between two gels. The trend is consistent with the trend of solid content of gastric digesta (Fig. 7-2).

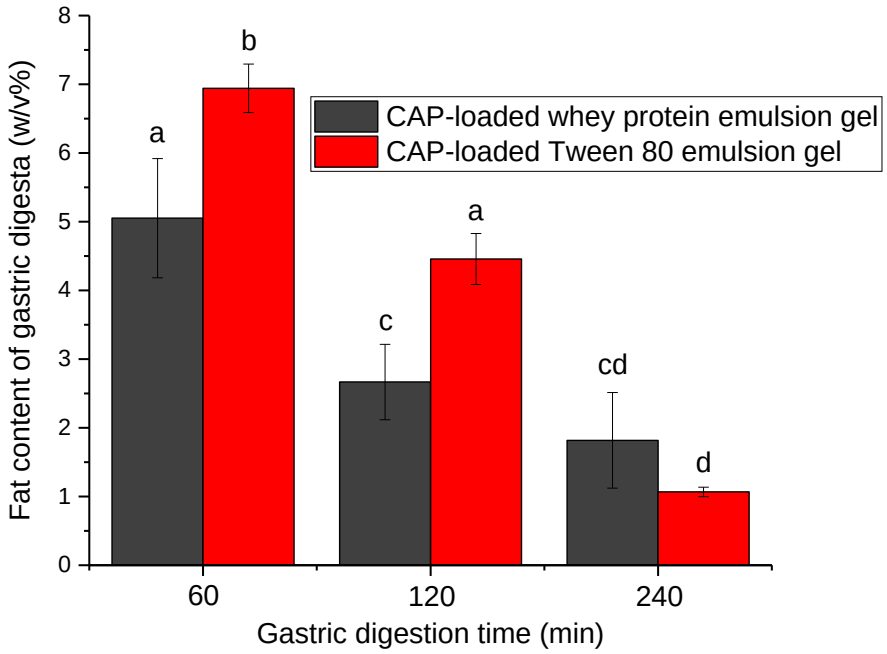


Figure 7-9 Oil content of gastric digesta emptied at 60, 120 and 240 min of digestion. Error bars represent standard deviations obtained from N = 3 independent experiments, each conducted in duplicate. Different lowercase letters indicate significant differences ($P < 0.05$).

7.3.3 Gel retention during *in vitro* gastric digestion

Figure 7-10 presents the gel retention in the HGS during 240 min of gastric digestion. CAP-loaded Tween 80 emulsion gel were emptied out faster than CAP-loaded whey protein emulsion gel ($P < 0.05$). The curves were fitted to a modified power exponential

function established by Siegel et al. (1988) for describing the gastric emptying behaviour of solid foods as Equation 7-1:

$$y(t) = 1 - (1 - e^{-kt})^{\beta} \qquad \textbf{Equation 7-1}$$

where $y(t)$ was the fractional gel retention at time t, k was the gastric emptying rate (min^{-1}), and β was the extrapolated y-intercept from the terminal portion of the curve. The parameters of the fitted curves are presented in Table 7-1.

CAP-loaded Tween 80 emulsion gel had significantly higher gastric emptying rate (k) and needed significantly less time to empty out 50% of the total solids (T_{half}) than CAP-loaded whey protein emulsion gel, which are mainly attributed to the significantly smaller masticated bolus particle size from CAP-loaded Tween 80 emulsion gel entering the stomach. Also, the particle size of the gastric digesta from CAP-loaded Tween 80 emulsion gel was much smaller than that from CAP-loaded whey protein emulsion gel during gastric digestion (Fig. 7-3). Since gastric emptying only allows particles smaller than 1 mm to pass through to the intestine, smaller particle size of the gastric digesta would generally lead to faster emptying rate. Similar results were reported by Koike et al. (2013) and Pera et al. (2002) investigating the effect of mastication efficiency on gastric emptying rate. In their study, they used the same food and asked the participants to chew the food either sufficiently or not; the masticated bolus particle size was controlled by the mastication efficiency and, therefore, did not consider the effect of food structure on mastication or gastric emptying. In our study, both gels were sufficiently masticated; the difference in masticated bolus particle size was caused by their structural difference due to difference in the type of emulsifier used.

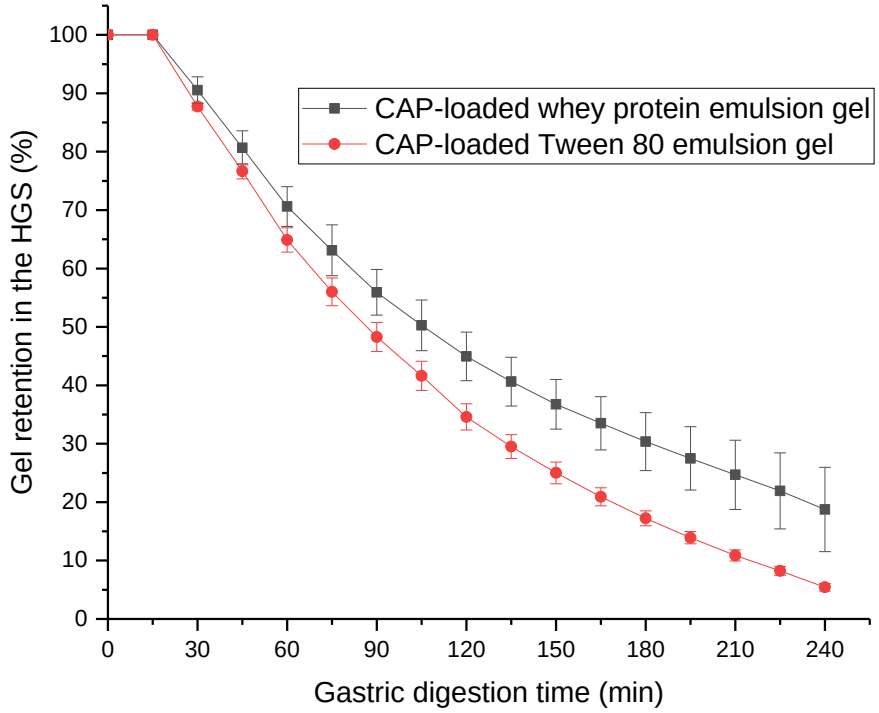


Figure 7-10 Gel retention in the HGS during gastric digestion. Error bars represent standard deviations obtained from N = 3 independent experiments.

Table 7-1 Parameters of the fitted power exponential function for gastric emptying. Results are shown as mean ± standard deviation of N = 3 independent experiments.

Parameters	CAP-loaded whey protein emulsion gel	CAP-loaded Tween 80 emulsion gel
$\boldsymbol{k}$ (min^{-1})	$0.00840^{a} \pm 0.00134$	$0.0132^{b} \pm 0.000487$
β	$1.371^{a} \pm 0.098$	$1.838^{b} \pm 0.033$
R^2	0.992 ± 0.006	0.998 ± 0.000
T_{half}^{*} (min)	$111.7^{a} \pm 13.9$	$87.6^{b} \pm 3.9$
T_{lag}^{**} (min)	$37.0^{a} \pm 5.2$	$46.1^{b} \pm 2.6$

*T_{half} is defined as the time needed to empty out half of the solids (i.e. $y(t) = 0.5$).

**T_{lag} was calculated as $ln\beta/k$ (Siegel et al., 1988).

a,bValues with different superscripts differ significantly ($P < 0.05$).

7.3.4 *In vitro* intestinal digestion and bioaccessibility of CAP

7.3.4.1 Physicochemical properties of gastric digesta emptied at 60, 120 and 240 min of digestion

Table 7-2 summarizes the pH, solid content, particle size, oil droplet size and oil content of the gastric digesta emptied at 60, 120 and 240 min of digestion from both gels. With increasing digestion time, the pH, solid content, gel particle size and oil content of gastric digesta gradually decreased for both gels; oil droplet size increased in gastric digesta at 240 min from both gels, indicating oil droplet coalescence. Gastric digesta emptied at 60, 120 and 240 min had distinct physicochemical properties, representing gastric digestion at initial, intermediate and final stage.

Table 7-2 pH, solid content, particle size, oil droplet size and oil content of gastric digesta emptied at 60, 120 and 240 min of digestion. Results are shown as mean ± standard deviation of N = 3 independent experiments.

	CAP-loaded whey protein emulsion gel			CAP-loaded Tween 80 emulsion gel		
Gastric digestion time	60 min	120 min	240 min	60 min	120 min	240 min
pH	$4.60^{a,x} \pm 0.34$	$3.57^{b,x} \pm 0.31$	$2.40^{c,x} \pm 0.23$	$4.41^{a,x} \pm 0.24$	$3.38^{b,x} \pm 0.08$	$2.27^{c,x} \pm 0.06$
Solid content (w/v%)	$10.7^{a,x} \pm 0.9$	$5.7^{b,x} \pm 0.4$	$3.6^{c,x} \pm 1.0$	$13.4^{a,y} \pm 0.9$	$8.0^{b,y} \pm 0.7$	$3.2^{c,x} \pm 0.2$
Particle size ($d_{4,3}$, µm)	$413.5^{a,x} \pm 45.2$	$335.2^{a,x} \pm 61.8$	$64.6^{b,x} \pm 123.2$	$184.5^{a,y} \pm 28.3$	$112.5^{b,y} \pm 11.8$	$10.8^{c,x} \pm 2.9$
Oil droplet size ($d_{4,3}$, µm)	$0.48^{a,x} \pm 0.01$	$0.49^{a,x} \pm 0.02$	$0.56^{b,x} \pm 0.03$	$0.51^{a,x} \pm 0.01$	$0.52^{a,x} \pm 0.01$	$1.01^{b,y} \pm 0.51$
Oil content (w/v%)	$5.0^{a,x} \pm 0.9$	$2.7^{b,x} \pm 0.5$	$1.8^{b,x} \pm 0.7$	$6.9^{a,y} \pm 0.4$	$4.4^{b,y} \pm 0.4$	$1.1^{c,x} \pm 0.1$

$^{a\text{-}c}$Values with different letters within the same gel type differ significantly ($P < 0.05$).

^{x,y}Values with different letters for the gastric digesta collected at the same digestion time differ significantly ($P < 0.05$).

7.3.4.2 Breakdown of gel particles during intestinal digestion

Figure 7-11 presents the changes in particle size distribution of gastric digesta emptied at 60, 120 and 240 min from both gels during 120 min of *in vitro* intestinal digestion. At 60 min of gastric digestion, the digesta from CAP-loaded whey protein emulsion gel showed a bimodal distribution with two peaks near 10 and 1000 μm; there was no peak in the range of 0.4 to 2 μm, indicating no oil droplet release. For CAP-loaded Tween 80 emulsion gel, the digesta also had a bimodal distribution with two peaks near 1 and 100 μm; the peak near 1 μm represents the oil droplets released during gastric digestion (Fig. 7-11 A1 and A2). For CAP-loaded whey protein emulsion gel, at 10 and 30 min of intestinal digestion, a new peak appeared in the range of 0.1 to 2 μm, indicating that a small portion of oil droplets was liberated from the solid gel particles; the peak near 1000 μm gradually decreased in volume while the peak near 10 μm increased significantly in volume, indicating the breakdown of gel particles during intestinal digestion. At 60 min of intestinal digestion, the peak near 1000 μm disappeared, the peak near 100 μm decreased in volume, and the peak near 1 μm increased in volume. At 90 min of intestinal digestion, the peak near 10 μm also disappeared and the peak near 1 μm increased slightly in volume, indicating the total disintegration of solid gel particles into individual oil droplets. At 120 min of intestinal digestion, the peak in the range of 0.04 to 2 μm slightly evolved into a bimodal pattern with a major peak near 1 μm and a small peak near 0.2 μm. The peak near 1 μm was likely to be the undigested oil droplets, while the peak near 0.2 μm could comprise undigested oil droplets and mixed micelles generated. For CAP-loaded Tween 80 emulsion gel, the breakdown of solid gel particles and release of oil droplets was much faster during intestinal digestion (Fig. 7-11 A2). At the end of intestinal digestion, the digesta had three peaks near 0.1, 1 and 10 μm. The small peak near 10 μm might be the coalesced oil droplets; the peak near 1 μm could be

the undigested oil droplets and the peak near 0.1 μm could be the undigested oil droplets and/or the mixed micelles and vesicles.

For gastric digesta emptied at 120 min, CAP-loaded whey protein emulsion gel had a bimodal distribution with two peaks near 10 and 1000 μm representing the solid gel particles and no oil droplet release (Fig. 7-11 B1). During intestinal digestion, with increasing digestion time, the solid gel particles gradually disintegrated with the release of free oil droplets; the oil droplets were gradually digested and formed mixed micelles and vesicles (indicated by the growth of the peak near 0.1 μm). CAP-loaded Tween 80 emulsion gel also had a bimodal distribution with two peaks near 1 and 100 μm representing oil droplets and solid gel particles respectively (Fig. 7-11 B2). CAP-loaded Tween 80 emulsion gel was also disintegrated faster than CAP-loaded whey protein emulsion gel, due to its smaller initial gel particle size and loose gel structure.

At 240 min of gastric digestion, oil droplet release was observed in the gastric digesta from both gels (Fig. 7-11 C1 and C2). During intestinal digestion, the remaining gel particles were quickly broken down and the oil droplets were completely released. The peaks in the range of 2 to 2000 μm from both gels possibly represent the solid gel particles at the beginning of intestinal digestion; however, during later stage, the peaks were most likely to be the coalesced oil droplets or air bubbles created during measurements because of the presence of bile salts in the samples. With increasing intestinal digestion time, the peak in the range of 0.04 to 2 μm slowly shifted to the left, indicating the digestion of oil droplets and the formation of mixed micelles and vesicles.

During intestinal digestion, CAP-loaded whey protein emulsion gel was disintegrated slower than CAP-loaded Tween 80 emulsion gel. The Tween-80-coated oil droplets had no interactions with protein matrix, and they appeared to be in flocculated state in the

emulsion gel (Fig. 5-2 in Chapter 5), which probably disrupted the protein network structure. Moreover, gastric digesta from CAP-loaded Tween 80 emulsion gel had significantly smaller initial gel particle size (Table 7-2), which contributed to its faster disintegration during intestinal digestion.

The disintegration of solid gel particles was much faster for gastric digesta emptied at 240 min than at 120 min and 60 min, for both gels. This was mainly because of the smaller initial gel particle size and lower solid content and oil content of the gastric digesta emptied at 240 min (Table 7-2).

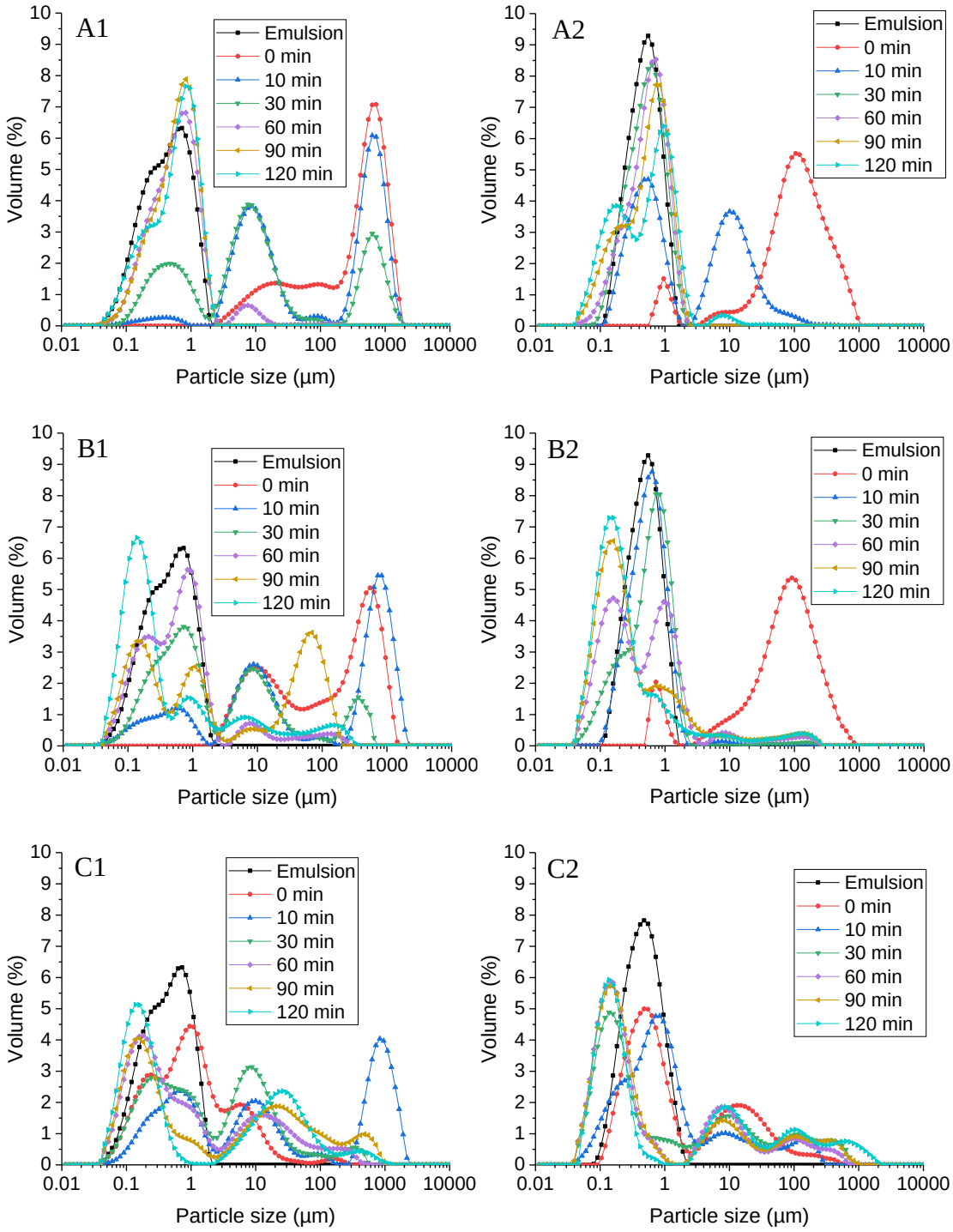


Figure 7-11 Changes in particle size distributions of gastric digesta during intestinal digestion (0, 10, 30, 60, 90 and 120 min). (A1), (B1) and (C1): gastric digesta from CAP-loaded whey protein emulsion gel emptied at 60, 120 and 240 min respectively; (A2), (B2) and (C2): gastric digesta from CAP-loaded Tween 80 emulsion gel emptied at 60, 120 and 240 min respectively.

7.3.4.3 Initial lipolysis rate

The initial lipolysis rate (i.e. the μmol of free fatty acid released per ml of digestion mixture per minute during the initial 2 min of intestinal digestion) calculated from Figure 7-11 is presented in Table 7-3. For CAP-loaded whey protein emulsion gel, no significant difference was found in initial lipolysis rate for gastric digesta emptied at different times; for CAP-loaded Tween 80 emulsion gel, gastric digesta emptied at 120 min had the highest initial lipolysis rate, followed by gastric digesta emptied at 240 min; gastric digesta emptied at 60 min had significantly lower initial lipolysis rate. Gastric digesta emptied at 60 and 240 min from CAP-loaded Tween 80 emulsion gel had significantly lower initial lipolysis rate than that from CAP-loaded whey protein emulsion gel; no significant difference was found between two gels for gastric digesta emptied at 120 min.

As discussed in Chapter 6, the initial lipolysis rate is influenced by multiple factors including the substrate-to-enzyme ratio, gel particle size, gel structure, etc. For CAP-loaded whey protein emulsion gel, the gastric digesta emptied at 60 min had higher oil content than the gastric digesta emptied at 120 and 240 min. This was probably because of the larger gel particle size of gastric digesta emptied at 60 min. For CAP-loaded Tween 80 emulsion gel, gastric digesta emptied at 60 min had the lowest initial lipolysis rate probably because its larger gel particle size and lower extent of oil droplet release; gastric digesta emptied at 120 min had higher initial lipolysis rate than gastric digesta emptied at 240 min probably because of its higher oil content, even though gastric digesta emptied at 240 min had smaller gel particle size.

For gastric digesta emptied at 60 min, CAP-loaded Tween 80 emulsion gel had significantly higher oil content, smaller gel particle size and release of oil droplets, as compared to CAP-loaded whey protein emulsion gel. However, gastric digesta from

CAP-loaded Tween 80 emulsion gel had significantly lower initial lipolysis rate. The presence of Tween 80 at the oil-water interface possibly inhibited or slowed down its replacement by bile salts and, therefore, delayed lipid digestion. Similar results were reported by Li and McClements (2011) where they reported the effect of the presence of small molecule surfactants on the *in vitro* gastrointestinal digestion of protein-stabilized emulsion, and found that there was a lag phase of free fatty acid release during intestinal digestion of protein-stabilized emulsion with added Tween 80; the length of the lag phase increased with increasing Tween 80 concentration.

For gastric digesta emptied at 120 min, the effect of Tween 80 on lipid digestion seemed to be less obvious as no significant difference was found between two gels. It could be because that the concentration of Tween 80 were lower in gastric digesta empted at 120 min than at 60 min, leading to higher ratio of bile salts-to-Tween 80. Meanwhile, the ratio of bile salts-to-lipids/inteface was also higher, which means that bile salts could be more effective at displacing Tween 80 from the interface. Therefore, no obvious lag phase was observed for gastric digesta emptied at 120 min.

For gastric digesta emptied at 240 min, CAP-loaded Tween 80 emulsion gel had significantly smaller gel particle size and higher extent of oil droplet release, but the initial lipolysis rate was lower, as compared to CAP-loaded whey protein emulsion gel. In Figure 7-6, apparent oil droplet coalescence was seen in CAP-loaded Tween 80 emulsion gel, leading to increased oil droplet size and decreased interface area, while CAP-loaded whey protein emulsion gel were more resistant against oil droplet coalescencence at the end of gastric digestion. Increased oil droplet size would generally lead to reduced lipolysis rate because of less interfacial area available for the lipolytic

reaction, which is in agreement with the report from Borreani, Leonardi, Moraga, Quiles, and Hernando (2019).

Table 7-3 Initial lipolysis rate ($\mu mol \cdot ml^{-1} \cdot min^{-1}$) of emptied gastric digesta during intestinal digestion. Results are shown as mean ± standard deviation of N = 3 independent experiments.

	CAP-loaded whey protein emulsion gel			CAP-loaded Tween 80 emulsion gel		
	60 min	120 min	240 min	60 min	120 min	240 min
Initial lipolysis rate ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	$0.99^{a,x}$ ± 0.36	$0.98^{a,x}$ ± 0.32	$0.97^{a,x}$ ± 0.17	$0.14^{a,y}$ ± 0.01	$1.15^{b,x}$ ± 0.17	$0.73^{c,y}$ ± 0.03

$^{a\text{-}c}$Values with different letters within the same gel type differ significantly ($P < 0.05$).
^{x,y}Values with different letters for the gastric digesta collected at the same digestion time differ significantly ($P < 0.05$).

7.3.4.4 Free fatty acid release profiles

Figure 7-12 shows the free fatty acid (FFA) release profiles per ml of digestion mixture during *in vitro* intestinal digestion of gastric digesta emptied at 60, 120 and 240 min from both gels. For CAP-loaded whey protein emulsion gel, the gastric digesta emptied at 60 min had significantly more FFA release per ml of digestion mixture than the gastric digesta emptied at 120 and 240 min (Fig. 7-12 A), probably because of its significantly higher oil content (Table 7-2). For CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 240 min had significantly lower FFA release per ml of digestion mixture because of its lower oil content; no significant difference was found between gastric digesta emptied at 60 and 120 min (Fig. 7-12 B). For both gels, the FFA release did not reach plateau at the end of intestinal digestion for gastric digesta emptied at 60 min; it took approximately 15 and 50 min to reach plateau for gastric digesta emptied at 120 and 240 min respectively.

For gastric digesta emptied at 60 min, the lipolysis rate for CAP-loaded whey protein emulsion gel gradually decreased with increasing digestion time during the first 45 min. After 45 min, the lipolysis rate was relatively constant until 120 min of intestinal digestion. This is presumably because of the gradual disintegration of solid gel particles and gradual release of oil droplets so that the ratio of substrate-to-enzyme remained relatively constant. For CAP-loaded Tween 80 emulsion gel, a lag phase of about 3 min was observed, followed by a rapid increase in lipolysis rate. The lipolysis rate was relatively constant during 5 to 25 min of digestion, which means that the concentration of the substrate was relatively constant, due to fast disintegration of gel particles and release of oil droplets. After 25 min, the lipolysis rate gradually decreased due to decreasing substrate concentration.

For gastric digesta emptied at 120 min, CAP-loaded Tween 80 emulsion gel had higher FFA release per ml of digestion mixture, probably because of its higher oil content than CAP-loaded whey protein emulsion gel. For gastric digesta emptied at 240 min, CAP-loaded Tween 80 emulsion gel had lower FFA release per ml of digestion mixture, even though the oil content was similar between two gels. The lower FFA release per ml of digestion mixture was probably caused by the inhibition effect of Tween 80 on lipid digestion.

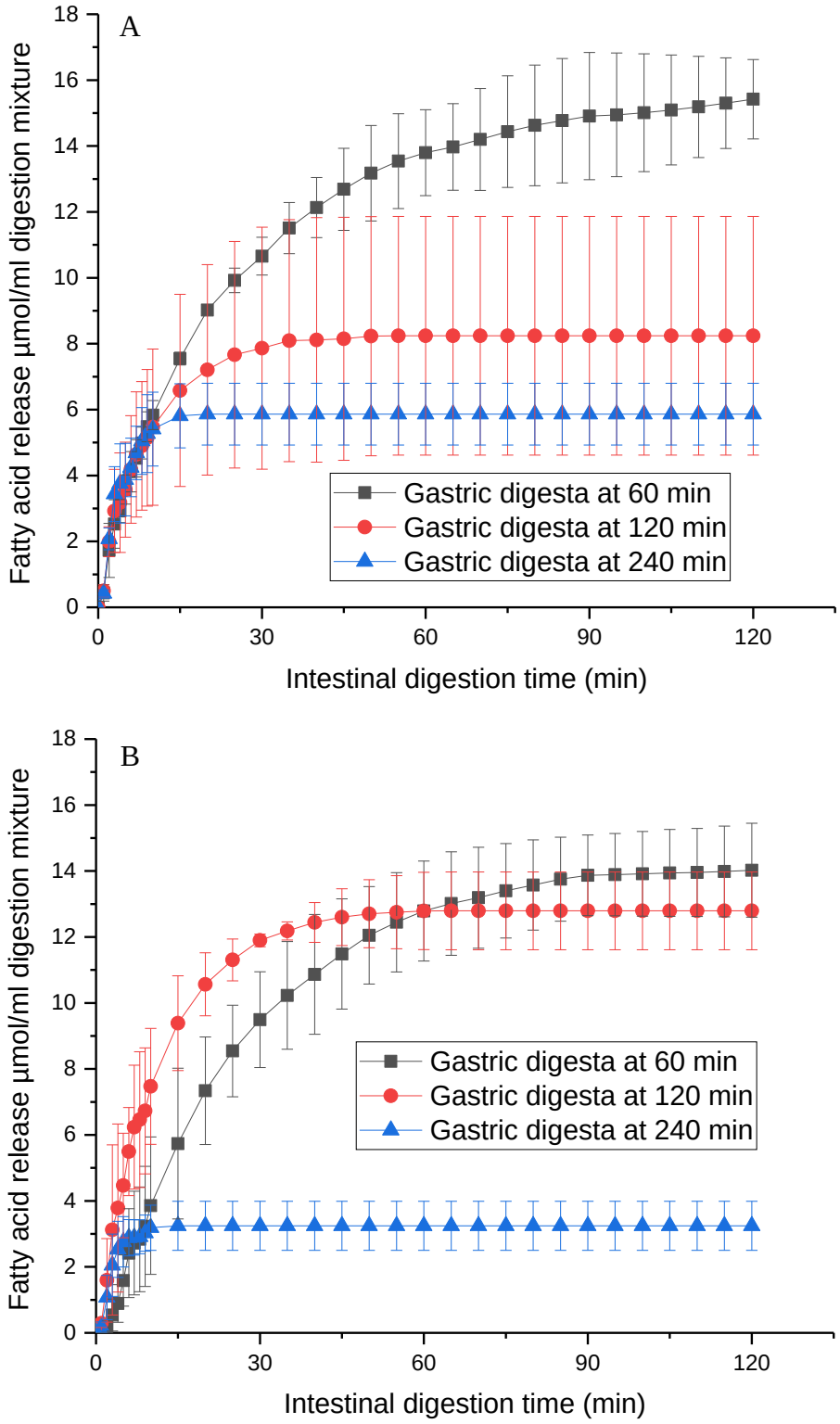


Figure 7-12 Free fatty acid release profile per ml of digestion mixture of gastric digesta emptied at 60, 120 and 240 min during 120 min of intestinal digestion. (A) CAP-loaded whey protein emulsion gel; (B) CAP-loaded Tween 80 emulsion gel. Error bars represent standard deviations obtained from N = 3 independent experiments.

Figure 7-13 shows the FFA release profiles per g of oil during *in vitro* intestinal digestion of gastric digesta emptied at 60, 120 and 240 min from both gels. FFA release per g of oil represents the extent of lipid digestion; no significant difference was found in the extent of lipid digestion for gastric digesta emptied at 60, 120 or 240 min from CAP-loaded whey protein emulsion gel. For CAP-loaded Tween 80 emulsion gel, no significant difference was found in the extent of lipid digestion for gastric digesta emptied at 120 and 240 min; gastric digesta emptied at 60 min had significantly lower extent of lipid digestion. For gastric digesta emptied at 60 and 240 min, CAP-loaded whey protein emulsion gel had higher extent of lipid digestion ($P < 0.05$); no significant difference was found in the gastric digesta emptied at 120 min between two gels.

Lipid digestion is considered as an interfacial process relying on the displacement of emulsifier / lipolytic products from the oil-water interface by bile salts and the adsorption of lipolytic enzymes to the interface (Golding & Wooster, 2010). The displacement of emulsifier from the interface by bile salts is essential for lipid digestion. Tween 80 seemed to exert certain resistance to its displacement by bile salts at the interface. Koukoura et al. (2019) studied the effect of using caseinate versus Tween 20 as emulsifier on the *in vitro* digestion behaviour of the emulsion and reported the gradual decrease of zeta-potential of the emusified oil droplets stabilized by Tween 20 during intestinal digestion. This result could support the suggestion that non-ionic small molecule surfactants such as Tween 20 or Tween 80 provide certain resistance. Another possible mechanism is that Tween 80 was able to form interfacial complexes with bile salts / lipolytic enzymes and, therefore, reduced the rate and extent of lipid digestion (Li & McClements, 2011).

Apart from the effect of Tween 80, the rate and extent of lipid digestion also depends on the ratio of bile salts and calcium to the substrate / lipolytic products or the interface area. The higher the ratio, the more effective the calcium and bile salts are at precipitating and displacing the lipolytic products (i.e. FFA, mono- and di-glycerides) from the interface, which would free the interface for further lipolysis and, therefore, increases the rate and extent of lipid digestion. This explains that due to the higher oil content of gastric digesta emptied at 60 min from CAP-loaded Tween 80 emulsion gel, the ratio of bile salts / calcium to substrate was lower, leading to lower rate and extent of lipid digestion, as compared to gastric digesta empted at 120 and 240 min from CAP-loaded Tween 80 emulsion gel. Similar results were reported by Li et al. (2011) that less oil content would generally lead to a higher extent of lipid digestion. Nevertheless, this effect was less evident in CAP-loaded whey protein emulsion gel where no significant difference was found between gastric digesta emptied at different times, even though the ratio was lower in the gastric digesta emptied at 60 min.

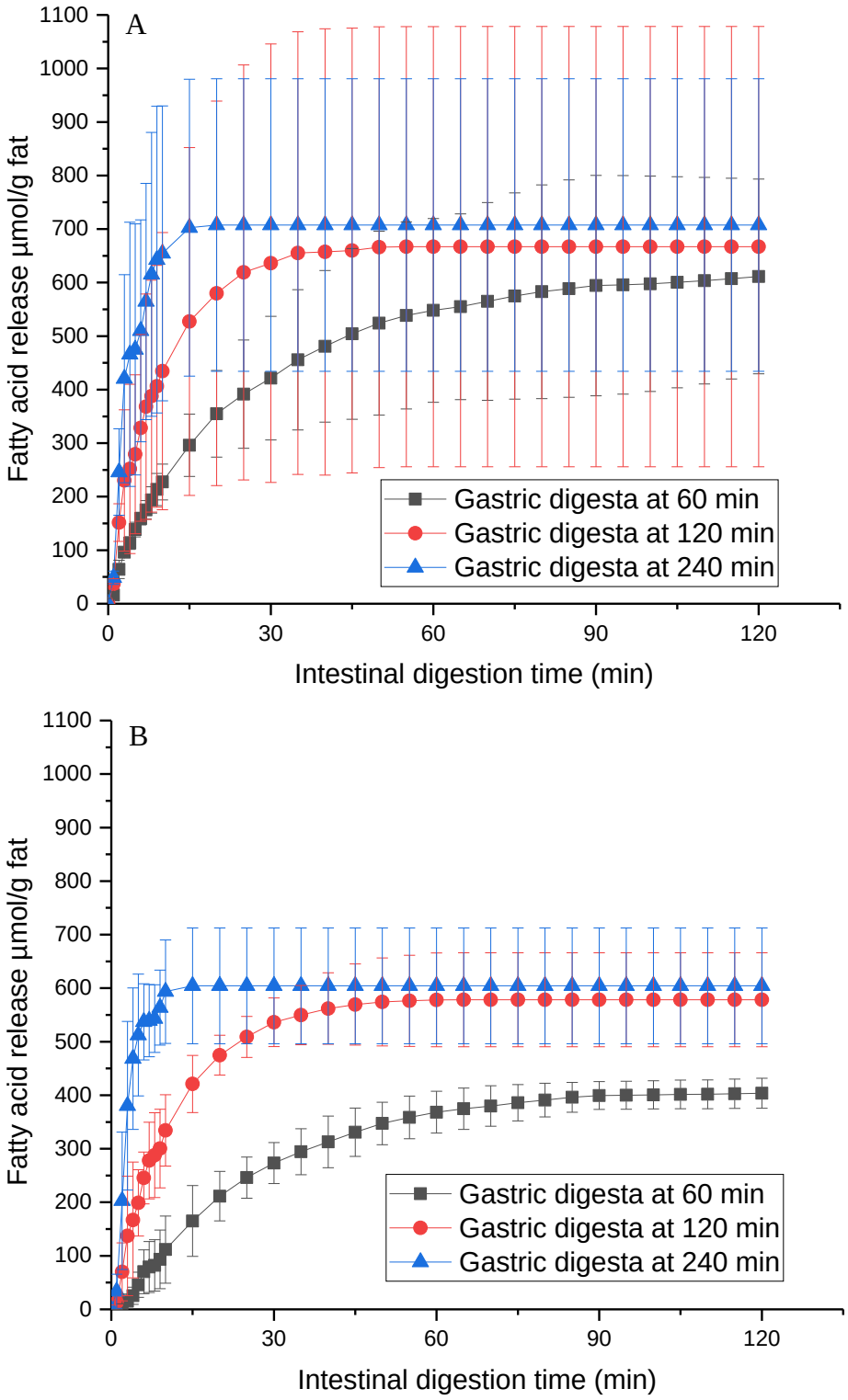


Figure 7-13 Free fatty acid release profile per g of fat of gastric digesta emptied at 60, 120 and 240 min during 120 min of intestinal digestion. (A) CAP-loaded whey protein emulsion gel; (B) CAP-loaded Tween 80 emulsion gel. Error bars represent standard deviations obtained from N = 3 independent experiments.

7.3.4.5 Bioaccessibility of CAP

The bioaccessibility of CAP after *in vitro* gastrointestinal digestion is shown in Figure 7-14. For both gels, the gastric digesta emptied at 60 min had significantly lower bioaccessibility of CAP than gastric digesta emptied at 120 and 240 min; no significant difference was found in bioaccessibility of CAP between gastric digesta emptied at 120 and 240 min. For gastric digesta emptied at 60 min, CAP-loaded Tween 80 emulsion gel had higher bioaccessibility of CAP than CAP-loaded whey protein emulsion gel ($P < 0.05$); no significant difference was found for gastric digesta emptied at 120 and 240 min between two gels.

The bioaccessibility of CAP depends on two steps: the release of CAP from food matrix during gastrointestinal digestion and the solubilization of CAP in the aqueous phase (in mixed micelles and vesicles). Since the lipophilic CAP molecules were dissolved in the oil droplets in the emulsion gel, the release of CAP molecules from the emulsion gel could involve the following steps: (1) movement of CAP molecules across the oil–aqueous interface; (2) movement through the gelled protein aqueous phase towards the surface of the gel particles; (3) movement across the solid–air interface or the solid–liquid interface (Mao et al., 2017). During gastrointestinal digestion, the CAP molecules could be released from the emulsion gel by diffusion through the solid gel particles following the three steps mentioned above, which does not rely on the release of oil droplets from protein matrix; however, the release of oil droplets would promote the release of CAP molecules. Moreover, they could be released due to lipid digestion, which largely relies on the liberation of free oil droplets from the solid gel particles. The breakdown of solid gel particles, the release of free oil droplets and hydrolysis of lipids would facilitate the release of CAP molecules. For different gel systems, the release

behaviour of CAP molecules could be different, depending on the rate of gel disintegration (affecting gel particle size), the rate and extent of oil droplet release, and the rate and extent of lipid digestion. In general, smaller gel particles, faster oil droplet release and higher extent of lipid digestion would lead to more CAP release from the lipid phase.

For CAP-loaded whey protein emulsion gel, gastric digesta emptied at different times had similar extent of lipid digestion (Fig. 7-13 A), however, the bioaccessibility of CAP was lower in gastric digesta emptied at 60 min ($P < 0.05$). A possible reason could be that the gastric digesta emptied at 60 min had significantly higher oil content and larger interface area; the lipid digestion did not reach plateau at 120 min of intestinal digestion, which means that there were still oil droplets left undigested. This is qualitatively confirmed by the particle size distribution presented in Fig. 7-11 A1 that at 120 min of intestinal digestion, the peak near 1 μm represents the undigested oil droplets. The role of bile salts in lipid digestion is that they displace surfactants from the oil-water interface, so that the co-lipase and lipase could anchor onto the interface and hydrolyse the lipids; they also displace the lipolytic products from the interface and help solubilize them in the aqueous phase by forming mixed micelles (Maldonado-Valderrama et al., 2011). Therefore, higher ratio of bile salts-to-interface would generally lead to higher extent of lipid digestion, and higher ratio of bile salts-to-CAP would generally promote the solubilization of CAP molecules. Most likely for gastric digesta emptied at 60 min, a substantial portion of the bile salts was still at the interface of the undigested oil droplets; therefore, the released CAP molecules were not well solubilized, leading to lower bioaccessibility of CAP. In Figure 7-11 A1, B1 and C1, we could see a clear difference in the formation of mixed micelles (represented by the peak near 0.1 μm) between gastric digesta emptied at 60 min or 120 / 240 min. The results indicate that the release of CAP

molecules from food matrix was not the limiting step determining the bioaccessibility of CAP, but the solubilization of CAP molecules.

For CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 60 min had significantly higher oil content, larger gel particle size, less extent of oil droplet release, and lower extent of lipid digestion, as compared to gastric digesta emptied at 120 and 240 min, which probably all contributed to its lower bioaccessibility of CAP. However, the higher oil content and lower extent of lipid digestion were believed to be the dominating factors, because the gel particles were quickly broken down into free oil droplets for gastric digesta emptied at different times, therefore the effects of gel particle size and rate and extent of oil droplet release were not evident. Higher extent of lipid digestion would lead to more release of CAP molecules and more lipolytic products generated; the lipolytic products would also participate in the formation of mixed micelles, which would help solubilize the CAP molecules been released.

For gastric digesta emptied at 60 min, CAP-loaded Tween 80 emulsion gel had significantly higher bioaccessibility of CAP than CAP-loaded whey protein emulsion gel. This means that more CAP molecules were released and solubilized during intestinal digestion of gastric digesta from CAP-loaded Tween 80 emulsion gel. CAP-loaded Tween 80 emulsion gel had significantly lower extent of lipid digestion than CAP-loaded whey protein emulsion gel (Fig. 7-13). The solid particles were broken down much faster and the oil droplets were released much faster from gel with inactive filler particles than gel with active filler particles, which would promote the release of CAP molecules from the food matrix. Moreover, Tween 80 as a small molecule surfactant, when displaced by bile salts from the interface, would also participate in the formation of mixed micelles and help solubilize the released CAP molecules in the aqueous phase and, therefore,

leading to higher bioaccessibility of CAP. Similar effect was observed for gastric digesta emptied at 120 and 240 min. The results are in agreement with the report from Mun et al. (2015) where they studied the bioaccessibility of β-carotene after *in vitro* digetion of emulsion stabilized by whey proteins or Tween 20 and found that Tween 20-stabilized emulsion had significantly higher bioaccessibility of β-carotene.

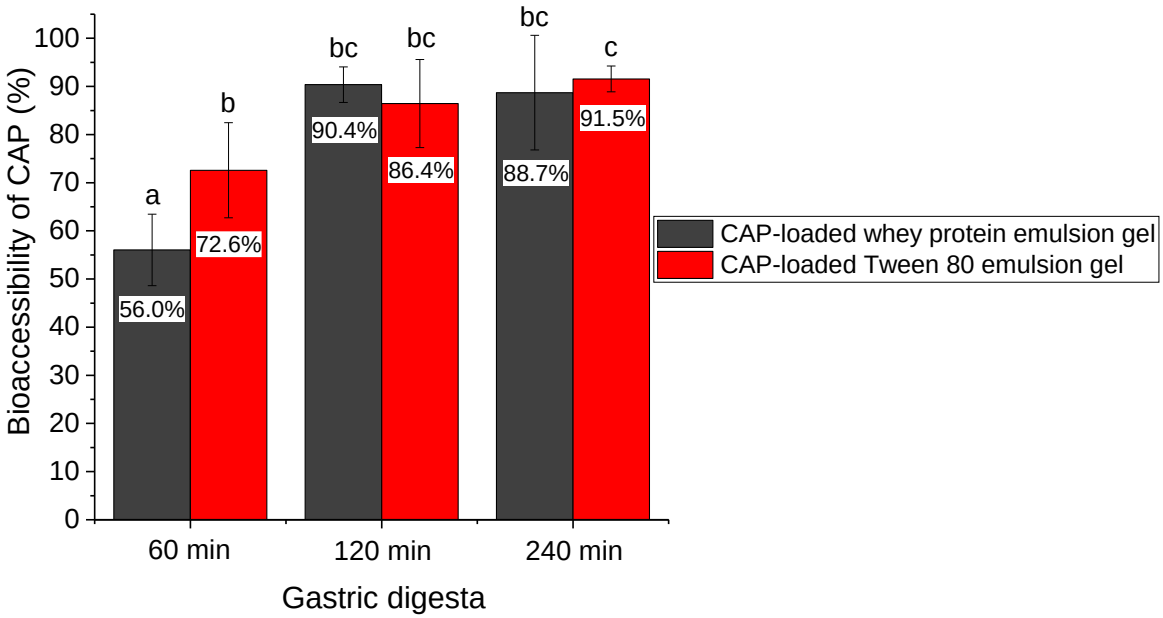


Figure 7-14 Bioaccessibility of CAP after *in vitro* gastrointestinal digestion. Error bars represent standard deviations obtained from N = 3 independent experiments, each conducted in duplicate. Different lowercase letters indicate significant differences between samples ($P < 0.05$).

7.4 Conclusions

In this chapter, the effect of using whey proteins versus Tween 80 as emulsifier on the simulated dynamic gastric digestion behaviour of emulsion gels containing CAP and the effect of the physicochemical characteristics and colloidal structure of gastric digesta emptied at different times on the *in vitro* intestinal digestion behaviour and bioaccessibility of CAP were examined. Results indicate that the CAP-loaded Tween 80 emulsion gel was emptied out significantly faster during gastric digestion than CAP-loaded whey protein emulsion gel, mainly due to its smaller masticated bolus particle size entering the stomach. The Tween-80-coated oil droplets were flocculated in the emulsion gel, disrupting the protein network structure; they had no interactions with the surrounding protein matrix and started to be released from protein matrix from the beginning of gastric digestion. On the contrary, the whey-protein-coated oil droplets had strong interactions with surrounding protein matrix mainly through hydrophobic interactions and disulphide binding, the thick protein layer around the oil droplets has protected them from liberation during gastric digestion. Minor oil droplet release was observed at the end of gastric digestion for CAP-loaded whey protein emulsion gel, because of protein hydrolysis and fast gel disintegration. Apparent oil droplet coalescence was observed at the end of gastric digestion for CAP-loaded Tween 80 emulsion gel, which was mainly attributed to the instability caused by the displacement of Tween 80 from the interface by lipolytic products produced during gastric digestion. Oil droplet coalescence was much less evident in CAP-loaded whey protein emulsion gel because the oil droplets were surrounded by thick protein layer.

A lag phase was observed at the beginning of intestinal digestion of gastric digesta from CAP-loaded Tween 80 emulsion gel, resulting in lower initial lipolysis rate, as

compared to CAP-loaded whey protein emulsion gel. In general, CAP-loaded Tween 80 emulsion gel had lower extent of lipid digestion than CAP-loaded whey protein emulsion gel. Because of the loose structure of CAP-loaded Tween 80 emulsion gel, the solid particles were broken down much faster and the oil droplets were liberated from gel matrix much faster than CAP-loaded whey protein emulsion gel during intestinal digestion, which have promoted the release of CAP molecules from food matrix. Meanwhile, the Tween 80 molecules, once displaced by bile salts from the interface, would also participate in the formation of mixed micelles and help solubilize the released CAP molecules, therefore, leading to improved bioaccessibility of CAP. Information obtained from this work could be useful in designing foods for delivery of lipophilic bioactive compounds. Using different type of emulsifier, the structure could be significantly altered, leading to distinct digestion behaviour in the GI tract, and eventually have an influence on the bioaccessibility of the incorporated bioactive compounds.

Chapter 8: Secretion of inflammatory mediators by human intestinal epithelial cells incubated with gastric digesta of emulsion gels containing capsaicinoids: Implication on gastric irritation

8.1 Abstract

This chapter developed an *in vitro* method to test gastric irritation by quantifying the secretion of interleukin-8 (IL-8) by human intestinal epithelial cells Caco-2 after incubation with gastric digesta of emulsion gels containing capsaicinoids (CAP) obtained from simulated dynamic gastric digestion. The emulsion gel structure was modified using different emulsifiers: whey proteins versus Tween 80. Results indicate that both the CAP and Tween 80 molecules were proinflammatory to Caco-2 cells and stimulated cells to produce IL-8. Gastric digesta from CAP-loaded Tween 80 emulsion gel (0.02 wt% CAP; Tween 80 as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl) stimulated significantly more IL-8 production than CAP-loaded whey protein emulsion gel (0.02 wt% CAP; whey proteins as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl), possibly because of the presence of Tween 80 and also, because more CAP molecules were released to the aqueous phase from CAP-loaded Tween 80 emulsion gel during gastric digestion. CAP-loaded Tween 80 emulsion gel had a loose gel structure; it was easily broken down into smaller pieces and had large amounts of oil droplet liberation from the protein matrix throughout gastric digestion, which would promote the release of CAP molecules into the aqueous phase, leading to higher IL-8 production. On the other hand, whey-protein-coated oil droplets had strong connections with surrounding

protein matrix and were well protected during gastric digestion; the release of CAP molecules was much less as compared to CAP-loaded Tween 80 emulsion gel. Results show that by modifying the structure of the foods, the gastric digestion behaviour can be modified, which would affect the release behaviour of CAP molecules and influence gastric irritation / inflammation.

8.2 Introduction

The bioactive compounds capsaicinoids (CAP), present in various peppers from the genus *Capsicum*, are responsible for causing sensory responses such as burning, irritation and pain when they get in contact with the human body. The sensory responses are closely related to their actions on a receptor protein called transient receptor potential vanilloid subtype 1 (TRPV-1) or vanilloid receptor subtype 1 (VR-1) (Frias & Merighi, 2016). TRPV-1 is highly expressed in a subset of sensory neurons which respond to mechanical, thermal and chemical stimuli; there is also evidence showing that TRPV-1 is expressed in non-neuronal cells, such as the epithelial cells of the gastrointestinal tract (Geppetti & Trevisani, 2004; Kato et al., 2003; Ward, Bayguinov, Won, Grundy, & Berthoud, 2003). Upon activation of TRPV-1, the cells release neuropeptides that induce neurogenic inflammation (Richardson & Vasko, 2002). Moreover, TRPV-1 is found to be upregulated in several human pathological conditions, such as gastrointestinal inflammation and ulcerative colitis (Geppetti & Trevisani, 2004; Yiangou et al., 2001). So, the inflammatory/irritation effect of CAP is possibly through the TRPV-1-dependent pathway.

The intestinal epithelium, comprising different cell types such as enterocytes, M cells, goblet cells, immune cells, etc. functions as a permeable barrier that plays an important role in modulating solute and fluid exchange and has direct consequences on nutrient

uptake and transportation. The mucosal integrity is challenged daily by external factors in the luminal environment, and the loss of intestinal barrier function can result in clinical and nutritional consequences such as inflammation and malnutrition (Ponce de León-Rodríguez et al., 2019). The human colon epithelial cells *in vitro* provide signals that are essential for initiating and amplifying acute mucosal inflammatory responses (Jung et al., 1995), and the Caco-2 cell line has been widely used to test the effects of food components on intestinal inflammation (Iftikhar et al., 2020; Ponce de León-Rodríguez et al., 2019).

Inflammation is a non-specific, harmonized reaction of the immune system, which can be stimulated by various internal (e.g. cell lysis) and external (e.g. microbial) factors (Iftikhar et al., 2020). The initial inflammatory responses include activation of macrophages resident in the gut tissue, which results in the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor α (TNF-α) or anti-inflammatory factors such as interleukin-10 (IL-10) (Kagnoff, 2014). The pro-inflammatory / anti-inflammatory effects of the food components on intestinal epithelium can be measured by quantifying these cytokines produced by Caco-2 cells.

Emulsion-based systems have been studied for the oral delivery of CAP, where CAP molecules are dissolved in emulsion droplets for the purposes of increased solubility, reduced mouth burn perception, reduced irritation and increased bioaccessibility (Lu et al., 2016; Luo et al., 2019, 2020; Zhu et al., 2015b). Lu et al. (2016) studied the gastric mucosa irritation of CAP-loaded nanoemulsion and free CAP water suspension by feeding them to rats, and then observing the stained histological tissues of gastric mucosa under the microscope. They found that CAP-loaded nanoemulsion alleviated the gastric mucosa irritation as compared to free CAP water suspension. However, limited

information is available on *in vitro* methods to test the irritation of CAP-loaded formulations during gastric digestion.

In this study, we aim to establish an *in vitro* method to quantify the gastric irritation by measuring the stimulated secretion of the inflammatory mediators IL-8 by Caco-2 cells after incubation with gastric digesta of CAP-loaded emulsion gels obtained from simulated dynamic gastric digestion. The structure of the CAP-loaded emulsion gel was modified using different emulsifiers: whey proteins versus Tween 80, to investigate how food structure and digestion behaviour affect gastric irritation.

The CAP-loaded whey protein emulsion gel $W_{0.5}$-100 (i.e. whey proteins as both emulsifier and gelling agent, oil droplet size $d_{4,3}$ of ~ 0.5 μm, 100 mM NaCl; characterized as gel with active filler particles) and the CAP-loaded Tween 80 emulsion gel $T_{0.5}$-100 (i.e. Tween 80 as emulsifier, whey proteins as gelling agent, oil droplet size $d_{4,3}$ of 0.5 μm, 100 mM NaCl; characterized as gel with inactive filler particles) were used to produce the gastric digesta samples used in this study. *In vitro* dynamic gastric digestion was carried out for 240 min in the Human Gastric simulator (HGS) to obtain the gastric digesta samples. In Chapter 7, we found that the CAP-loaded Tween 80 emulsion gel had much smaller gel particle size and much higher oil droplet release from gel matrix during gastric digestion. Therefore, it is hypothesized that the gastric digesta from CAP-loaded Tween 80 emulsion gel would have higher release of CAP molecules from gel matrix, which would cause higher IL-8 production.

8.3 Results and discussion

8.3.1 Physiochemical characteristics of emptied gastric digesta

The *in vitro* dynamic gastric digestions of CAP-loaded whey protein emulsion gel, CAP-loaded Tween 80 emulsion gel, whey protein emulsion gel without CAP and Tween 80 emulsion gel without CAP were performed in a Human Gastric Simulator for 240 min. The simulated gastric fluid (SGF, pH 1.5) was added into the stomach chamber at a rate of 2.5 ml/min. Gastric emptying only started after 30 min because of the existence of the lag phase during gastric digestion of solid foods (Siegel et al., 1988). Starting from 30 min onwards, 45 ml of the gastric content was emptied out in every 15 min to reach a gastric emptying rate of 3 ml/min. Gastric digesta collected at 0 min consisted of the *in vitro* masticated bolus (160 g grinded gel mixed with 50 ml simulated salivary fluid) as well as 70 ml fasting SGF. Gastric digesta collected at 0, 30, 60, 90, 120, 150, 180, 210 and 240 min of digestion were used in this study. Table 8-1 summarizes the physiochemical properties, including pH, solid content (w/v%), particle size ($d_{4,3}$, μm) and oil droplet size ($d_{4,3}$, μm) of gastric digesta from CAP-loaded whey protein emulsion gel (0.02 wt% CAP; whey proteins as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl), CAP-loaded Tween 80 emulsion gel (0.02 wt% CAP; Tween 80 as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl), whey protein emulsion gel without CAP (whey proteins as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl) and Tween 80 emulsion gel without CAP (Tween 80 as emulsifier, oil droplet size $d_{4,3}$ ~ 0.5 μm, 100 mM NaCl), respectively, as a function of digestion time. Emulsion gels without CAP were used as controls.

Within the gel pairings with or without CAP, none of the four parameters measured showed significant differences, indicating that with both whey protein and Tween 80

emulsion gels, the incorporation of CAP in emulsion droplets had no significant effect on pH, solid content, particle size or oil droplet size over time during the digestive process in the model stomach. The pH did not differ significantly between the two gel types (i.e. whey proteins versus Tween 80 as emulsifier of the emulsion gel). Gastric digesta from Tween 80 emulsion gels had higher solid content initially compared to whey protein emulsion gels, but this difference was lost after 180 min of digestion (Chapter 7, Fig. 7-2). The average particle size of the gastric digesta from Tween 80 emulsion gels was about half that of the whey protein emulsion gels at 30 min of digestion, mainly due to its smaller masticated bolus particle size entering the stomach; the gap grew progressively greater as digestion progressed, indicating that Tween 80 emulsion gels were disintegrated faster than the whey protein emulsion gels during gastric digestion. Oil droplet size did not change measurably during digestion of whey protein emulsion gels, while it increased at the end by 50 – 100% during digestion of Tween 80 emulsion gels, indicating that oil droplet coalescence was more severe in Tween 80 emulsion gels. More detailed discussion on the different digestion behaviours between CAP-loaded whey protein emulsion gel and CAP-loaded Tween 80 emulsion gel can be found in Chapter 7.

Table 8-1 Physiochemical properties of gastric digesta from whey protein emulsion gel without CAP, CAP-loaded whey protein emulsion gel, Tween 80 emulsion gel without CAP and CAP-loaded Tween 80 emulsion gel, as a function of digestion time. Results are presented as mean ± standard deviation of N = 3 independent experiments (continued)

	whey protein emulsion gel without CAP	CAP-loaded whey protein emulsion gel	Tween 80 emulsion gel without CAP	CAP-loaded Tween 80 emulsion gel	whey protein emulsion gel without CAP	CAP-loaded whey protein emulsion gel	Tween 80 emulsion gel without CAP	CAP-loaded Tween 80 emulsion gel
Time point	pH	pH	pH	pH	Solid content	Solid content	Solid content	Solid content
0	$5.58^{a} \pm 0.23$	$5.88^{a} \pm 0.27$	$5.62^{a} \pm 0.06$	$5.70^{a} \pm 0.12$	n/a	n/a	n/a	n/a
30	$4.96^{a} \pm 0.28$	$5.26^{a} \pm 0.36$	$4.89^{a} \pm 0.23$	$5.06^{a} \pm 0.22$	$10.2^{a} \pm 1.4$	$10.9^{a} \pm 1.3$	$13.9^{b} \pm 0.1$	$14.0^{b} \pm 0.7$
60	$4.36^{a} \pm 0.31$	$4.60^{a} \pm 0.34$	$4.25^{a} \pm 0.16$	$4.41^{a} \pm 0.24$	$9.8^{a} \pm 0.0$	$10.7^{a} \pm 0.9$	$11.2^{b} \pm 0.0$	$13.4^{b} \pm 0.9$
90	$3.82^{a} \pm 0.31$	$4.03^{a} \pm 0.35$	$3.66^{a} \pm 0.18$	$3.79^{a} \pm 0.10$	$6.6^{a} \pm 0.2$	$7.3^{a} \pm 0.7$	$8.5^{b} \pm 0.0$	$8.8^{b} \pm 0.1$
120	$3.42^{a} \pm 0.28$	$3.57^{a} \pm 0.31$	$3.26^{a} \pm 0.18$	$3.38^{a} \pm 0.08$	$5.4^{a} \pm 0.7$	$5.7^{a} \pm 0.4$	$6.4^{b} \pm 0.1$	$8.0^{b} \pm 0.7$
150	$3.07^{a} \pm 0.30$	$3.21^{a} \pm 0.31$	$2.92^{a} \pm 0.17$	$3.05^{a} \pm 0.07$	$4.0^{a} \pm 0.3$	$4.2^{a} \pm 0.6$	$5.2^{b} \pm 0.2$	$5.1^{b} \pm 0.2$
180	$2.77^{a} \pm 0.28$	$2.89^{a} \pm 0.29$	$2.61^{a} \pm 0.17$	$2.76^{a} \pm 0.04$	$3.4^{a} \pm 0.1$	$3.6^{a} \pm 0.3$	$4.2^{a} \pm 0.2$	$4.2^{a} \pm 0.3$
210	$2.52^{a} \pm 0.28$	$2.64^{a} \pm 0.27$	$2.38^{a} \pm 0.17$	$2.50^{a} \pm 0.06$	$3.4^{a} \pm 0.5$	$3.2^{a} \pm 0.6$	$3.6^{a} \pm 0.1$	$3.4^{a} \pm 0.2$
240	$2.32^{a} \pm 0.27$	$2.41^{a} \pm 0.23$	$2.17^{a} \pm 0.14$	$2.27^{a} \pm 0.06$	$3.6^{a} \pm 0.2$	$3.6^{a} \pm 1.0$	$2.8^{a} \pm 0.0$	$3.2^{a} \pm 0.2$

Table 8-1 continued

	whey protein emulsion gel without CAP	CAP-loaded whey protein emulsion gel	Tween 80 emulsion gel without CAP	CAP-loaded Tween 80 emulsion gel	whey protein emulsion gel without CAP	CAP-loaded whey protein emulsion gel	Tween 80 emulsion gel without CAP	CAP-loaded Tween 80 emulsion gel
Time point	Particle size	Particle size	Particle size	Particle size	Oil droplet size	Oil droplet size	Oil droplet size	Oil droplet size
0	n/a	n/a	n/a	n/a	$0.50^{a} \pm 0.02$	$0.50^{a} \pm 0.02$	$0.50^{a} \pm 0.03$	$0.51^{a} \pm 0.00$
30	$476.4^{a} \pm 44.6$	$428.2^{a} \pm 19.2$	$186.7^{b} \pm 54.5$	$210.5^{b} \pm 68.0$	$0.48^{a} \pm 0.02$	$0.49^{a} \pm 0.01$	$0.51^{a} \pm 0.01$	$0.52^{a} \pm 0.01$
60	$457.9^{a} \pm 11.0$	$413.5^{a} \pm 45.2$	$157.7^{b} \pm 40.4$	$184.5^{b} \pm 28.3$	$0.48^{a} \pm 0.01$	$0.48^{a} \pm 0.01$	$0.51^{a} \pm 0.02$	$0.51^{a} \pm 0.01$
90	$403.0^{a} \pm 18.7$	$376.8^{a} \pm 40.9$	$128.2^{b} \pm 31.0$	$172.5^{b} \pm 19.4$	$0.49^{a} \pm 0.01$	$0.48^{a} \pm 0.02$	$0.52^{a} \pm 0.02$	$0.53^{a} \pm 0.02$
120	$390.0^{a} \pm 14.3$	$335.2^{a} \pm 61.8$	$109.6^{b} \pm 55.0$	$112.5^{b} \pm 11.8$	$0.49^{a} \pm 0.03$	$0.49^{a} \pm 0.02$	$0.51^{a} \pm 0.00$	$0.52^{a} \pm 0.01$
150	$334.8^{a} \pm 29.3$	$244.8^{a} \pm 39.2$	$71.0^{b} \pm 46.6$	$112.1^{b} \pm 37.5$	$0.50^{a} \pm 0.01$	$0.49^{a} \pm 0.04$	$0.52^{a} \pm 0.00$	$0.53^{a} \pm 0.02$
180	$241.0^{a} \pm 40.0$	$249.7^{a} \pm 79.6$	$49.0^{b} \pm 25.1$	$62.3^{b} \pm 22.5$	$0.50^{a} \pm 0.01$	$0.50^{a} \pm 0.01$	$0.53^{b} \pm 0.00$	$0.55^{b} \pm 0.01$
210	$241.2^{a} \pm 41.6$	$181.5^{ab} \pm 138.7$	$23.5^{b} \pm 10.9$	$71.1^{b} \pm 43.7$	$0.53^{a} \pm 0.02$	$0.53^{a} \pm 0.03$	$0.69^{b} \pm 0.03$	$0.71^{b} \pm 0.19$
240	$21.6^{a} \pm 24.7$	$64.6^{a} \pm 123.2$	$6.2^{a} \pm 1.5$	$10.8^{a} \pm 2.9$	$0.55^{a} \pm 0.02$	$0.56^{a} \pm 0.03$	$0.76^{b} \pm 0.02$	$1.01^{b} \pm 0.51$

^{a,b}Values with different superscripts within the same time point differ significantly by group ($P < 0.05$).

8.3.2 *In vitro* cytotoxicity of gastric digesta on Caco-2 cells

The pH of the gastric digesta was either adjusted to 7.0 or left unchanged after emptying from the stomach, in order to study the effect of the pH of gastric digesta on *in vitro* cytotoxicity. Gastric digesta was sterilized under ultraviolet (UV) light for 30 min, then diluted in cell complete growth medium with a dilution factor of 10 before application to Caco-2 cells cultured on the plastic surface of 96 well plates. The incubation time was 24 h. MTT assay was performed to determine the *in vitro* cytotoxicity of gastric digesta on Caco-2 cells. Results were expressed as percentage of viable cells in ratio to the negative control (i.e. Caco-2 cells incubated with cell complete growth medium alone).

Figure 8-1 presents the results of viability of Caco-2 cells after 24 h incubation with gastric digesta collected at different digestion times from four emulsion gels, where the pH of gastric digesta was left unadjusted. Whey protein emulsion gels with and without CAP showed similar bimodal cytotoxic patterns. The initial digesta at 0 and 30 min of digestion were not cytotoxic. At 60, 90, and 120 min, the digesta resulted in nearly 100% cytotoxicity. After this, the 150 – 240 min time points returned to having no significant cytotoxicity. This is unlikely to be due to the parameters previously measured (pH, solid content, particle size, or oil droplet size) as none of these showed a bimodal pattern but rather steadily decreased or remained unchanged over time.

There were significant differences in cytotoxic effects only in the final two time points between whey protein emulsion gel without CAP versus with CAP. The gastric digesta from whey protein emulsion gel without CAP collected at 210 and 240 min resulted in slightly more viable cells compared to the CAP-loaded whey protein emulsion gel and this was statistically significant. A possible reason could be that the particle size

of gastric digesta decreased drastically at 210 and 240 min of digestion (Table 8-1), with the release of free oil droplets from protein matrix (Figure 7-4 A in Section 7.3.2.2, Chapter 7: particle size distributions of gastric digesta emptied at different times from CAP-loaded whey protein emulsion gel). This in turn may have allowed the CAP molecules to be released into the aqueous phase, where they would have come in contact and stimulated the Caco-2 cells. The cytotoxicity of capsaicin on Caco-2 cells behaves in a dose-dependent manner. Isoda, Han, Tominaga, and Maekawa (2001) studied the cytotoxicity of capsaicin on Caco-2 cells by measuring the lactate dehydrogenase (LDH) release after incubation with different concentrations of capsaicin. They found that when capsaicin was present at above 200 μM, the cells released significantly more LDH, indicating its toxicity to the cells. Similar results were reported by Tsukura et al. (2007) where they studied the cytotoxicity of capsaicin on Caco-2 monolayers by cell viability assay using WST-1 reagent and reported that when capsaicin was present at above 200 μM, cell viability decreased significantly; when capsaicin was below 100 μM, it showed no toxicity to Caco-2 cells. We estimated the CAP concentrations presented in each well as a function of digestion time, based on the solid content results presented in Table 8-1 and the CAP concentration presented to cells from gastric digesta collected at 0 min of digestion was about 40 μM and about 7 μM at 240 min of digestion. The CAP levels were quite low, however, the slight differences between whey protein emulsion gels with or without CAP seemed to be caused by the presence of the extra CAP molecules, since other factors during digestion were the same (i.e. the digestive enzymes, decrease in pH, etc.).

For gastric digesta from Tween 80 emulsion gels with or without CAP, the cell viability showed a decreasing trend with increasing digestion time. Gastric digesta from Tween 80 emulsion gels with or without CAP did not cause any cytotoxicity from 0 –

120 min of digestion and indeed showed an increase in cell number; however, cytotoxicity/viability is extrapolated from optical density, and it is likely that the gel particles themselves contributed to optical density, causing an artificial increase in the apparent number of viable cells. Even though the results presented here already subtracted the readings from the blank control wells (i.e. wells without cells, incubated with gastric digesta), the gel pieces may have adhered to the cells during incubation and may not have been sufficiently taken out during cytotoxicity measurements. For wells without cells, the gastric digesta was relatively easily and sufficiently taken out, causing the density differences of gel particles between wells with or without cells, leading to the artificial increase in optical density.

Some cytotoxicity was caused by the CAP-loaded Tween 80 emulsion gel at 150 min of digestion. From 180 – 240 min of digestion, both gel types reduced cell viability by approximately 70%. This pattern is unlikely to be due to pH; although the pH of these digesta also did decrease over time, the whey protein emulsion gels had similar pH levels and they were not cytotoxic at the later time points. It may be due to the release of Tween 80 into the digestive fluid, as this surfactant has been shown to be cytotoxic to Caco-2 cells at concentrations of 0.1% to 0.25% (Lu et al., 2014; Shah, Palamakula, & Khan, 2004). We estimated the concentrations of Tween 80 presented in each well as a function of digestion time, based on the solid content results presented in Table 8-1 and the approximate Tween 80 concentrations presented to cells from gastric digesta were 0.12% at 0 min of digestion, 0.05% at 120 min of digestion and 0.02% at 240 min of digestion. The cytotoxic effect of Tween 80 to Caco-2 cells was more apparent during 180 to 240 min of digestion, because at these digestion time points, the gel particles were broken down into much smaller pieces, with more oil droplet liberated from the protein matrix.

Therefore, the chances of Tween 80 also being released and contacting with Caco-2 cells would have increased and stimulated cell death.

No significant difference was found in cell viability between Tween 80 emulsion gels with and without CAP except for gastric digesta collected at 150 min of digestion. At this time point, the gel without CAP caused no cytotoxicity whereas with its CAP-loaded counterpart caused approximately 50% cytotoxicity. The gels did not differ significantly at this time point for pH, solid content, or the size of particles or oil droplets. It is possible that, as with the whey protein gel with CAP at the later time points, CAP was released from the CAP-loaded Tween 80 emulsion gel at 150 min of digestion. After this time point, the CAP cytotoxicity may have been masked by the general cytotoxicity caused by the release of Tween 80 from both Tween 80 emulsion gels as described above.

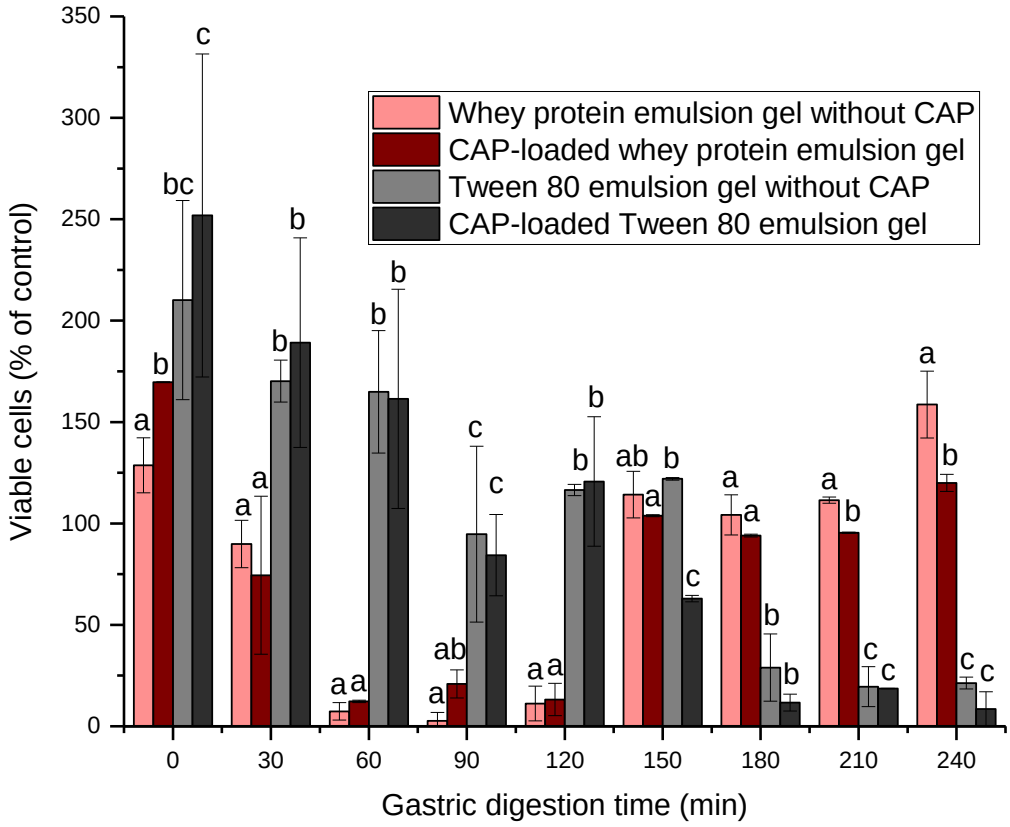


Figure 8-1 Viability of Caco-2 cells as determined by MTT assay after 24 h incubation with gastric digesta emptied at different digestion times. pH of gastric digesta was not adjusted. Gastric digesta was diluted 1:10 with cell complete growth medium before application to Caco-2 cells. Error bars represent standard deviations of N = 2 independent experiments, each conducted in duplicate. Different lowercase letters indicate significant differences between samples within the same digestion time point ($P < 0.05$).

Figure 8-2 presents the results of viability of Caco-2 cells after 24 h incubation with gastric digesta, where the pH of gastric digesta was immediately adjusted to 7.0 after emptying from the stomach. Adjusting the pH of gastric digesta to 7.0 would inactivate the digestive enzymes. Also, the neutral pH would be favoured by Caco-2 cells in terms of cell viability.

Comparing Fig. 8-1 and Fig. 8-2, there were clear differences that could only be attributed to the pH adjustment of gastric digesta. Gastric digesta from CAP-loaded whey protein emulsion gel continued to cause a very high degree of cytotoxicity at the 60 – 120 min time points, and this effect was further carried over to 150 min of digestion. However, whey protein emulsion gel without CAP did not cause significant cytotoxicity at any time point. This finding is as inexplicable as the original finding that these gels were cytotoxic during the middle time points of digestion but not the early and late time points. The Tween 80 emulsion gels again showed a purported increase in viable cells at the 0 – 60 min time points; as above, it can be speculated that this is an artefact of the gel particles themselves contributing to optical density. However, in contrast to the findings with the pH unadjusted samples, Figure 8-2 shows that at 150 min there was no difference between Tween 80 emulsion gels with or without CAP; moreover, at the 180 – 240 min the Tween 80 emulsion gel without CAP showed increasing levels of cytotoxicity whereas the CAP-loaded Tween 80 emulsion gel had no cytotoxic effects. This likely rules out the above speculations that in the pH unadjusted digesta, cytotoxicity at the late time points was due to CAP and/or Tween-80 being released into the solution. It is unclear why adjusting the pH altered the cytotoxicity of the whey protein gel without CAP and the Tween 80 gel with CAP, but not their respective counterparts. However, without further exploration of these findings, it is difficult to explain these phenomena.

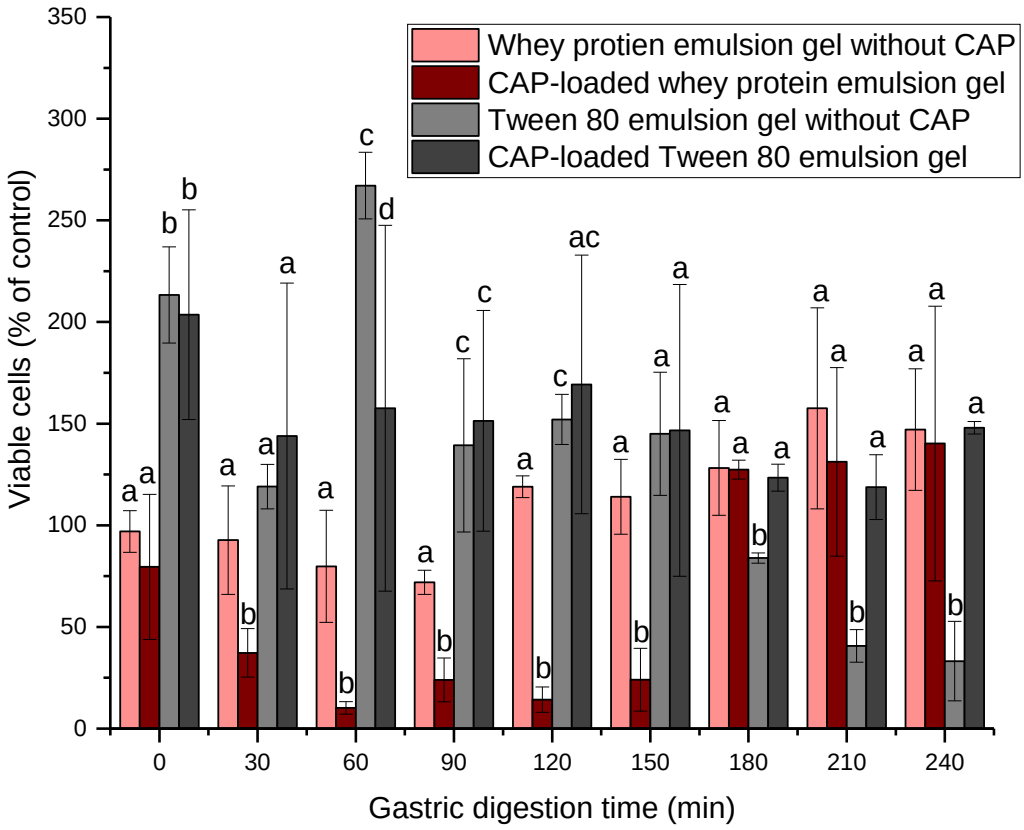


Figure 8-2 Viability of Caco-2 cells as determined by MTT assay after 24 h incubation with gastric digesta emptied at different digestion times. pH of gastric digesta was adjusted to 7.0. Gastric digesta was diluted for 10 times with cell complete growth medium before application on Caco-2 cells. Error bars represent standard deviations of N = 2 independent experiments, each conducted in duplicate. Different lowercase letters indicate significant differences between samples within the same digestion time point ($P < 0.05$).

8.3.3 Effect of gastric digesta on human interleukin-8 production by Caco-2 cells

Figure 8-3 shows the production of interleukin-8 (IL-8) by Caco-2 cells after 24 h incubation with diluted gastric digesta samples emptied at different times from whey protein emulsion gel without CAP, CAP-loaded whey protein emulsion gel, Tween 80 emulsion gel without CAP and CAP-loaded Tween 80 emulsion gel, where the pH of gastric digesta was left unadjusted. Gastric digesta was diluted in cell growth medium by a factor of 10, the same concentration as was used for the cell viability assay described above, as used to treat Caco-2 cells for 24 hours. Caco-2 cells were also incubated with 2ng/ml interleukin 1 beta (IL-1β) or growth medium alone as positive and negative controls, respectively. IL-1β is a pro-inflammatory cytokine protein that would stimulate intestinal epithelial cells to produce IL-8 (Fusunyan, Quinn, Ohno, MacDermott, & Sanderson, 1998). IL-8 production by Caco-2 cells after incubation with 2ng/ml IL-1β and complete growth medium was 823.4 ± 279.3 (positive control) and 12.4 ± 6.5 ng/ml (negative control), respectively. The results from positive and negative controls indicate that the Caco-2 cells were responding normally to stimulation and could produce high concentrations of IL-8 within the selected time period. Caco-2 cells were also incubated with a solution of CAP in growth medium at CAP concentration of 50 µg/ml (~ 160 µM). The result of percentage cell viability was 71.4 ± 28.7%, and the IL-8 production was 75.5 ± 36.7 ng/ml, indicating that CAP had a low level of cytotoxicity to Caco-2 cells and was also able to stimulate Caco-2 cells to produce IL-8.

From Fig. 8-3 A (i.e. IL-8 production per well), it is apparent that gastric digesta from CAP-loaded whey protein emulsion gel was able to stimulate Caco-2 cells to produce more IL-8 than gastric digesta emptied at the same times from whey protein emulsion gel without CAP, although IL-8 production overall was low. When normalized to the

number of viable cells (IL-8 concentration divided by the percentage of viable cells remaining after treatment) in Figure 8-3 B, the CAP-loaded whey protein emulsion gel again elicited little to no IL-8 production throughout the digestion period, and the whey protein emulsion gel without CAP induced no IL-8 production. This suggests that the components causing cytotoxicity in these gastric digesta samples, whether active digestive enzymes or aversive pH, do not themselves upregulate IL-8 production, and further that none of the components elicit significant IL-8 even when the cells remain fully viable. Only the presence of CAP molecules appeared to induce any IL-8 production, and this was minimal.

In contrast, gastric digesta from Tween 80 emulsion gels with or without CAP at their natural pH did induce IL-8 production (Fig. 8-3 B). Gastric digesta from Tween 80 emulsion gel without CAP elicited low levels of IL-8 during the first two hours of digestion even though the cells remained fully viable at these times. At and after 180 min, cell viability was compromised but IL-8 production on a per-cell basis rose to moderate levels. The counterpart gastric digesta from CAP-loaded Tween 80 emulsion gel induced moderate IL-8 production during 0 – 150 min of digestion, when the cells remained fully viable at these times. At and after 180 min, cell viability was also compromised but IL-8 production on a per-cell basis increased to very high concentrations. However it cannot be determined from these existing data whether the lower number of viable cells in these treatments produced 10-fold more IL-8 on a per-cell basis, or if the full complement of cells produced moderate IL-8 similar to the earlier time points and subsequently lost viability. The latter explanation is not unlikely given that IL-8 protein in Caco-2 cell conditioned medium peaks at 18 – 24 hours post-stimulation but has been shown to be present in measurable levels within 8 hours of stimulation (Parlesak, Haller, Brinz, Baeuerlein, & Bode, 2004).

Across the spectrum of assessments, gastric digesta from Tween 80 emulsion gels with unadjusted pH induced more IL-8 production than the matching whey protein emulsion gels, indicating that the matrix did influence inflammation. Moreover, gastric digesta from CAP-loaded Tween 80 emulsion gel stimulated more IL-8 secretion than gastric digesta collected at the same times from CAP-loaded whey protein emulsion gel ($P < 0.05$) and produced the highest overall level of IL-8 on a per-cell basis. This could be the combined effects of the presence of Tween 80 molecules and that there were possibly more CAP molecules released to aqueous phase and exposed to Caco-2 cells in gastric digesta from CAP-loaded Tween 80 emulsion gel. For both types of gel, the inclusion of CAP increased the per-cell production of IL-8, demonstrating that CAP itself induces IL-8; this was particularly apparent for the CAP-loaded Tween 80 emulsion gel. As discussed earlier, Tween 80, as a small-molecule surfactant, is also a proinflammatory factor and toxic substance when presented to Caco-2 cells at sufficient concentrations. It is stated in literature that the exposure of surfactants, such as Tween 20, Tween 60, Tween 85, sucrose esters and etc., to tissues / cells could cause cell damage or cell death and / or trigger the immune response of tissues / cells to secrete inflammation markers such as IL-8 (Lémery et al., 2015; Monteiro-Riviere, Inman, Wang, & Nemanich, 2005).

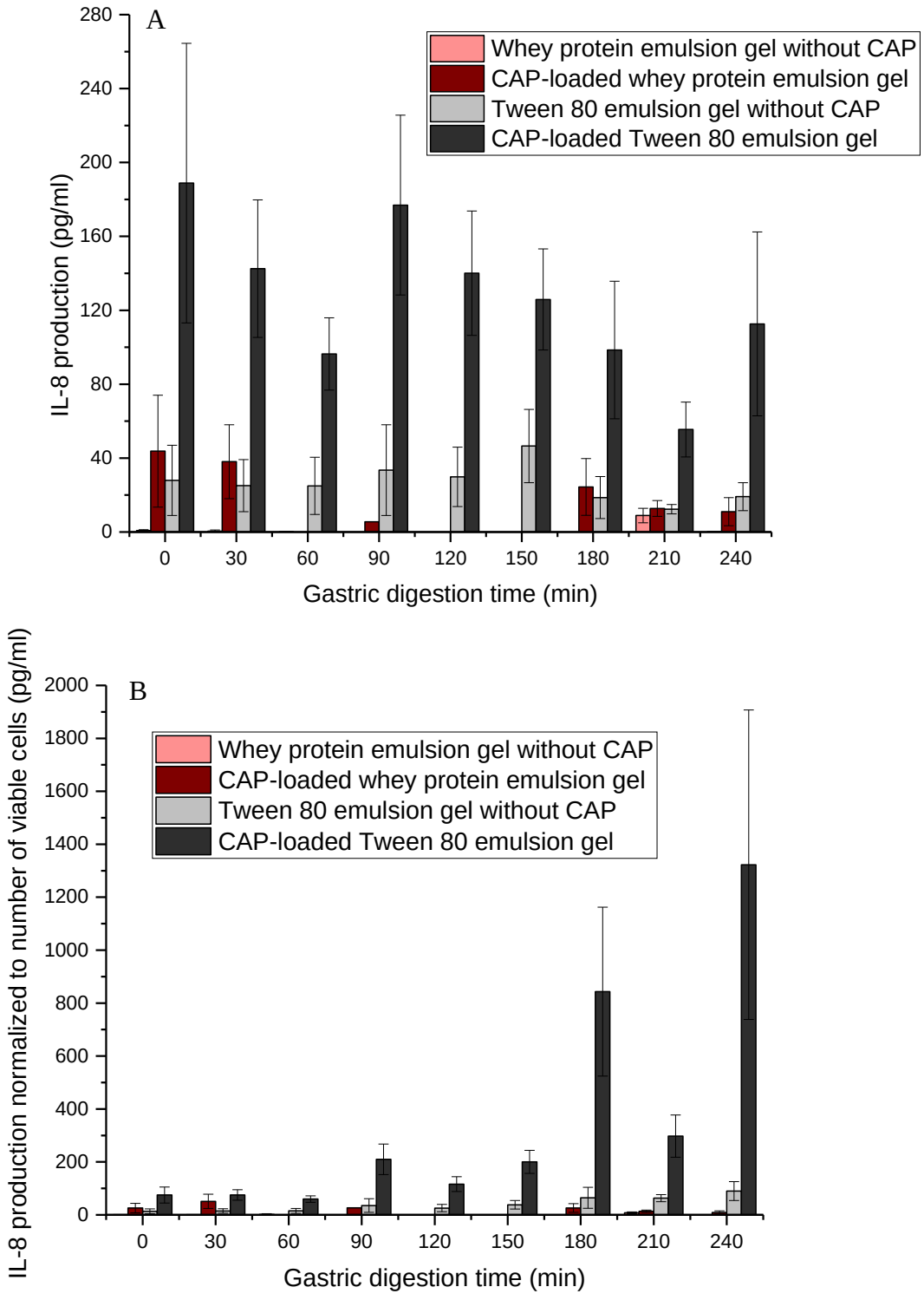


Figure 8-3 Effect of gastric digesta collected at different digestion times on IL-8 production by Caco-2 cells. The pH of gastric digesta was left unadjusted. Error bars represent standard deviations of N = 2 independent experiments, each conducted in duplicate. A) IL-8 production per well; B) IL-8 production normalized to the number of viable cells.

The same assessments were carried out for gastric digesta adjusted to pH 7.0 (Fig. 8-4 A and B). Figure 8-4 A presents the non-normalised IL-8 production by Caco-2 cells after 24 h incubation with gastric digesta, where the pH of gastric digesta was immediately adjusted to 7.0 after emptying from the stomach. Figure 8-4 B shows these data normalised to the relative number of viable cells present (as measured and shown in Figure 8-2).

In Figure 8-4 B, the digesta from whey protein emulsion gels, with or without CAP, induced low to moderate levels of IL-8 in Caco-2 cells on a per-cell basis in a pattern similar to the digesta not adjusted for pH (Fig. 8-3 B). However, the levels of IL-8 did appear higher when the pH of gastric digesta was adjusted compared to non-adjusted samples, and higher with the CAP-loaded whey protein emulsion gel compared to no CAP.

For gastric digesta from Tween 80 emulsion gels adjusted for pH, normal cell viability had been retained throughout, except for the gastric digesta from Tween 80 emulsion gel without CAP at the final two time points. Figure 8-4 A shows that IL-8 production was induced at fairly constant levels throughout the digestion time period. When normalised to viable cell number (Figure 8-4 B), the IL-8 production on a per-cell basis was altered only for the 210 and 240 min time points for Tween 80 emulsion gel without CAP. This suggests that IL-8 production was induced in the full complement of cells prior to apoptosis/necrosis being induced, as discussed above. For this reason, the remaining data in this chapter are presented without being normalized to cell number.

Gastric digesta from Tween 80 emulsion gels with pH adjusted resulted in little cytotoxicity (Fig. 8-2) yet, though cell numbers were similar to whey protein emulsion gel without CAP, digesta from Tween 80 emulsion gel induced more IL-8 (Fig. 8-4 A

and B). This suggests that the Tween 80 emulsion gel components can themselves induce IL-8. IL-8 induced from gastric digesta from CAP-loaded Tween 80 emulsion gel was approximately twice as high as that from its counterpart Tween 80 emulsion gel without CAP. Given that the only difference between these two gels is the incorporation of CAP molecules, it can be inferred that CAP was responsible for the differing levels of IL-8 production between the Tween 80 emulsion gels with or without CAP. This would also imply that the same level of CAP was present and able to stimulate the cells starting at time 0 and remaining constant throughout the entire digestive period. However, this is more likely due to differences in the final dilution factor (i.e. dilution of gastric contents due to continuous gastric secretion and emptying), which is discussed in more detail below. A comparison between Fig. 8-3 B and Fig. 8-4 B shows that IL-8 production was similar for gastric digesta from Tween 80 emulsion gel without CAP regardless of pH adjustment; however, adjusting the pH did lower IL-8 production somewhat with the CAP-loaded Tween 80 emulsion gel.

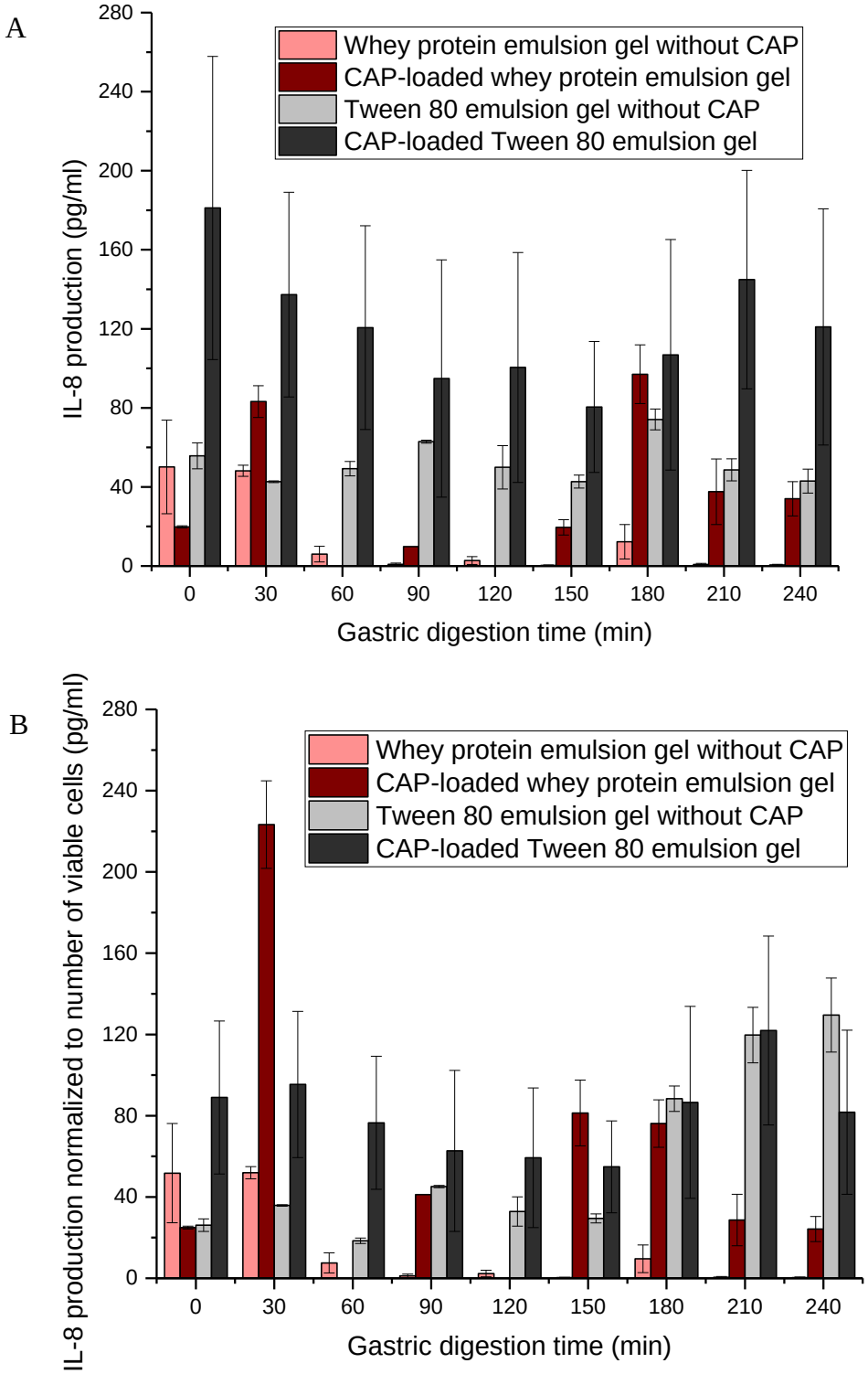


Figure 8-4 Effect of gastric digesta emptied at different digestion times on IL-8 production by Caco-2 cells. The pH of gastric digesta was adjusted to 7.0. Error bars represent standard deviations of N = 2 independent experiments, each conducted in duplicate. A) IL-8 production per well; B) IL-8 production normalized to the number of viable cells.

Since the solid content of gastric digesta gradually decreased with increasing digestion time due to gradual dilution, the IL-8 production was recalculated by multiplying a dilution factor to normalize the solid content of gastric digesta emptied at different times and from different gels. The dilution facotors (DF) for gastric digesta at different digestion times from four emulsion gels are calculated based on Equation 8-1:

$$DF = \frac{m_0}{m_i} \qquad \textbf{Equation 8-1}$$

where, m_0 was the solid content of gastric digesta collected at 0 min of digestion from CAP-loaded Tween 80 emulsion gel. At the beginning of gastric digestion, 160 g grinded CAP-loaded Tween 80 emulsion gel (solid content: 51.2 g) was mixed with 50 ml SSF and 70 ml fasting SGF. The volume of the mixture in the stomach at 0 min of digestion was about 270 ml. Therefore, the calculated theoretical m_0 was 19.0 w/v%. Similarly, the calculated theoretical value of solid content of gastric digesta at 0 min of digestion from whey protein emulsion gel with or without CAP was 17.8 w/v% (i.e. 48 g solids in 270 ml mixture). m_i was the solid content of gastric digesta emptied at other times from all gels (presented in Table 8-1). The results of the dilution factors are presented in Table 8-2.

Table 8-2 Dilution factors for gastric digesta at different digestion times from whey protein emulsion gels with or without CAP and Tween 80 emulsion gels with or without CAP. Results are presented as mean ± standard deviation of N = 3 independent experiments.

Gastric digestion time point	Whey protein emulsion gel without CAP	CAP-loaded whey protein emulsion gel	Tween 80 emulsion gel without CAP	CAP-loaded Tween 80 emulsion gel
0	1.07 ± 0.00	1.07 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
30	1.88 ± 0.18	1.59 ± 0.00	1.36 ± 0.01	1.34 ± 0.06
60	1.94 ± 0.00	1.67 ± 0.01	1.69 ± 0.00	1.40 ± 0.08
90	2.85 ± 0.06	2.62 ± 0.07	2.23 ± 0.00	2.15 ± 0.03
120	3.52 ± 0.33	3.62 ± 0.20	2.98 ± 0.04	2.33 ± 0.17
150	4.81 ± 0.28	4.83 ± 0.14	3.67 ± 0.08	3.78 ± 0.08
180	5.58 ± 0.07	5.65 ± 0.52	4.46 ± 0.14	4.64 ± 0.28
210	5.69 ± 0.61	5.76 ± 0.50	5.31 ± 0.12	5.52 ± 0.27
240	5.33 ± 0.26	4.73 ± 0.27	6.83 ± 0.05	6.00 ± 0.38

The normalized IL-8 production was calculated based on Fig. 8-3 A and Table 8-2 and presented in Figure 8-5. The pH of gastric digesta was not adjusted to 7.0. Since the results in Fig. 8-5 were normalized according to the solid content of gastric digesta, the differences between samples came from their differences in composition, gel particle size and colloidal structure of the gastric digesta. Gastric digesta from whey protein emulsion gel without CAP stimulated the lowest IL-8 production among four emulsion gels, while gastric digesta from CAP-loaded Tween 80 emulsion gel stimulated significantly more IL-8 production than gastric digesta collected at the same digestion times from other emulsion gels. The elevated IL-8 production in CAP-loaded whey protein emulsion gel in comparison to the whey protein emulsion gel without CAP came from the presence of the CAP molecules; the effect of CAP molecules on IL-8 production was higher towards the end of gastric digestion, possibly because the particle size of the gastric digesta was

smaller, and a proportion of the oil droplets was liberated from the protein matrix, resulting in higher release of CAP molecules from the food matrix. Similar results were observed between Tween 80 emulsion gels with or without CAP. The difference in normalized IL-8 production between Tween 80 emulsion gels with or without CAP was higher than the difference between whey protien emulsion gels with or without CAP. This indicates that there were possibily more CAP molecules been released to the aqueous phase during gastric digestion from CAP-loaded Tween 80 emulsion gel than CAP-loaded whey protein emulsion gel. However, IL-8 production from Tween 80 emulsion gel without CAP was higher than that from either whey protein emulsion gels, suggesting that the Tween 80 itself may induce minor inflammation; this has never been assessed in Caco-2 cells but it has been shown that Tween 80 can induce low levels of IL-8 production directly in keratinocytes (Coquette, Berna, Vandenbosch, Rosdy, & Poumay, 1999) and indirectly in blood cells (Kolter, Ott, Hauer, Reimold, & Fricker, 2015).

With increasing digestion time, the normalized IL-8 production showed an increasing trend for CAP-loaded Tween 80 emulsion gel; no such trend was found for other emulsion gels. As discussed previously in Chapter 5 and Chapter 7, CAP-loaded Tween 80 emulsion gel had a loose network structure; the Tween-80-coated oil droplets had no interactions with the whey protein matrix, also, the oil droplets appeared to be in a flocculated state in the protein matrix. During digestion, CAP-loaded Tween 80 emulsion gel was broken down into smaller pieces more easily and released large amounts of oil droplets, as compared to CAP-loaded whey protein emulsion gel. The smaller gel particles, loose network structure and greater release of oil droplets of CAP-loaded Tween 80 emulsion gel led to much higher possibility of CAP molecules being released into the aqueous phase, as compared to CAP-loaded whey protein emulsion gel.

Moreover, with increasing digestion time, the gel particles became smaller, and the extent of oil droplet release also increased; therefore, the release of CAP molecules into the aqueous increased with increasing digestion time, leading to the increasing trend in normalized IL-8 production. On the other hand, the oil droplets were mostly kept in the solid gel particles during gastric digestion of CAP-loaded whey protein emulsion gel. Since CAP molecules were dissolved in oil droplets, the extent of oil droplet release would affect the release of CAP molecules into the aqueous phase. Also, larger gel particle size of CAP-loaded whey protein emuslion gel during gastric digestion would also lead to less contact area of the gastric digesta with Caco-2 cells, and therefore, leading to lower inflammatory effect.

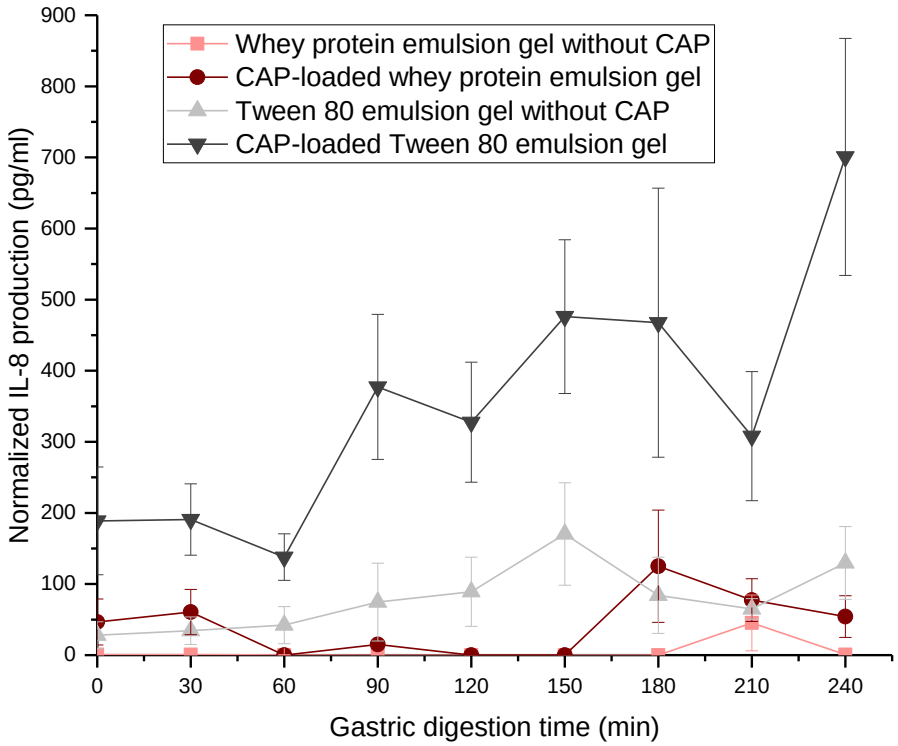


Figure 8-5 IL-8 production by Caco-2 cells after 24 h incubation with gastric digesta collected at different digestion times from whey protein emulsion gel without CAP, CAP-loaded whey protein emulsion gel, Tween 80 emulsion gel without CAP and CAP-loaded Tween 80 emulsion gel, normalised by dilution factor to control for digesta content. The pH of gastric digesta was not adjusted to 7.0. Error bars represent standard deviations of N = 2 independent experiments, each conducted in duplicate.

8.4 Conclusions

In this chapter, we investigated gastric irritation using an *in vitro* method by measuring the IL-8 production by Caco-2 cells after incubation with gastric digesta of emulsion gels containing capsaicinoids (CAP) obtained from simulated dynamic gastric digestion. The emulsion gel structure was modified using different emulsifiers: whey proteins versus Tween 80. Emulsion gels without CAP were also used as controls. Results indicate that the presence of CAP molecules in CAP-loaded emulsion gels was able to stimulate Caco-2 cells to produce significantly more IL-8, as compared to emulsion gels without CAP, indicating that CAP molecules are proinflammatory factors to Caco-2 cells and could stimulate the immune response of cells to secrete at least one inflammatory mediator. Gastric digesta from Tween 80 emulsion gel without CAP stimulated more IL-8 production than whey protein emulsion gel without CAP, possibly because of the presence of Tween 80, which is also a proinflammatory factor. Moreover, gastric digesta from CAP-loaded Tween 80 emulsion gel stimulated significantly more IL-8 production than CAP-loaded whey protein emulsion gel. This is the result of the combined effects from the presence of Tween 80 molecules and that there were possibly more CAP molecules released to the aqueous phase from CAP-loaded Tween 80 emulsion gel during gastric digestion. The Tween-80-coated oil droplets appeared to be in a flocculated state in the whey protein matrix; they had no interactions with the protein matrix, resulting in a loose gel structure. CAP-loaded Tween 80 emulsion gel was easily broken down into smaller gel pieces and had large amounts of oil droplet liberation from protein matrix throughout gastric digestion, which would promote the release of CAP molecules into the aqueous phase and, therefore, leading to higher IL-8 production. On the other hand, whey-protein-coated oil droplets had strong connections with surrounding protein matrix and were well protected during gastric digestion; the release

of CAP molecules would be much less as compared to CAP-loaded Tween 80 emulsion gel. Results demonstrate that by modifying the structure of the foods, the gastric digestion behaviour can be modified, which would then affect the release behaviour of CAP molecule and influence gastric irritation.

Information obtained from this study provides an *in vitro* method for testing gastric irritation / inflammation of CAP-loaded food formulations using Caco-2 cells. Also, this study provides knowledge on designing food structure for the incorporation of bioactive compounds in foods, and providing controlled release of the bioactive compounds. However, the findings presented in this chapter are preliminary, and it would be of interest to further explore the interactions between CAP, gel structures and its components during digestion on the inflammatory response in gut enterocytes.

Chapter 9: Overall conclusions, discussion and future recommendations

9.1 Overall conclusions and discussion

In this project, a series of experiments were performed on the digestion of emulsion gels containing capsaicinoids (CAP), including *in vivo* oral processing, *in vitro* dynamic gastric digestion and *in vitro* intestinal digestion. The aim was to investigate the effect of emulsion gel structure on its disintegration and digestion behaviour as well as the release behaviour of capsacinoids in the mouth and the gastrointestinal tract.

CAP-loaded emulsion gels with different structures and rheological properties were formulated by varying oil droplet size, ionic strength and type of emulsifier. In general, with decreasing oil droplet size, gel strength gradually increased, because smaller oil droplets could be better incorporated into protein networks. However, at low ionic strength (i.e. 10 mM NaCl), the gel strength decreased with decreasing oil droplet size ($d_{4,3}$) from 1 μm to 0.5 and 0.2 μm. A possible explanation was that at low ionic strength, the electrostatic repulsion was relatively high. With smaller oil droplet size, the number of oil droplets and interfacial area were higher, indicating higher proportion of proteins was adsorbed at the oil-water interface and less proteins were present in the aqueous phase. The adsorbed and partially unfolded proteins at the interface may not be available to participate in gel formation because of the high electrostatic repulsion between oil droplets, leading to fewer cross-links formed between proteins and therefore lower gel strength. Also, gel strength increased with increasing NaCl concentration from 10 mM to 100 mM, because at high ionic strength (i.e. 100 mM NaCl), the electrostatic repulsion between whey proteins were reduced, therefore more cross-links could be formed

between protein molecules during heat-set gel formation. Whey protein or small molecule surfactant Tween 80 was used as emulsifier to form gels with active filler particles and gels with inactive filler particles, respectively. The Tween-80-coated oil droplets had no interactions with whey protein matrix; they appeared to be in a flocculated state in the protein matrix, disrupting the gel structure, leading to a drastic decrease in gel strength.

Oral processing is the initial stage of food digestion. It is a complicated process and is influenced by many factors, such as food mechanical properties, the presence of excess liquids, serving temperature, etc. In this project, two key factors were identified to determine the degree of gel fragmentation during oral processing. The first factor was the extent of oil droplet release during mastication. Because of the loose structure of the gels with inactive filler particles, the Tween-80-coated oil droplets were easily liberated from the protein matrix under mechanical compression and shearing during mastication (oil droplet release > 49%) and this led to significantly smaller masticated bolus particle size. The released oil droplets also facilitated lubrication of gel particles and bolus formation, leading to reduced chewing cycles. For gels with active filler particles, the oil droplets were well protected by thick protein layer against liberation during mastication. The second factor affecting the degree of fragmentation was fracture properties. Generally, lower fracture strain would lead to higher degree of fragmentation. The fracture strain indicates the brittleness of the gel. The lower the fracture strain, the more brittle the gel, and therefore easier for the gel to be broken down into smaller particles at small deformation. On the contrary, gels with higher fracture strain were more elastic and appeared to be less fragmented during oral processing. Gels with low fracture stress and high fracture strain were least fragmented. They were easily deformable in the human mouth and the bolus produced had smoother surfaces; because of their high deformability

and smooth surfaces, the big particles were safe for swallowing and did not require further fragmentation. Gels with high fracture stress and low fracture strain were most fragmented for the purpose of safe swallowing.

Other than particle size reduction, oral processing also contributes to the sensory perception and appreciation. We investigated the mouth burn perception of CAP-loaded emulsion gels in relation to their structure and in-mouth breakdown behaviour and found that the degree of fragmentation was the dominating factor determining mouth burn perception. A higher degree of fragmentation led to greater surface exposure (i.e. new surface area) during mastication and therefore to higher mouth burn perception. The large amounts of oil droplet release during mastication also enhanced mouth burn perception, especially for gels with inactive filler particles. This effect was less prominent for gels with active filler particles, because the extent of oil droplet release was much less (values < 8%).

The digestion and disintegration of food particles in the stomach was also studied in relation to emulsion gel structure. Gels formed at high ionic strength (100 mM or 200 mM NaCl), i.e. the hard gel, had a more compact particulate gel structure and more crosslinks between whey proteins, which may have hindered the cleavage sites of pepsin, leading to slower disintegration of gel particles and slower hydrolysis of whey proteins during gastric digestion. Gel formed at low ionic strength (10 mM NaCl), i.e. the soft gel, was disintegrated faster and mostly disintegrated into free oil droplets at the end of gastric digestion with the occurrence of oil droplet coalescence. Gel with inactive filler particles was observed to have more oil droplet release from the protein matrix during gastric digestion, because of the loose connection between Tween-80-coated oil droplets and whey protein matrix.

Gastric digestion is a dynamic process with continuous gastric secretion and emptying. Gastric emptying, modulated by the pyloric sphincter, only allows particles smaller than 1 mm to pass through to the duodenum. The rate of gastric emptying is greatly impacted by the disintegration behaviour in the stomach as well as the initial particle size of the masticated food bolus entering the stomach. It was found that even though the hard gel was disintegrated slower than the soft gel during gastric digestion, it was emptied out faster due to the smaller masticated bolus particle size of the hard gel. The gastric emptying rate of the soft gel increased at later stage of gastric digestion due to the rapid disintegration of gel particles. Gel with inactive filler particles had smaller masticated bolus particle size and it was disintegrated faster during gastric digestion because of its loose gel structure, leading to its significantly faster rate of gastric emptying. Results indicate that sufficient mastication of food in the oral phase would significantly facilitate gastric emptying.

When CAP molecules are released from the food matrix during digestion, it could cause undesirable pain, irritation, inflammation or even damage the cells or tissues, which limit the ingestion of capsaicinoids at high doses. Whether incorporating capsaicinoids in emulsion gel could reduce irritation, prevent cell damage and how gel structure affects the level of irritation during gastric digestion were investigated in Chapter 8. An *in vitro* method to quantify the gastric irritation of CAP-loaded food formulations during gastric digestion was developed, by incubating Caco-2 cells with gastric digesta of CAP-loaded emulsion gels obtained from simulated dynamic gastric digestion. The stimulated secretion of interleukin-8 (IL-8) was measured. Results suggest that Caco-2 cells had immune responses to CAP-loaded samples by secreting significant amounts of IL-8, while no such response was observed in samples without CAP. This confirms that CAP molecules are inflammatory factors to Caco-2 cells. Also, the gastric

digesta from gel with inactive filler was able to stimulate more IL-8 production than the gel with active filler particles. Two possible reasons were identified. Firstly, it was found that Tween 80 was also a proinflammatory factor to Caco-2 cells and could stimulate IL-8 secretion. Secondly, gels with active or inactive filler particles had distinct behaviour during gastric digestion, which may have caused different release behaviours of CAP. Gel with inactive filler particles was significantly more disintegrated and released large amounts of oil droplets during gastric digestion, due to its loose gel structure. Since CAP molecules were dissolved in the oil droplets, both the large amounts of oil droplet release and the smaller gel particle size would promote the release of CAP molecules into the aqueous phase, which may have possibly caused the elevated IL-8 production.

Gastric emptying into the small intestine is a dynamic and continuous process, controlled by the pyloric sphincter. The food chyme leaving the stomach at different digestion times and from gels with different structures would have distinct characteristics, which may have an impact on the digestion behaviour and the bioaccessibility of the nutrients in the intestine. Properties such as particle size, solid content, fat content, the extent of oil droplet release and gel structure have an impact on the intestinal digestion behaviour and the bioaccessibility of nutrients. The extent of lipid digestion is influenced by a number of factors: the ratio of enzyme to substrate, the ratio of bile salts / calcium to substrate / lipolytic products, the interfacial area, etc. At higher enzyme to substrate ratios, the enzyme would be more effective at hydrolysing the lipids. The higher the ratio of bile salts / calcium to substrate / lipolytic products, the more effective the calcium and bile salts are at precipitating and displacing the lipolytic products from the interface and free the interface for further lipolysis. The higher the interfacial area (i.e. smaller oil droplet size, smaller gel particle size), the more exposure of substrate to lipolytic enzymes, leading to higher extent of lipid digestion. Moreover, the presence of Tween

80 in the gel with inactive filler particles decreased the rate and extent of lipolysis, because Tween 80 had certain resistance against replacement by bile salts from the interface.

The bioaccessibility of capsaicinoids was generally positively correlated with the extent of lipid digestion. A higher extent of lipid digestion would lead to more release of capsaicnoids from the food matrix; also, more lipolytic products would be produced and participate in micelle formation that help solubilize the released capsaicinoids. Moreover, the presence of Tween 80 molecules in the gel with inactive filler particles would also participate in the formation of mixed micelles and help solubilize the released capsaicinoids molecules, leading to improved bioaccessibility of capsaicinoids.

Overall, emulsion gel structure was the key factor affecting its disintegration and digestion behaviour as well as the release behaviour of capsaicinoids during the three main stages of food digestion, i.e. the mouth, stomach and small intestine. By controlling food structure, we could tailor the breakdown and digestion behaviour of the food in human body, and eventually control or modulate the release behaviour of targeted nutrients. Moreover, in this project, the effect of breakdown behaviour in the mouth on the subsequent gastrointestinal digestion was considered, which were often neglected in previous studies. Food digestion is a whole and sequential process, the previous stage of digestion would affect the next stages of digestion significantly. This should be taken into consideration when assessing the nutritional values of a food product. Information obtained from this project demonstrate that the release behaviour of targeted nutrients could be controlled via the food structure design.

9.2 Recommendations for future work

9.2.1 Caco-2 cell work

Caco-2 cell monolayer can be used to investigate cellular uptake of nutrients after intestinal digestion, to indicate the bioaccessibility / bioavailability of the nutrient. For capsaicinoids, it is worthwhile to measure the transepithelial electrical resistance (TEER) values of the cell monolayer, since capsaicianoids are known to be able to reversibly open the tight junctions and decrease TEER.

The *in vitro* method developed in Chapter 8 for measuring gastric irritation using Caco-2 cells can be further validated with more comprehensive work.

9.2.2 Animal and human studies

The human gastric simulator and *in vitro* intestinal digestion have certain constraints and are not able to simulate all the aspects of the real physiological conditions of the human body. Food digestion in the human body is a much more complicated process modulated by neural and hormonal responses. Therefore, the *in vitro* digestion methods should be validated by carrying out *in vivo* studies.

The oral delivery of nutrients can also be measured in animal or human studies by measuring the concentration of the nutrient or its metabolites in the blood stream after ingesting the food. Human studies are the gold standard, but when ethical and financial costs are a consideration, cannulated pigs can be employed as an excellent model of human digestion, absorption and nutrient metabolism. Results from *in vivo* studies can be used to correlate with results form *in vitro* studies.

In Chapter 8, we established an *in vitro* method to quantify gastric irritation of CAP-loaded food formulations, by measuring the stimulated IL-8 production by Caco-2 cells after incubation with gastric digesta obtained from *in vitro* dynamic gastric digestion. This *in vitro* method can be validated by performing *in vivo* studies and measure the *in vivo* responses of gastric cells / tissues to CAP-loaded foods during gastric digestion.

9.2.3 Gastric lipid digestion and its effect on intestinal digestion

In our study, fungal lipase was used during gastric digestion to represent gastric lipase. Gels with different structures could have different rate and extent of lipolysis during gastric digestion, and this may also influence intestinal digestion and bioaccessibility of bioactive compounds.

9.2.4 Sensory perception and digestion behaviour of differently structured emulsion gels induced for delivery of capsaicinoids

Apart from the heat-set whey protein emulsion gel, the cold-set gel formation could be induced by calcium, acid and enzymes. The addition of polysaccharides such as pectin and alginate would also modify the gel structure. Moreover, the composition of the emulsion gel (i.e. protein or oil concentration) could be modified to manipulate the gel structure. It is worth to investigate the sensory perception and digestion behaviour of differently structured emulsion gels for delivery of bioactive compounds such as capsaicinoids at macro- and micro-scales, in order to gain a systemic understanding on how food structure affects sensory perception, breakdown, protein hydrolysis, lipid digestion and bioaccessibility of bioactive compounds.

9.2.5 Effect of capsaicinoids on lipid digestion

It is reported in literature that capsaicinoids (CAP) have anti-obesity effect. It is worthwhile to investigate the possible mechanisms of how capsaicinoids-loaded food formulations regulate lipid digestion and find out the effective CAP concentrations required. This study can be established in *in vitro* systems first, then clinical studies can be conducted, by feeding human participants with CAP-loaded versus CAP-free foods and measure the postprandial lipaemic response in blood.

9.2.6 Modelling food breakdown and digestion in the human body for predicting the sensory perception and nutritional value of a food product

Food breakdown and digestion behaviour is closely related to a few factors such as food composition, structure, and rheological properties, and has a dominating effect on the sensory perception and nutrient release behaviour of the food product. By modelling food breakdown and digestion in relation to its composition, structure, etc., it is helpful in predicting the sensory perception as well as evaluating the nutritional value of the food.

9.2.7 Effect of capsaicinoids on gut microbiota

Capsaicinoids could regulate the composition and population of the gut microbiota, which would have an effect on the gut-brain axis. It is worth to investigate how capsaicinoids regulate gut microbiota composition and population; this could also be linked to the anti-obesity effect of capsaicinoids.

Bibliography

Agrawal, K. R., Lucas, P. W., Prinz, J. F., & Bruce, I. C. (1997). Mechanical properties of foods responsible for resisting food breakdown in the human mouth. *Archives of Oral Biology, 42*(1), 1–9.

Aguilera, J. M. (1995). Gelation of whey proteins: Chemical and rheological changes during phase transition in food. *Food technology (Chicago), 49*(10), 83–89.

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry, 132*(2), 799–807.

Ahrens Jr, E. H., Blankenhorn, D. H., & Hirsch, J. (1956). Measurement of the human intestinal length *in vivo* and some causes of variation. *Gastroenterology, 31*(3), 274–284.

Ako, K., Durand, D., Nicolai, T., & Becu, L. (2009a). Quantitative analysis of confocal laser scanning microscopy images of heat-set globular protein gels. *Food Hydrocolloids*, *23*(4), 1111–1119.

Ako, K., Nicolai, T., Durand, D., & Brotons, G. (2009b). Micro-phase separation explains the abrupt structural change of denatured globular protein gels on varying the ionic strength or the pH. *Soft Matter*, *5*(20), 4033–4041.

American Society of Agricultural and Biological Engineers. (2008). Method of determining and expressing fineness of feed materials by sieving. Standard ANSI/ASAE S319.4. St Joseph, MI: American Society of Agricultural and Biological Engineers.

American Spice Trade, A. (1985). *Official analytical methods of the American Spice Trade Association*: Third edition. Englewood Cliffs, NJ : The Association, 1985.

Amerine, M. A., Pangborn, R. M., & Roessler, E. B. (2013). *Principles of sensory evaluation of food*: Elsevier.

Anderberg, E. K., & Artursson, P. (1993). Epithelial transport of drugs in cell culture. VIII: Effects of sodium dodecyl sulfate on cell membrane and tight junction permeability in human intestinal epithelial (Caco-2) cells. *Journal of Pharmaceutical Sciences, 82*(4), 392–398.

Anderson, D. J. (1956). Measurement of stress in mastication. I. *Journal of Dental Research, 35*(5), 664–670.

Angle, C. W., Clarke, B., & Dabros, T. (2017). Dewatering kinetics and viscoelastic properties of kaolin as tailings model under compressive pressures. *Chemical Engineering Research and Design, 118*, 286–293.

Arora, S., Ali, J., Ahuja, A., Khar, R. K., & Baboota, S. (2005). Floating drug delivery systems: A review. *AAPS PharmSciTech, 6*(3), E372–E390.

Artursson, P. (1990). Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorbtive (Caco-2) cells. *Journal of Pharmaceutical Sciences, 79*(6), 476–482.

Artursson, P., Palm, K., & Luthman, K. (2012). Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Advanced Drug Delivery Reviews, 64*(SUPPL.), 280–289.

ASTM. (1981). Guidelines for the selection and training of sensory panel members. In: ASTM Committee E–18.

Atkinson, P. J., Dickinson, E., Horne, D. S., & Richardson, R. M. (1995). Neutron reflectivity of adsorbed β-casein and β-lactoglobulin at the air/water interface. *Journal of the Chemical Society, Faraday Transactions, 91*(17), 2847–2854.

Baek, I., Linforth, R. S. T., Blake, A., & Taylor, A. J. (1999). Sensory perception is related to the rate of change of volatile concentration in-nose during eating of model gels. *Chemical Senses, 24*(2), 155–160.

Bakker, J., Brown, W. E., Hills, B. M., Boudaud, N., Wilson, C. E., & Harrisson, M. (1996). Effect of the food matrix on flavour release and perception. In A. Taylor, & D. S. Mottram (Eds.), *Flavour science: recent developments* (pp. 369–374). Cambridge: Royal Society of Chemistry.

Barbé, F., Ménard, O., Le Gouar, Y., Buffière, C., Famelart, M. H., Laroche, B., Le Feunteun, S., Dupont, D., & Rémond, D. (2013). The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids. *Food Chemistry, 136*(3–4), 1203–1212.

Barnes, H. A., Hutton, J. F., & Walters, K. (1989). Rheology of suspensions. *An introduction to rheology* (pp. 115–139). Amsterdam: Elsevier.

Baron, R. F., & Penfield, M. P. (1996). Capsaicin heat intensity - Concentration, carrier, fat level, and serving temperature effects. *Journal of Sensory Studies*, *11*(4), 295–316.

Barrangou, L. M., Drake, M. A., Daubert, C. R., & Foegeding, E. A. (2006). Sensory texture related to large-strain rheological properties of agarglycerol gels as a model food. *Journal of Texture Studies, 37*(3), 241–262.

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2015). *Neuroscience: Exploring the brain: Fourth edition*.

Beggs, S., Liu, X. J., Kwan, C., & Salter, M. W. (2010). Peripheral nerve injury and TRPV1-expressing primary afferent C-fibers cause opening of the blood-brain barrier. *Molecular Pain, 6:74*.

Benini, L., Castellani, G., Brighenti, F., Heaton, K. W., Brentegani, M. T., Casiraghi, M. C., Sembenini, C., Pellegrini, N., Fioretta, A., Minniti, G., Porrini, M., Testolin, G., & Vantini, I. (1995). Gastric emptying of a solid meal is accelerated by the removal of dietary fibre naturally present in food. *Gut, 36*(6), 825–830.

Benjamin, O., Silcock, P., Leus, M., & Everett, D. W. (2012). Multilayer emulsions as delivery systems for controlled release of volatile compounds using pH and salt triggers. *Food Hydrocolloids, 27*(1), 109–118.

Benmoussa, M., Suhendra, B., Aboubacar, A., & Hamaker, B. R. (2006). Distinctive sorghum starch granule morphologies appear to improve raw starch digestibility. *Starch/Staerke, 58*(2), 92–99.

Bennett, D. J., & Kirby, G. W. (1968). Constitution and biosynthesis of capsaicin. *Journal of the Chemical Society C: Organic*, 442–446.

Bezelgues, J. B., Serieye, S., Crosset-Perrotin, L., & Leser, M. E. (2008). Interfacial and foaming properties of some food grade low molecular weight surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 331*(1–2), 56–62.

Bilecen, D., Scheffler, K., Seifritz, E., Bongartz, G., & Steinbrich, W. (2000). Hydro-MRI for the visualization of gastric wall motility using RARE magnetic resonance imaging sequences. *Abdominal Imaging, 25*(1), 30–34.

Bíró, T., Brodie, C., Modarres, S., Lewin, N. E., Ács, P., & Blumberg, P. M. (1998a). Specific vanilloid responses in C6 rat glioma cells. *Molecular Brain Research, 56*(1–2), 89–98.

Bíró, T., Maurer, M., Modarres, S., Lewin, N. E., Brodie, C., Ács, G., Ács, P., Paus, R., & Blumberg, P. M. (1998b). Characterization of functional vanilloid receptors expressed by mast cells. *Blood, 91*(4), 1332–1340.

Boger, D. V. (2009). Rheology and the resource industries. *Chemical Engineering Science, 64*(22), 4525–4536.

Bordoni, A., Laghi, L., Babini, E., Di Nunzio, M., Picone, G., Ciampa, A., Valli, V., Danesi, F., & Capozzi, F. (2014). The foodomics approach for the evaluation of protein bioaccessibility in processed meat upon *in vitro* digestion. *Electrophoresis, 35*(11), 1607–1614.

Bornhorst, G. M., & Singh, R. P. (2013). Kinetics of *in vitro* bread bolus digestion with varying oral and gastric digestion parameters. *Food Biophysics, 8*(1), 50–59.

Borreani, J., Leonardi, C., Moraga, G., Quiles, A., & Hernando, I. (2019). How do different types of emulsifiers/stabilizers affect the *in vitro* intestinal digestion of o/w emulsions? *Food Biophysics, 14*(3), 313–325.

Bortnowska, G. (2012). Effects of pH and ionic strength of NaCl on the stability of diacetyl and (–)-α-pinene in oil-in-water emulsions formed with food-grade emulsifiers. *Food Chemistry, 135*(3), 2021–2028.

Bourne, M. (2002). Relationship between rheology and food texture. In *Engineering and Food for the 21st Century* (pp. 291–306). New York: CRC Press.

Bourne, M. (2004). Relation between texture and mastication. *Journal of Texture Studies, 35*(2), 125–143.

Boutin, C., Giroux, H. J., Paquin, P., & Britten, M. (2007). Characterization and acid-induced gelation of butter oil emulsions produced from heated whey protein dispersions. *International Dairy Journal, 17*(6), 696–703.

Brandt, M. A., Skinner, E. Z., & Coleman, J. A. (1963). Texture profile method. *Journal of Food Science, 28*(4), 404–409.

Brener, W., Hendrix, T. R., & McHugh, P. R. (1983). Regulation of the gastric emptying of glucose. *Gastroenterology, 85*(1), 76–82.

Brew, K. (2013). α-Lactalbumin. In *Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, 4th Edition* (pp. 121–273).

Brown, J. F., Hanson, P. J., & Whittle, B. J. R. (1992). Nitric oxide donors increase mucus gel thickness in rat stomach. *European Journal of Pharmacology*, *223*(1), 103–104.

Brown, J. F., Keates, A. C., Hanson, P. J., & Whittle, B. J. R. (1993). Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *265*(3 28–3), G418–G422.

Brownlow, S., Morais Cabral, J. H., Cooper, R., Flower, D. R., Yewdall, S. J., Polikarpov, I., North, A. C. T., & Sawyer, L. (1997). Bovine β-lactoglobulin at 1.8 Å resolution - Still an enigmatic lipocalin. *Structure*, *5*(4), 481–495.

Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from hat-denatured whey. *Trends in Food Science and Technology*, *9*(4), 143–151.

Bryant, C. M., & McClements, D. J. (2000). Influence of NaCl and $CaCl_2$ on cold-set gelation of heat-denatured whey protein. *Journal of Food Science*, *65*(5), 801–804.

Buettner, A., Beer, A., Hannig, C., & Settles, M. (2001). Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging-consequences for retronasal aroma stimulation. *Chemical Senses*, *26*(9), 1211–1219.

Bujacz, A. (2012). Structures of bovine, equine and leporine serum albumin. *Acta Crystallographica Section D: Biological Crystallography*, *68*(10), 1278–1289.

Burton-Freeman, B. (2000). Dietary fiber and energy regulation. *Journal of Nutrition, 130*(2 SUPPL.), 272S–275S.

Calbet, J. A. L., & MacLean, D. A. (1997). Role of caloric content on gastric emptying in humans. *Journal of Physiology, 498*(2), 553–559.

Camilleri, M. (2006). Integrated upper gastrointestinal response to food intake. *Gastroenterology, 131*(2), 640–658.

Camilleri, M., & Prather, C. M. (1994). Axial forces during gastric emptying in health and models of disease. *Digestive Diseases and Sciences, 39*(12 Supplement), 14S–17S.

Cardello, A. V., Matas, A., & Sweeney, J. (1982). The standard scales of texture: rescaling by magnitude estimation. *Journal of Food Science, 47*, 1738–1740.

Carey, M. C., Small, D. M., & Bliss, C. M. (1983). Lipid digestion and absorption. *Annual Review of Physiology, 45*, 651–677.

Carr, J., Baloga, D., Guinard, J. X., Lawter, L., Marty, C., & Squire, C. (1996). The effect of gelling agent type and concentration on flavor release in model systems. In *Flavor–food interactions , ACS Symposium Series* (Vol. 633, pp. 98–108). Washington, DC: American Chemical Society.

Cecil, J. E., Francis, J., & Read, N. W. (1999). Comparison of the effects of a high-fat and high-carbohydrate soup delivered orally and intragastrically on gastric emptying, appetite, and eating behaviour. *Physiology and Behavior, 67*(2), 299–306.

Cerf-Ducastel, B., van de Moortele, P. F., MacLeod, P., Le Bihan, D., & Faurion, A. (2001). Interaction of gustatory and lingual somatosensory perceptions at the cortical level in the human: A functional magnetic resonance imaging study. *Chemical Senses, 26*(4), 371–383.

Chanda, S., Bashir, M., Babbar, S., Koganti, A., & Bley, K. (2008). *In vitro* hepatic and skin metabolism of capsaicin. *Drug Metabolism and Disposition*, *36*(4), 670–675.

Cheison, S. C., Leeb, E., Letzel, T., & Kulozik, U. (2011). Influence of buffer type and concentration on the peptide composition of trypsin hydrolysates of β-lactoglobulin. *Food Chemistry*, *125*(1), 121–127.

Chen, J. (2009). Food oral processing - A review. *Food Hydrocolloids*, *23*(1), 1–25.

Chen, J., & Dickinson, E. (1995). Protein/surfactant interfacial interactions Part 3. Competitive adsorption of protein + surfactant in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *101*(1), 77–85.

Chen, J., & Dickinson, E. (1998a). Viscoelastic properties of protein-stabilized emulsions: Effect of protein-surfactant interactions. *Journal of Agricultural and Food Chemistry*, *46*(1), 91–97.

Chen, J., & Dickinson, E. (1998b). Viscoelastic properties of heat-set whey protein emulsion gels. *Journal of Texture Studies*, *29*(3), 285–304.

Chen, J., & Dickinson, E. (1999). Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. *Colloids and Surfaces B: Biointerfaces*, *12*(3–6), 373–381.

Chen, J., Dickinson, E., & Edwards, M. (1999). Rheology of acid-induced sodium caseinate stabilized emulsion gels. *Journal of Texture Studies*, *30*(4), 377–396.

Chen, J., Dickinson, E., Langton, M., & Hermansson, A. M. (2000). Mechanical properties and microstructure of heat-set whey protein emulsion gels: Effect of emulsifiers. *LWT - Food Science and Technology*, *33*(4), 299–307.

Chen, J., Khandelwal, N., Liu, Z., & Funami, T. (2013). Influences of food hardness on the particle size distribution of food boluses. *Archives of Oral Biology*, *58*(3), 293–298.

Chen, R. Y. Z., & Guth, P. H. (1995). Interaction of endogenous nitric oxide and CGRP in sensory neuron-induced gastric vasodilation. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 268*(5 31–5), G791–G796.

Chirayath, M. V., Gajdzik, L., Hulla, W., Graf, J., Cross, H. S., & Peterlik, M. (1998). Vitamin D increases tight-junction conductance and paracellular Ca^{2+} transport in Caco-2 cell cultures. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 274*(2 37–2), G389–G396.

Choi, C. H., Jung, Y. K., & Oh, S. H. (2010). Autophagy induction by capsaicin in malignant human breast cells is modulated by p38 and extracellular signal-regulated mitogen-activated protein kinases and retards cell death by suppressing endoplasmic reticulum stress-mediated apoptosis. *Molecular Pharmacology, 78*(1), 114–125.

Chow, J., Norng, M., Zhang, J., & Chai, J. (2007). TRPV6 mediates capsaicin-induced apoptosis in gastric cancer cells-Mechanisms behind a possible new "hot" cancer treatment. *Biochimica et Biophysica Acta - Molecular Cell Research, 1773*(4), 565–576.

Christensen, C. M. (1980). Effects of solution viscosity on perceived saltiness and sweetness. *Perception & Psychophysics, 28*(4), 347–353.

Collins, L. M. C., & Dawes, C. (1987). The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *Journal of Dental Research, 66*(8), 1300–1302.

Collins, M. D., Wasmund, L. M., & Bosland, P. W. (1995). Improved method for quantifying capsaicinoids in Capsicum using high-performance liquid chromatography. *HortScience, 30*(1), 137–139.

Collins, P. J., Horowitz, M., Cook, D. J., Harding, P. E., & Shearman, D. J. (1983). Gastric emptying in normal subjects - a reproducible technique using a single scintillation camera and computer system. *Gut, 24*(12), 1117–1125.

Contreras-Padilla, M., & Yahia, E. M. (1998). Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. *Journal of Agricultural and Food Chemistry, 46*(6), 2075–2079.

Coquette, A., Berna, N., Vandenbosch, A., Rosdy, M., & Poumay, Y. (1999). Differential expression and release of cytokines by an *in vitro* reconstructed human epidermis following exposure to skin irritant and sensitizing chemicals. *Toxicology in Vitro, 13*(6), 867–877.

Courraud, J., Berger, J., Cristol, J. P., & Avallone, S. (2013). Stability and bioaccessibility of different forms of carotenoids and vitamin A during *in vitro* digestion. *Food Chemistry, 136*(2), 871–877.

Creusot, N., Gruppen, H., van Koningsveld, G. A., de Kruif, C. G., & Voragen, A. G. J. (2006). Peptide-peptide and protein-peptide interactions in mixtures of whey protein isolate and whey protein isolate hydrolysates. *International dairy journal, 16*(8), 840–849.

Curtis, H., & Barnes, N. S. (1994). *Invitation to biology*: Macmillan.

Dalgleish, D. G. (1997). Adsorption of protein and the stability of emulsions. *Trends in Food Science and Technology, 8*(1), 1–6.

Dalgleish, D. G. (2003). Food emulsions: Their structures and properties. In *Food Emulsions*: CRC Press.

Danielsson, I., & Lindman, B. (1981). The definition of microemulsion. *Colloids and Surfaces, 3*(4), 391–392.

Darwiche, G., Björgell, O., & Almér, L. O. (2003). The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. *BMC Gastroenterology, 3:12*.

Davenport, H. W. (2010). Gastrointestinal physiology, 1895–1975: motility. *Comprehensive Physiology*, 1–101.

de Feijter, J. A., & Benjamins, J. (1982). Soft-particle model of compact macromolecules at interfaces. *Journal of Colloid and Interface Science, 90*(1), 289–292.

Delie, F., & Rubas, W. (1997). A human colonic cell line sharing similarities with enterocytes as a model to examine oral absorption: Advantages and limitations of the Caco-2 model. *Critical Reviews in Therapeutic Drug Carrier Systems, 14*(3), 221–286.

Delwiche, J. (2004). The impact of perceptual interactions on perceived flavor. *Food Quality and Preference, 15*(2), 137–146.

Demetriades, K., Coupland, J. N., & McClements, D. J. (1997). Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. *Journal of Food Science, 62*(3), 462–467.

Derry, S., Lloyd, R., Moore, R. A., & McQuay, H. J. (2009). Topical capsaicin for chronic neuropathic pain in adults. *Cochrane Database of Systematic Reviews*, (4).

Devezeaux de Lavergne, M., Strijbosch, V. M. G., van den Broek, A. W. M., van de Velde, F., & Stieger, M. (2016). Uncoupling the impact of fracture properties and composition on sensory perception of emulsion-filled gels. *Journal of Texture Studies, 47*(2), 92–111.

Devezeaux de Lavergne, M., van de Velde, F., van Boekel, M. A. J. S., & Stieger, M. (2015a). Dynamic texture perception and oral processing of semi-solid food gels:

Part 2: Impact of breakdown behaviour on bolus properties and dynamic texture perception. *Food Hydrocolloids, 49*, 61–72.

Devezeaux de Lavergne, M., van Delft, M., van de Velde, F., van Boekel, M. A. J. S., & Stieger, M. (2015b). Dynamic texture perception and oral processing of semi-solid food gels: Part 1: Comparison between QDA, progressive profiling and TDS. *Food Hydrocolloids, 43*, 207–217.

Devraj, R., Williams, H. D., Warren, D. B., Mullertz, A., Porter, C. J. H., & Pouton, C. W. (2013). *In vitro* digestion testing of lipid-based delivery systems: Calcium ions combine with fatty acids liberated from triglyceride rich lipid solutions to form soaps and reduce the solubilization capacity of colloidal digestion products. *International Journal of Pharmaceutics, 441*(1–2), 323–333.

Di Lorenzo, C., Williams, C. M., Hajnal, F., & Valenzuela, J. E. (1988). Pectin delays gastric emptying and increases satiety in obese subjects. *Gastroenterology, 95*(5), 1211–1215.

Dias, C. B., Zhu, X., Thompson, A. K., Singh, H., & Garg, M. L. (2019). Effect of the food form and structure on lipid digestion and postprandial lipaemic response. *Food and Function, 10*(1), 112–124.

Dickinson, E. (1998). Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society - Faraday Transactions, 94*(12), 1657–1669.

Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets. *Food Hydrocolloids, 28*(1), 224–241.

Dickinson, E., & Chen, J. (1999). Heat-set whey protein emulsion gels: Role of active and inactive filler particles. *Journal of Dispersion Science & Technology, 20*(1/2), 197.

Dickinson, E., Goddard, E., & Ananthapadmanabhan, K. (1993). Interactions of Surfactants with Polymers and Proteins. *CRC, Boca Raton*, 295.

Dickinson, E., & Matsumura, Y. (1994). Proteins at liquid interfaces: Role of the molten globule state. *Colloids and Surfaces B: Biointerfaces, 3*(1–2), 1–17.

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *Journal of Agricultural and Food Chemistry, 44*(6), 1371–1377.

Dill, K. A., & Bromberg, S. (2003). *Molecular driving forces: statistical thermodynamics in chemistry and biology*. New York, NY: Garland Science.

Dimitrova, T. D., & Leal-Calderon, F. (2001). Bulk elasticity of concentrated protein-stabilized emulsions. *Langmuir, 17*(11), 3235–3244.

Donnan, F. G. (1924). The theory of membrane equilibria. *Chemical Reviews, 1*(1), 73–90.

Donnerer, J., Amann, R., Schuligoi, R., & Lembeck, F. (1990). Absorption and metabolism of capsaicinoids following intragastric administration in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology, 342*(3), 357–361.

Doty, R. L., & Laing, D. G. (2003). Psychophysical measurement of human olfactory function, including odorant mixture assessment. *Handbook of Olfaction and Gustation*, 203–228.

Dou, D., Ahmad, A., Yang, H., & Sarkar, F. H. (2011). Tumor cell growth inhibition is correlated with levels of capsaicin present in hot peppers. *Nutrition and Cancer, 63*(2), 272–281.

Driver, J., & Spence, C. (2000). Multisensory perception: Beyond modularity and convergence. *Current Biology, 10*(20), R731–R735.

Eaglesham, A., Herrington, T. M., & Penfold, J. (1992). A neutron reflectivity study of a spread monolayer of bovine serum albumin. *Colloids and Surfaces*, *65*(1), 9–16.

El-Nokaly, M., & Cornell, D. (1991). *Microemulsions and emulsions in foods*: ACS Publications.

Engelen, L., Fontijn-Tekamp, A., & van der Bilt, A. (2005). The influence of product and oral characteristics on swallowing. *Archives of Oral Biology*, *50*(8), 739–746.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*(SUPPL. 2), S33–S50.

Evans, D. F., & Wennerström, H. (1994). *The colloidal domain: where physics, chemistry, biology, and technology meet*: VCH Publishers.

Faisant, N., BuléOn, H., Colonna, P., & Champ, M. (1995). Digestion of raw banana starch in the small intestine of healthy humans: Structural features of resistant starch. *British Journal of Nutrition*, *73*(1), 111–123.

Faussone-Pellegrini, M. S., Taddei, A., Bizzoco, E., Lazzeri, M., Vannucchi, M. G., & Bechi, P. (2005). Distribution of the vanilloid (capsaicin) receptor type 1 in the human stomach. *Histochemistry and Cell Biology*, *124*(1), 61–68.

Feldman, M., Cryer, B., McArthur, K. E., Huet, B. A., & Lee, E. (1996). Effects of aging and gastritis on gastric acid and pepsin secretion in humans: A prospective study. *Gastroenterology*, *110*(4), 1043–1052.

Fellows, C. M., & Doherty, W. O. S. (2006). Insights into bridging flocculation. *Macromolecular Symposia*, *231*, 1–10.

Feng, Y., Zhu, Y., Wan, J., Yang, X., Firempong, C. K., Yu, J., & Xu, X. (2018). Enhanced oral bioavailability, reduced irritation and increased hypolipidemic

activity of self-assembled capsaicin prodrug nanoparticles. *Journal of Functional Foods, 44*, 137–145.

Ferrua, M. J., & Singh, R. P. (2010). Modeling the fluid dynamics in a human stomach to gain insight of food digestion. *Journal of Food Science, 75*(7), R151–R162.

Fine, K. D., Santa Ana, C. A., Porter, J. L., & Fordtran, J. S. (1995). Effect of changing intestinal flow rate on a measurement of intestinal permeability. *Gastroenterology*, *108*(4), 983–989.

Flory, P. (1953). Principle of polymer chemistry. *New York: Ithaca: Cornell University*.

Foegeding, E. A. (2007). Rheology and sensory texture of biopolymer gels. *Current Opinion in Colloid and Interface Science, 12*(4–5), 242–250.

Foegeding, E. A., Daubert, C. R., Drake, M. A., Essick, G., Trulsson, M., Vinyard, C. J., & van de Velde, F. (2011). A comprehensive approach to understanding textural properties of semi- and soft-solid foods. *Journal of Texture Studies, 42*(2), 103–129.

Foegeding, E. A., & Drake, M. A. (2007). Invited review: Sensory and mechanical properties of cheese texture. *Journal of Dairy Science*, *90*(4), 1611–1624.

Fogh, J., & Trempe, G. (1975). New human tumor cell lines. In *Human tumor cells in vitro* (pp. 115–159): Springer.

Fontijn-Tekamp, F. A., van der Bilt, A., Abbink, J. H., & Bosman, F. (2004). Swallowing threshold and masticatory performance in dentate adults. *Physiology and Behavior, 83*(3), 431–436.

Fordtran, J. S., & Walsh, J. H. (1973). Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *The Journal of Clinical Investigation, 52*(3), 645–657.

Foster, K. D., Woda, A., & Peyron, M. A. (2006). Effect of texture of plastic and elastic model foods on the parameters of mastication. *Journal of Neurophysiology, 95*(6), 3469–3479.

Fox, P. F., & McSweeney, P. L. H. (2009). *Advanced dairy chemistry* (Vol. 2).

French, S. J., & Read, N. W. (1994). Effect of guar gum on hunger and satiety after meals of differing fat content: Relationship with gastric emptying. *American Journal of Clinical Nutrition, 59*(1), 87–91.

Frias, B., & Merighi, A. (2016). Capsaicin, nociception and pain. *Molecules, 21*(6), 797.

Fuhrmann, P. L., Kalisvaart, L. C. M., Sala, G., Scholten, E., & Stieger, M. (2019). Clustering of oil droplets in o/w emulsions enhances perception of oil-related sensory attributes. *Food Hydrocolloids, 97*, 105215.

Fullard, L., Lammers, W., Wake, G. C., & Ferrua, M. J. (2014). Propagating longitudinal contractions in the ileum of the rabbit-Efficiency of advective mixing. *Food and Function, 5*(11), 2731–2742.

Fusunyan, R. D., Quinn, J. J., Ohno, Y., MacDermott, R. P., & Sanderson, I. R. (1998). Butyrate enhances interleukin (IL)-8 secretion by intestinal epithelial cells in response to IL-1β and lipopolysaccharide. *Pediatric Research, 43*(1), 84–90.

Ganong, W. F. (1995). *Review of medical physiology*: Mcgraw-hill.

Geppetti, P., & Trevisani, M. (2004). Activation and sensitisation of the vanilloid receptor: Role in gastrointestinal inflammation and function. *British Journal of Pharmacology, 141*(8), 1313–1320.

Gibbs, C. H., Mahan, P. E., Lundeen, H. C., Brehnan, K., Walsh, E. K., & Holbrook, W. B. (1981). Occlusal forces during chewing and swallowing as measured by sound transmission. *The Journal of Prosthetic Dentistry, 46*(4), 443–449.

Gillette, M. H., Appel, C. E., & Lego, M. C. (1984). A new method for sensory evaluation of red pepper heat. *Journal of Food Science, 49*(4), 1028–1033.

Gleeson, J. P., Brayden, D. J., & Ryan, S. M. (2017). Evaluation of PepT1 transport of food-derived antihypertensive peptides, Ile-Pro-Pro and Leu-Lys-Pro using *in vitro, ex vivo* and *in vivo* transport models. *European Journal of Pharmaceutics and Biopharmaceutics, 115*, 276–284.

Gleeson, J. P., Frías, J. M., Ryan, S. M., & Brayden, D. J. (2018). Sodium caprate enables the blood pressure-lowering effect of Ile-Pro-Pro and Leu-Lys-Pro in spontaneously hypertensive rats by indirectly overcoming PepT1 inhibition. *European Journal of Pharmaceutics and Biopharmaceutics, 128*, 179–187.

Goff, H. D. (1997). Colloidal aspects of ice cream – A review. *International Dairy Journal, 7*(6–7), 363–373.

Golding, M., & Wooster, T. J. (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid and Interface Science, 15*(1–2), 90–101.

Gomes, A., Costa, A. L. R., & Cunha, R. L. (2018). Impact of oil type and WPI/Tween 80 ratio at the oil-water interface: Adsorption, interfacial rheology and emulsion features. *Colloids and Surfaces B: Biointerfaces, 164*, 272–280.

Govindarajan, V. S., Narasimhan, S., & Dhanaraj, S. (1977). Evaluation of spices and oleoresins. II. Pungency of capsicum by scoville heat units - A standardized procedure. *Journal of Food Science and Technology, 14*(1), 28–34.

Govindarajan, V. S., & Salzer, U. J. (1986). Capsicum - production, technology, chemistry, and quality. Part II. Processed products, standards, world production and trade. *C R C Critical Reviews in Food Science and Nutrition, 23*(3), 207–288.

Gregson, R. A. M. (1963). The effect of psychological conditions on preference for taste mixtures. *Food Technology, 17*(3), 44.

Guinard, J. X., & Marty, C. (1995). Time-intensity measurement of flavor release from a model gel system: effect of gelling agent type and concentration. *Journal of Food Science, 60*(4), 727–730.

Guo, Q. (2015). *Behaviour of emulsion gels in the human mouth and simulated gastrointestinal tract.* (Doctor of Philosophy), Massey University, Palmerston North, Manawatu, New Zealand.

Guo, Q., Ye, A., Bellissimo, N., Singh, H., & Rousseau, D. (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research, 68*, 109-118.

Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2013). The breakdown properties of heat-set whey protein emulsion gels in the human mouth. *Food Hydrocolloids, 33*(2), 215–224.

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

Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2014b). Effect of gel structure on the gastric digestion of whey protein emulsion gels. *Soft Matter, 10*(8), 1214–1223.

Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2016). Impact of colloidal structure of gastric digesta on *in-vitro* intestinal digestion of whey protein emulsion gels. *Food Hydrocolloids, 54*, 255–265.

Guo, Q., Ye, A., Lad, M., Ferrua, M., Dalgleish, D., & Singh, H. (2015). Disintegration kinetics of food gels during gastric digestion and its role on gastric emptying: An *in vitro* analysis. *Food and Function, 6*(3), 756–764.

Gwartney, E. A., Larick, D. K., & Foegeding, E. A. (2004). Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. *Journal of Food Science, 69*, S333–S339.

Hansson, A., Giannouli, P., & van Ruth, S. (2003). The influence of gel strength on aroma release from pectin gels in a model mouth and *in vivo*, monitored with proton-transfer-reaction mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*(16), 4732–4740.

Harris, J. I., Cole, R. D., & Pon, N. G. (1956). The kinetics of acid hydrolysis of dipeptides. *The Biochemical Journal, 62*(1), 154–159.

Haynes, C. A., & Norde, W. (1994). Globular proteins at solid/liquid interfaces. *Colloids and Surfaces B: Biointerfaces, 2*(6), 517–566.

Haynes, C. A., & Norde, W. (1995). Structures and stabilities of adsorbed proteins. *Journal of Colloid and Interface Science, 169*(2), 313–328.

Heaney, R. P. (2001). Factors influencing the measurement of bioavailability, taking calcium as a model. *Journal of Nutrition, 131*(4 SUPPL.), 1344S–1348S.

Hidalgo, I. J., Raub, T. J., & Borchardt, R. T. (1989). Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology, 96*(2), 736–749.

Hiiemae, K., Heath, M. R., Heath, G., Kazazoglu, E., Murray, J., Sapper, D., & Hamblett, K. (1996). Natural bites, food consistency and feeding behaviour in man. *Archives of Oral Biology, 41*(2), 175–189.

Hiiemae, K. M., & Palmer, J. B. (1999). Food transport and bolus formation during complete feeding sequences on foods of different initial consistency. *Dysphagia, 14*(1), 31–42.

Hills, B. P., & Harrison, M. (1995). Two-film theory of flavour release from solids. *International Journal of Food Science & Technology, 30*(4), 425–436.

Himeno, S., Tarui, S., Kanayama, S., Kuroshima, T., Shinomura, V., Hayashi, C., Tateishi, K., Imagawa, K., Hashimura, E., & Hamaoka, T. (1983). Plasma cholecystokinin responses after ingestion of liquid meal and intraduodenal infusion of fat, amino acids, or hydrochloric acid in man: Analysis with region specific radioimmunoassay. *The American Journal of Gastroenterology, 78*(11), 703–707.

Hirose, M. (1993). Molten globule state of food proteins. *Trends in Food Science and Technology, 4*(2), 48–51.

Hoebler, C., Lecannu, G., Belleville, C., Devaux, M. F., Popineau, Y., & Barry, J. L. (2002). Development of an *in vitro* system simulating bucco-gastric digestion to assess the physical and chemical changes of food. *International Journal of Food Sciences and Nutrition, 53*(5), 389–402.

Hofmann, A. F., & Roda, A. (1984). Physicochemical properties of bile acids and their relationship to biological properties: An overview of the problem. *Journal of Lipid Research, 25*(13), 1477–1489.

Holt, S., Reid, J., Taylor, T. V., Tothill, P., & Heading, R. C. (1982). Gastric emptying of solids in man. *Gut, 23*(4), 292–296.

Holzer, P., Lippe, I. T., Jocic, M., Wachter, C., Erb, R., & Heinemann, A. (1993). Nitric oxide-dependent and oxide-independent hyperemia due to calcitonin-gene-related peptide in the rat stomach. *British Journal of Pharmacology, 110*(1), 404–410.

Hong, S. M., Tanaka, M., Koyanagi, R., Shen, W., & Matsui, T. (2016). Structural design of oligopeptides for intestinal transport model. *Journal of Agricultural and Food Chemistry, 64*(10), 2072–2079.

Hoover, R. (2000). Acid-treated starches. *Food Reviews International, 16*(3), 369–392.

Hoover, R., & Zhou, Y. (2003). *In vitro* and *in vivo* hydrolysis of legume starches by α-amylase and resistant starch formation in legumes - A review. *Carbohydrate Polymers, 54*(4), 401–417.

Horne, D. S., Atkinson, P. J., Dickinson, E., Pinfield, V. J., & Richardson, R. M. (1998). Neutron reflectivity study of competitive adsorption of β-lactoglobulin and nonionic surfactant at the air-water interface. *International Dairy Journal, 8*(2), 73–77.

Hu, D. E., Easton, A. S., & Fraser, P. A. (2005). TRPV1 activation results in disruption of the blood-brain barrier in the rat. *British Journal of Pharmacology, 146*(4), 576–584.

Huffman, V. L., Schadle, E. R., Villalon, B., & Burns, E. E. (1978). Volatile components and pungency in fresh and processed jalapeno peppers. *Journal of Food Science, 43*(6), 1809–1811.

Humberstone, A. J., & Charman, W. N. (1997). Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Advanced Drug Delivery Reviews, 25*(1), 103–128.

Humphrey, S. P., & Williamson, R. T. (2001). A review of saliva: Normal composition, flow, and function. *Journal of Prosthetic Dentistry, 85*(2), 162–169.

Hunt, J. A., & Dalgleish, D. G. (1996). The effect of the presence of KCl on the adsorption behaviour of whey protein and caseinate in oil-in-water emulsions. *Food Hydrocolloids, 10*(2), 159–165.

Hunt, J. N., & Stubbs, D. F. (1975). The volume and energy content of meals as determinants of gastric emptying. *The Journal of Physiology, 245*(1), 209–225.

Hutchings, J. B., & Lillford, P. J. (1988). The perception of food texture-the philosophy of the breakdown path. *Journal of Texture Studies, 19*(2), 103–115.

Hutchings, S. C., Foster, K. D., Bronlund, J. E., Lentle, R. G., Jones, J. R., & Morgenstern, M. P. (2011). Mastication of heterogeneous foods: peanuts inside two different food matrices. *Food Quality* and Preference, *22*(4), 332–339.

Ibekwe, V. C., Fadda, H. M., McConnell, E. L., Khela, M. K., Evans, D. F., & Basit, A. W. (2008). Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive ileo-colonic release systems. *Pharmaceutical Research, 25*(8), 1828–1835.

Iftikhar, M., Iftikhar, A., Zhang, H., Gong, L., & Wang, J. (2020). Transport, metabolism and remedial potential of functional food extracts (FFEs) in Caco-2 cells monolayer: A review. *Food Research International, 136*.

Ikeda, S. (2003). Heat-induced gelation of whey proteins observed by rheology, atomic force microscopy, and Raman scattering spectroscopy. *Food Hydrocolloids, 17*(4), 399–406.

Ikeda, S., & Foegeding, E. A. (1999). Dynamic viscoelastic properties of thermally induced whey protein isolate gels with added lecithin. *Food Hydrocolloids, 13*(3), 245–254.

Ikeda, S., Foegeding, E. A., & Hagiwara, T. (1999). Rheological study on the fractal nature of the protein gel structure. *Langmuir, 15*(25), 8584–8589.

Ikeda, S., & Morris, V. J. (2002). Fine-stranded and particulate aggregates of heat-denatured whey proteins visualized by atomic force microscopy. *Biomacromolecules, 3*(2), 382–389.

ISO. (1993). Sensory analysis - General guidance for the selection, training, and monitoring of assessors - Part I: Selected assessors. In: International Organization for Standarization.

ISO. (1999). Sensory analysis - Methodology - Magnitude estimation method. In (pp. 20): International Organization for Standardization.

Isoda, H., Han, J., Tominaga, M., & Maekawa, T. (2001). Effects of capsaicin on human intestinal cell line Caco-2. *Cytotechnology, 36*(1–3), 155–161.

Ito, K., Nakazato, T., Yamato, K., Miyakawa, Y., Yamada, T., Hozumi, N., Segawa, K., Ikeda, Y., & Kizaki, M. (2004). Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: Implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. *Cancer Research, 64*(3), 1071–1078.

Iwai, K., Suzuki, T., Fujiwake, H., & Oka, S. (1979). Simultaneous microdetermination of capsaicin and its four analogues by using high-performance liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography A, 172*(1), 303–311.

Jalabert-Malbos, M. L., Mishellany-Dutour, A., Woda, A., & Peyron, M. A. (2007). Particle size distribution in the food bolus after mastication of natural foods. Food Quality and Preference, *18*(5), 803–812.

Jampen, S., Britt, I. J., Yada, S., & Tung, M. A. (2001). Rheological properties of gellan gels containing filler particles. *Journal of Food Science, 66*(2), 289–293.

Jenkins, G. N. (1978). The physiology and biochemistry of the mouth. In: Philadelphia: Lippincott.

Joyner , H. S., & Daubert, C. R. (2017). Rheological principles for food analysis. In S. S. Nielsen (Ed.), *Food analysis* (pp. 511–527). Cham: Springer International Publishing.

Jung, H. C., Eckmann, L., Yang, S. K., Panja, A., Fierer, J., Morzycka-Wroblewska, E., & Kagnoff, M. F. (1995). A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *Journal of Clinical Investigation, 95*(1), 55–65.

Kagnoff, M. F. (2014). The intestinal epithelium is an integral component of a communications network. *Journal of Clinical Investigation, 124*(7), 2841–2843.

Kaiser, M., Pereira, S., Pohl, L., Ketelhut, S., Kemper, B., Gorzelanny, C., Galla, H. J., Moerschbacher, B. M., & Goycoolea, F. M. (2015). Chitosan encapsulation modulates the effect of capsaicin on the tight junctions of MDCK cells. *Scientific Reports, 5:10048*.

Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J. B., & Reppas, C. (2006). Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharmaceutical Research, 23*(1), 165–176.

Kato, S., Aihara, E., Nakamura, A., Xin, H., Matsui, H., Kohama, K., & Takeuchi, K. (2003). Expression of vanilloid receptors in rat gastric epithelial cells: Role in cellular protection. *Biochemical Pharmacology, 66*(6), 1115–1121.

Kawada, T., Suzuki, T., Takahashi, M., & Iwai, K. (1984). Gastrointestinal absorption and metabolism of capsaicin and dihydrocapsaicin in rats. *Toxicology and Applied Pharmacology, 72*(3), 449–456.

Kawada, T., Watanabe, T., Katsura, K., Takami, H., & Iwai, K. (1985). Formation and metabolism of pungent principle of capsicum fruits. XV. Microdetermination of

capsaicin by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography A, 329*(C), 99–105.

Kerrigan, D. D., Mangnall, Y. F., Read, N. W., & Johnson, A. G. (1991). Influence of acid-pepsin secretion on gastric emptying of solids in humans: studies with cimetidine. *Gut, 32*(11), 1295–1297.

Kerstens, S., Murray, B. S., & Dickinson, E. (2006). Microstructure of β-lactoglobulin-stabilized emulsions containing non-ionic surfactant and excess free protein: Influence of heating. *Journal of Colloid and Interface Science, 296*(1), 332–341.

Kilara, A., & Vaghela, M. N. (2004). Whey proteins. In *Proteins in Food Processing* (pp. 72–99).

Kim, C. S., Park, W. H., Park, J. Y., Kang, J. H., Kim, M. O., Kawada, T., Yoo, H., Han, I. S., & Yu, R. (2004). Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor γ in HT-29 human colon cancer cells. *Journal of Medicinal Food, 7*(3), 267–273.

Kim, J. D., Kim, J. M., Pyo, J. O., Kim, S. Y., Kim, B. S., Yu, R., & Han, I. S. (1997). Capsaicin can alter the expression of tumor forming-related genes which might be followed by induction of apoptosis of a Korean stomach cancer cell line, SNU-1. *Cancer Letters, 120*(2), 235–241.

Kim, K. H., Renkema, J. M. S., & Van Vliet, T. (2001). Rheological properties of soybean protein isolate gels containing emulsion droplets. *Food Hydrocolloids, 15*(3), 295–302.

Kim, Y. M., Hwang, J. T., Kwak, D. W., Lee, Y. K., & Park, O. J. (2007) Involvement of AMPK signaling cascade in capsaicin-induced apoptosis of HT-29 colon cancer cells. In*: Vol. 1095. Annals of the New York Academy of Sciences* (pp. 496–503).

Kinsella, J. E., & Whitehead, D. M. (1989) Proteins in whey: Chemical, physical, and functional properties. In*: Vol. 33. Advances in Food and Nutrition Research* (pp. 343–438).

Kohyama, K., Gao, Z., Watanabe, T., Ishihara, S., Nakao, S., & Funami, T. (2017). Relationships between mechanical properties obtained from compression test and electromyography variables during natural oral processing of gellan gum gels. *Journal of Texture Studies, 48*(1), 66–75.

Koike, S., Sujino, T., Ohmori, H., Shimazaki, K., Fukuyama, E., Kanai, T., Hibi, T., Ono, T. (2013). Gastric emptying rate in subjects with malocclusion examined by [13C] breath test. *Journal of Oral Rehabilitation, 40*(8), 574–581.

Kolter, M., Ott, M., Hauer, C., Reimold, I., & Fricker, G. (2015). Nanotoxicity of poly(n-butylcyano-acrylate) nanoparticles at the blood–brain barrier, in human whole blood and *in vivo*. *Journal of Controlled Release, 197*, 165–179.

Kong, F., Oztop, M. H., Paul Singh, R., & McCarthy, M. J. (2013). Effect of boiling, roasting and frying on disintegration of peanuts in simulated gastric environment. *LWT - Food Science and Technology*, *50*(1), 32–38.

Kong, F., & Singh, R. P. (2009). Modes of disintegration of solid foods in simulated gastric environment. *Food Biophysics, 4*(3), 180–190.

Kong, F., & Singh, R. P. (2010). A human gastric simulator (HGS) to study food digestion in human stomach. *Journal of Food Science, 75*(9), E627–E635.

Kong, F., & Singh, R. P. (2011). Solid loss of carrots during simulated gastric digestion. *Food Biophysics, 6*(1), 84–93.

Konturek, S. J., Brzozowski, T., Majka, J., Pytko-Polonczyk, J., & Stachura, J. (1993). Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *European Journal of Pharmacology, 239*(1–3), 215–217.

Korel, F., Bağdatlioğlu, N., Balaban, M. Ö., & Hişil, Y. (2002). Ground red peppers: Capsaicinoids content, scoville scores, and discrimination by an electronic nose. *Journal of Agricultural and Food Chemistry, 50*(11), 3257–3261.

Kosuge, S., & Furuta, M. (1970). Studies on the pungent principle of capsicum. Part XIV. Chemical constitution of the pungent principle. *Agricultural and Biological Chemistry, 34*(2), 248–256.

Koukoura, E., Panagiotopoulou, M., Pavlou, A., Karageorgiou, V., Fatouros, D. G., Vasiliadou, C., & Ritzoulis, C. (2019). *In vitro* digestion of caseinate and Tween 20 emulsions. *Food Biophysics, 14*(1), 60–68.

Krog, N. J., & Vang Sparsø, F. (2003). Food emulsifiers: Their chemical and physical properties. In *Food Emulsions*: CRC Press.

Kulmyrzaev, A., Bryant, C., & McClements, D. J. (2000). Influence of sucrose on the thermal denaturation, gelation, and emulsion stabilization of whey proteins. *Journal of Agricultural and Food Chemistry, 48*(5), 1593–1597.

Kuwata, K., Hoshino, M., Era, S., Batt, C. A., & Goto, Y. (1998). α→β transition of β-lactoglobulin as evidenced by heteronuclear NMR. *Journal of Molecular Biology, 283*(4), 731–739.

Langley, K. R., & Green, M. L. (1989). Compression strength and fracture properties of model particulate food composites in relation to their microstructure and particle-matrix interaction. Journal of Texture Studies, 20(2), 191–207.

Lawless, H. T., Hartono, C., & Hernandez, S. (2000). Thresholds and suprathreshold intensity functions for capsaicin in oil and aqueous based carriers. *Journal of Sensory Studies, 15*(4), 437–447.

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices*: Springer Science & Business Media.

Lawless, H. T., Rozin, P., & Shenker, J. (1985). Effects of oral capsaicin on gustatory, olfactory and irritant sensations and flavor identification in humans who regularly or rarely consume chili pepper. *Chemical Senses, 10*(4), 579–589.

Leclère, C. J., Champ, M., Boillot, J., Guille, G., Lecannu, G., Molis, C., Bornet, F., Krempf, M., Delort-Laval, J., & Galmiche, J. P. (1994). Role of viscous guar gums in lowering the glycemic response after a solid meal. *American Journal of Clinical Nutrition, 59*(4), 914–921.

Lee, H. A., Choi, S. J., & Moon, T. W. (2006). Characteristics of sodium caseinate- and soy protein isolate-stabilized emulsion-gels formed by microbial transglutaminase. *Journal of Food Science, 71*(6), C352–C357.

Leelahuta, Y., Glinsukon, T., & Wangpanish, W. (1983). *In vitro* capsaicin metabolism in the rat, mouse and hamster: A preliminary report. *Toxicon, 21*(SUPPL. 3), 245–248.

Leidy, H. J., Apolzan, J. W., Mattes, R. D., & Campbell, W. W. (2010). Food form and portion size affect postprandial appetite sensations and hormonal responses in healthy, nonobese, older adults. *Obesity, 18*(2), 293–299.

Leman, J., & Kinsella, J. E. (1989). Surface activity, film formation, and emulsifying properties of milk proteins. *Critical Reviews in Food Science and Nutrition, 28*(2), 115–138.

Lémery, E., Briançon, S., Chevalier, Y., Bordes, C., Oddos, T., Gohier, A., & Bolzinger, M. A. (2015). Skin toxicity of surfactants: Structure/toxicity relationships. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 469*, 166–179.

Li, Y., Hu, M., & McClements, D. J. (2011). Factors affecting lipase digestibility of emulsified lipids using an *in vitro* digestion model: Proposal for a standardised pH-stat method. *Food Chemistry*, *126*(2), 498–505.

Li, Y., & McClements, D. J. (2010). New mathematical model for interpreting pH-stat digestion profiles: Impact of lipid droplet characteristics on *in vitro* digestibility. *Journal of Agricultural and Food Chemistry*, *58*(13), 8085–8092.

Li, Y., & McClements, D. J. (2011). Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions. *European Journal of Pharmaceutics and Biopharmaceutics*, *79*(2), 423–431.

Liang, L., Leung Sok Line, V., Remondetto, G. E., & Subirade, M. (2010). *In vitro* release of α-tocopherol from emulsion-loaded β-lactoglobulin gels. *International Dairy Journal*, *20*(3), 176–181.

Liao, D., Gregersen, H., Hausken, T., Gilja, O. H., Mundt, M., & Kassab, G. (2004). Analysis of surface geometry of the human stomach using real-time 3-D ultrasonography *in vivo*. *Neurogastroenterology and Motility*, *16*(3), 315–324.

Liddle, R. A., Green, G. M., Conrad, C. K., & Williams, J. A. (1986). Proteins but not amino acids, carbohydrates, or fats stimulate cholecystokinin secretion in the rat. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *251*(2).

Lin, Q., Liang, R., Zhong, F., Ye, A., & Singh, H. (2018). Effect of degree of octenyl succinic anhydride (OSA) substitution on the digestion of emulsions and the bioaccessibility of β-carotene in OSA-modified-starch-stabilized-emulsions. *Food Hydrocolloids*, *84*, 303–312.

Lindmark, T., Kimura, Y., & Artursson, P. (1998). Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids

in Caco-2 cells. *Journal of Pharmacology and Experimental Therapeutics*, *284*(1), 362–369.

Liu, F., & Tang, C.-H. (2011). Cold, gel-like whey protein emulsions by microfluidisation emulsification: Rheological properties and microstructures. *Food Chemistry*, *127*(4), 1641–1647.

Liu, K., Stieger, M., van der Linden, E., & van de Velde, F. (2015). Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. *Food Hydrocolloids*, *44*, 244–259.

Lo, Y. C., Yang, Y. C., Wu, I. C., Kuo, F. C., Liu, C. M., Wang, H. W., Kuo, C. H., Wu, J. Y., & Wu, D. C. (2005). Capsaicin-induced cell death in a human gastric adenocarcinoma cell line. *World Journal of Gastroenterology*, *11*(40), 6254–6257.

Lu, M., Cao, Y., Ho, C. T., & Huang, Q. (2016). Development of organogel-derived capsaicin nanoemulsion with improved bioaccessibility and reduced gastric mucosa irritation. *Journal of Agricultural and Food Chemistry*, *64*(23), 4735–4741.

Lu, Y., Wang, Y. Y., Yang, N., Zhang, D., Zhang, F. Y., Gao, H. T., Rong, W. T., Yu, S. Q., Xu, Q. (2014). Food emulsifier polysorbate 80 increases intestinal absorption of di-(2-ethylhexyl) phthalate in rats. *Toxicological sciences : an official journal of the Society of Toxicology*, *139* 10.1093/toxsci/kfu055.

Lucas, P. W., Prinz, J. F., Agrawal, K. R., & Bruce, I. C. (2002). Food physics and oral physiology. *Food Quality and Preference*, *13*(4), 203–213.

Ludy, M. J., & Mattes, R. D. (2012). Comparison of sensory, physiological, personality, and cultural attributes in regular spicy food users and non-users. *Appetite*, *58*(1), 19–27.

Lund, J. P. (1991). Mastication and its control by the brain stem. *Critical Reviews in Oral Biology and Medicine, 2*(1), 33–64.

Luo, N., Ye, A., Wolber, F. M., & Singh, H. (2019). Structure of whey protein emulsion gels containing capsaicinoids: impact on in-mouth breakdown behaviour and sensory perception. *Food Hydrocolloids, 92*, 19–29.

Luo, N., Ye, A., Wolber, F. M., & Singh, H. (2020). In-mouth breakdown behaviour and sensory perception of emulsion gels containing active or inactive filler particles loaded with capsaicinoids. *Food Hydrocolloids, 108.*

Luo, X. J., Peng, J., & Li, Y. J. (2011). Recent advances in the study on capsaicinoids and capsinoids. *European Journal of Pharmacology, 650*(1), 1–7.

Macierzanka, A., Böttger, F., Lansonneur, L., Groizard, R., Jean, A. S., Rigby, N. M., Cross, K., Wellner, N., & MacKie, A. R. (2012). The effect of gel structure on the kinetics of simulated gastrointestinal digestion of bovine β-lactoglobulin. *Food Chemistry, 134*(4), 2156–2163.

MacKie, A. R., Wilde, P. J., Wilson, D. R., & Clark, D. C. (1993). Competitive effects in the adsorbed layer of oil-in-water emulsions stabilised by β-lactoglobulin-Tween 20 mixtures. *Journal of the Chemical Society, Faraday Transactions, 89*(15), 2755–2759.

MacRitchie, F., & Owens, N. F. (1969). Interfacial coagulation of proteins. *Journal of Colloid and Interface Science, 29*(1), 66–71.

Madenci, D., & Egelhaaf, S. U. (2010). Self-assembly in aqueous bile salt solutions. *Current Opinion in Colloid and Interface Science, 15*(1-2), 109–115.

Maggi, C. A., & Meli, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *General Pharmacology, 19*(1), 1–43.

Malagelada, J.-R., & Azpiroz, F. (2010). Determinants of gastric emptying and transit in the small intestine. In *Comprehensive Physiology*: John Wiley & Sons, Inc.

Malagelada, J. R., Go, V. L. W., & Summerskill, W. H. J. (1979). Different gastric, pancreatic, and biliary responses to solid-liquid or homogenized meals. *Digestive Diseases and Sciences, 24*(2), 101–110.

Malagelada, J. R., Longstreth, G. F., Summerskill, W. H. J., & Go, V. L. W. (1976). Measurement of gastric functions during digestion of ordinary solid meals in man. *Gastroenterology, 70*(2), 203–210.

Malaki Nik, A., Wright, A. J., & Corredig, M. (2011). Impact of interfacial composition on emulsion digestion and rate of lipid hydrolysis using different *in vitro* digestion models. *Colloids and Surfaces B: Biointerfaces, 83*(2), 321–330.

Maldonado-Valderrama, J., Wilde, P., Macierzanka, A., & MacKie, A. R. (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science, 165*(1), 36–46.

Malone, M. E., & Appelqvist, I. A. M. (2003). Gelled emulsion particles for the controlled release of lipophilic volatiles during eating. *Journal of Controlled Release, 90*(2), 227–241.

Mao, L., Roos, Y. H., Biliaderis, C. G., & Miao, S. (2017). Food emulsions as delivery systems for flavor compounds: a review. *Critical Reviews in Food Science and Nutrition, 57*, 3173–3187.

Mao, L., Roos, Y. H., & Miao, S. (2014). Study on the rheological properties and volatile release of cold-set emulsion-filled protein gels. *Journal of Agricultural and Food Chemistry, 62*, 11420–11428.

Marciani, L., Gowland, P. A., Fillery-Travis, A., Manoj, P., Wright, J., Smith, A., Young, P., Moore, R., & Spiller, R. C. (2001a). Assessment of antral grinding of a model

solid meal with echo-planar imaging. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *280*(5 43–5), G844–G849.

Marciani, L., Gowland, P. A., Spiller, R. C., Manoj, P., Moore, R. J., Young, P., & Fillery-Travis, A. J. (2001b). Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *280*(6 43–6), G1227–G1233.

Marciani, L., Young, P., Wright, J., Moore, R., Coleman, N., Gowland, P. A., & Spiller, R. C. (2001c). Antral motility measurements by magnetic resonance imaging. *Neurogastroenterology and Motility*, *13*(5), 511–518.

Matsumura, Y., Mitsui, S., Dickinson, E., & Mori, T. (1994). Competitive adsorption of α-lactalbumin in the molten globule state. *Topics in Catalysis*, *8*(6), 555–566.

Mattes, R. D., & Campbell, W. W. (2009). Effects of food form and timing of ingestion on appetite and energy intake in lean young adults and in young adults with obesity. *Journal of the American Dietetic Association*, *109*(3), 430–437.

McClements, D. J. (2009). Biopolymers in Food Emulsions. In *Modern Biopolymer Science* (pp. 129–166).

McClements, D. J. (2000). Comments on viscosity enhancement and depletion flocculation by polysaccharides. *Food Hydrocolloids*, *14*(2), 173–177.

McClements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter*, *8*(6), 1719–1729.

McClements, D. J. (2015). *Food emulsions: principles, practices, and techniques*: CRC press.

McClements, D. J., Monahan, F. J., & Kinsella, J. E. (1993). Effect of emulsion droplets on the rheology of whey protein isolate gels. *Journal of Texture Studies (USA)*(4), 411.

Medicis, S., & Hiiemae, K. (1998). *Natural bite sizes for common foods.* Paper presented at the Journal of Dental Research.

Mehalebi, S., Nicolai, T., & Durand, D. (2008). The influence of electrostatic interaction on the structure and the shear modulus of heat-set globular protein gels. *Soft Matter, 4*(4), 893–900.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007a). Factors influencing sensory verdicts. In *Sensory evaluation techniques* (4th ed., pp. 39–44). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007b). Introduction to sensory techniques. In *Sensory evaluation techniques* (4th ed., pp. 1–5). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007c). Measuring responses. In *Sensory evaluation techniques* (4th ed., pp. 45–61). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007d). Overall difference tests: does a sensory difference exist between samples. In *Sensory evaluation techniques* (4th ed., pp. 63–104). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007e). Selection and training of panel members. In *Sensory evaluation techniques* (pp. 141–172). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007f). *Sensory evaluation techniques* (4th ed.). Boca Raton, FL 33487–2742: CRC Press, Taylor & Francis Group.

Meilgaard, M. C., & Reid, D. S. (1979). Determination of personal and group thresholds and the use of magnitude estimation in beer flavour chemistry. *Progress in Flavour Research*, 67–73.

Meyer, J. H., Dressman, J., Fink, A., & Amidon, G. (1985). Effect of size and density on canine gastric emptying of nondigestible solids. *Gastroenterology, 89*(4), 805–813.

Meyer, J. H., MacGregor, I. L., Gueller, R., Martin, P., & Cavalieri, R. (1976). 99mTc-Tagged chicken liver as a marker of solid food in the human stomach. *The American Journal of Digestive Diseases, 21*(4), 296–304.

Meyer, J. H., Ohashi, H., Jehn, D., & Thomson, J. B. (1981). Size of liver particles emptied from the human stomach. *Gastroenterology, 80*(6), 1489–1496.

Meyer, J. H., Thomson, J. B., Cohen, M. B., Shadchehr, A., & Mandiola, S. A. (1979). Sieving of solid food by the canine stomach and sieving after gastric surgery. *Gastroenterology, 76*(4), 804–813.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Maclerzanka, A., MacKie, A. R., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food-an international consensus. *Food and Function, 5*(6), 1113–1124.

Mioche, L. (2004). Mastication and food texture perception: Variation with age. *Journal of Texture Studies, 35*(2), 145–158.

Monteiro-Riviere, N. A., Inman, A. O., Wang, Y. Y., & Nemanich, R. J. (2005). Surfactant effects on carbon nanotube interactions with human keratinocytes. *Nanomedicine: Nanotechnology, Biology, and Medicine, 1*(4), 293–299.

Moran, T. H., & McHugh, P. R. (1982). Cholecystokinin suppresses food intake by inhibiting gastric emptying. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 11*(3), R491–R497.

Mori, A., Lehmann, S., O'Kelly, J., Kumagai, T., Desmond, J. C., Pervan, M., McBride, W. H., Kizaki, M., & Koeffler, H. P. (2006). Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. *Cancer Research, 66*(6), 3222–3229.

Morr, C. V., & Ha, E. Y. W. (1993). Whey protein concentrates and isolates: Processing and functional properties. *Critical Reviews in Food Science and Nutrition*, *33*(6), 431–476.

Mosca, A. C., Rocha, J. A., Sala, G., van de Velde, F., & Stieger, M. (2012). Inhomogeneous distribution of fat enhances the perception of fat-related sensory attributes in gelled foods. *Food Hydrocolloids, 27*(2), 448–455.

Moskowitz, H. R. (1977). Magnitude estimation: Notes on what, how, when, and why to use it. *Journal of Food Quality, 1*(3), 195–227.

Mózsik, G., Szolcsányi, J., & Dömötör, A. (2007). Capsaicin research as a new tool to approach of the human gastrointestinal physiology, pathology and pharmacology. *Inflammopharmacology, 15*(6), 232–245.

Mukherjee, T., Squillantea, E., Gillespieb, M., & Shao, J. (2004). Transepithelial electrical resistance is not a reliable measurement of the Caco-2 monolayer integrity in transwell. *Drug Delivery: Journal of Delivery and Targeting of Therapeutic Agents, 11*(1), 11–18.

Mukhopadhyay, S., & Maitra, U. (2004). Chemistry and biology of bile acids. *Current Science*, *87*(12), 1666–1683.

Mun, S., Decker, E. A., & McClements, D. J. (2007). Influence of emulsifier type on *in vitro* digestibility of lipid droplets by pancreatic lipase. *Food Research International*, *40*(6), 770–781.

Mun, S., Kim, Y. R., Shin, M., & McClements, D. J. (2015). Control of lipid digestion and nutraceutical bioaccessibility using starch-based filled hydrogels: Influence of starch and surfactant type. *Food Hydrocolloids*, *44*, 380–389.

Nasrawi, C. W., & Pangborn, R. M. (1990). Temporal effectiveness of mouth-rinsing on capsaicin mouth-burn. *Physiology and Behavior*, *47*(4), 617–623.

Nelson, E. K. (1919). The constitution of capsaicin, the pungent principle of capsicum. *Journal of the American Chemical Society*, *41*(7), 1115–1121.

Nicolai, T., Britten, M., & Schmitt, C. (2011). β-Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids*, *25*(8), 1945–1962.

Nigsch, F., Klaffke, W., & Miret, S. (2007). *In vitro* models for processes involved in intestinal absorption. *Expert Opinion on Drug Metabolism and Toxicology*, *3*(4), 545–556.

Nishihara, K., Nozawa, Y., Nakano, M., Ajioka, H., & Matsuura, N. (2002). Sensitizing effects of lafutidine on CGRP-containing afferent nerves in the rat stomach. *British Journal of Pharmacology*, *135*(6), 1487–1494.

Nyemb, K., Guérin-Dubiard, C., Dupont, D., Jardin, J., Rutherfurd, S. M., & Nau, F. (2014). The extent of ovalbumin *in vitro* digestion and the nature of generated peptides are modulated by the morphology of protein aggregates. *Food Chemistry*, *157*, 429–438.

O' Dwyer, S. P., O' Beirne, D., Ní Eidhin, D., Hennessy, A. A., & O' Kennedy, B. T. (2013a). Formation, rheology and susceptibility to lipid oxidation of multiple emulsions (O/W/O) in table spreads containing omega-3 rich oils. *LWT - Food Science and Technology, 51*(2), 484–491.

O'Dwyer, S. P., O'Beirne, D., Ní Eidhin, D., Hannon, J. A., & O'Kennedy, B. T. (2013b). Oxidative stability of tuna fat spreads (O/W/O emulsions) using conventional lipid oxidation methods, SPME-GC/MS and sensory analysis. *European Food Research and Technology, 237*(3), 385–398.

O'Mahony, M. (1986). Sensory adaptation. *Journal of Sensory Studies, 1*(3-4), 237–258.

Oh, S. H., Kim, Y. S., Lim, S. C., Hou, Y. F., Chang, I. Y., & You, H. J. (2008). Dihydrocapsaicin (DHC), a saturated structural analog of capsaicin, induces autophagy in human cancer cells in a catalase-regulated manner. *Autophagy, 4*(8), 1009–1019.

øktedalen, O., Nesland, A., Opstad, P. K., & Berstad, A. (1988). The influence of prolonged physical stress on gastric juice components in healthy man. *Scandinavian Journal of Gastroenterology*, *23*(9), 1132–1136.

Olausson, E. A., Alpsten, M., Larsson, A., Mattsson, H., Andersson, H., & Attvall, S. (2008). Small particle size of a solid meal increases gastric emptying and late postprandial glycaemic response in diabetic subjects with gastroparesis. *Diabetes Research and Clinical Practice, 80*(2), 231–237.

Onuki, A. (1989). Theory of pattern formation in gels: Surface folding in highly compressible elastic bodies. *Physical Review A, 39*(11), 5932–5948.

Oppermann, A. K. L., Verkaaik, L. C., Stieger, M., & Scholten, E. (2017). Influence of double (w1/o/w2) emulsion composition on lubrication properties. *Food and Function, 8*(2), 522–532.

Pafumi, Y., Lairon, D., De La Porte, P. L., Juhel, C., Storch, J., Hamosh, M., & Armand, M. (2002). Mechanisms of inhibition of triacylglycerol hydrolysis by human gastric lipase. *Journal of Biological Chemistry, 277*(31), 28070–28079.

Pangborn, R. M. (1979). Physiological and psychological misadventures in sensory measurement or the crocodiles are coming. *Sensory Evaluation Methods for the Practicing Food Technologist*, 2–1.

Pangborn, R. M. (1984). Sensory techniques of food analysis. *Food Analysis. Principles and Techniques, 1*, 37ff.

Pangborn, R. M., Guinard, J. X., & Meiselman, H. L. (1989). Evaluation of bitterness of caffeine in hot chocolate drink by category, graphic, and ratio scaling. *Journal of Sensory Studies, 4*(1), 31–53.

Paphangkorakit, J., & Osborn, J. W. (1997). The effect of pressure on a maximum incisal bite force in man. *Archives of Oral Biology, 42*(1), 11–17.

Parlesak, A., Haller, D., Brinz, S., Baeuerlein, A., & Bode, C. (2004). Modulation of cytokine release by differentiated CACO-2 cells in a compartmentalized coculture model with mononuclear leucocytes and nonpathogenic bacteria. *Scandinavian Journal of Immunology, 60*(5), 477–485.

Patton, J. S., & Carey, M. C. (1979). Watching fat digestion. *Science, 204*(4389), 145–148.

Peng, W., Jiang, X. Y., Zhu, Y., Omari-Siaw, E., Deng, W. W., Yu, J. N., Xu, X. M., Zhang, W. M. (2015). Oral delivery of capsaicin using MPEG-PCL nanoparticles. *Acta Pharmacologica Sinica, 36*(1), 139–148.

Pera, P., Bucca, C., Borro, P., Bernocco, C., De Lillo, A., & Carossa, S. (2002). Influence of mastication on gastric emptying. *Journal of Dental Research, 81*(3), 179–181.

Peram, M. R., Loveday, S. M., Ye, A., & Singh, H. (2013). *In vitro* gastric digestion of heat-induced aggregates of β-lactoglobulin. *Journal of Dairy Science, 96*(1), 63–74.

Pérez–Juan, M., Flores, M., & Toldrá, F. (2007). Effect of ionic strength of different salts on the binding of volatile compounds to porcine soluble protein extracts in model systems. *Food Research International, 40*(6), 687–693.

Permyakov, E. A., & Berliner, L. J. (2000). α-Lactalbumin: structure and function. *FEBS Letters, 473*(3), 269–274.

Pershing, L. K., Reilly, C. A., Corlett, J. L., & Crouch, D. J. (2004). Effects of vehicle on the uptake and elimination kinetics of capsaicinoids in human skin *in vivo*. *Toxicology and Applied Pharmacology, 200*(1), 73–81.

Peyron, M. A., Lassauzay, C., & Woda, A. (2002). Effects of increased hardness on jaw movement and muscle activity during chewing of visco-elastic model foods. *Experimental Brain Research, 142*(1), 41–51.

Peyron, M. A., Mishellany, A., & Woda, A. (2004). Particle size distribution of food boluses after mastication of six natural foods. *Journal of Dental Research, 83*(7), 578–582.

Pinto, M., Robine Leon, S., & Appay, M. D. (1983). Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biology of the Cell, 47*(3), 323–330.

Plou, F. J., Ferrer, M., Nuero, O. M., Calvo, M. V., Alcalde, M., Reyes, F., & Ballesteros, A. (1998). Analysis of Tween 80 as an esterase/lipase substrate for lipolytic activity assay. *Biotechnology Techniques, 12*(3), 183–186.

Poll, L., & Flink, J. M. (1984). Aroma analysis of apple juice: influence of salt addition on headspace volatile composition as measured by gas chromatography and corresponding sensory evaluations. *Food Chemistry, 13*(3), 193–207.

Ponce de León-Rodríguez, M. D. C., Guyot, J. P., & Laurent-Babot, C. (2019). Intestinal *in vitro* cell culture models and their potential to study the effect of food components on intestinal inflammation. *Critical Reviews in Food Science and Nutrition, 59*(22), 3648–3666.

Popescu, M., Chiutu, L., Mircioiu, C., & Dima, Ş. (2013). Capsaicin microemulsions: Preparation, characterization and *in vitro* release study. *Farmacia, 62*(1), 58–68.

Poste, L. M., Mackie, D. A., Butler, G., & Larmond, E. (1991). *Laboratory methods for sensory analysis of food*. Ottawa, Canada: Canada Communication Group.

Pouzot, M., Durand, D., & Nicolai, T. (2004). Influence of the ionic strength on the structure of heat-set globular protein gels at pH 7. β-Lactoglobulin. *Macromolecules, 37*(23), 8703–8706.

Pouzot, M., Nicolai, T., Visschers, R. W., & Weijers, M. (2005). X-ray and light scattering study of the structure of large protein aggregates at neutral pH. *Food Hydrocolloids, 19*(2), 231–238.

Prakash, U. N. S., & Srinivasan, K. (2010). Beneficial influence of dietary spices on the ultrastructure and fluidity of the intestinal brush border in rats. *British Journal of Nutrition, 104*(1), 31–39.

Prescott, J., & Stevenson, R. J. (1995). Effects of oral chemical irritation on tastes and flavors in frequent and infrequent users of chili. *Physiology and Behavior, 58*(6), 1117–1127.

Prinz, J. F., & Lucas, P. W. (1995). Swallow thresholds in human mastication. *Archives of Oral Biology, 40*(5), 401–403.

Qin, B. Y., Bewley, M. C., Creamer, L. K., Baker, H. M., Baker, E. N., & Jameson, G. B. (1998). Structural basis of the tanford transition of bovine β-lactoglobulin. *Biochemistry*, *37*(40), 14014–14023.

Quesada-Pérez, M., Maroto-Centeno, J. A., Forcada, J., & Hidalgo-Alvarez, R. (2011). Gel swelling theories: The classical formalism and recent approaches. *Soft Matter*, *7*(22), 10536–10547.

Rabe, S., Krings, U., & Berger, R. G. (2003). Initial dynamic flavour release from sodium chloride solutions. *European Food Research and Technology*, *218*(1), 32–39.

Rabišková, M., & Valášková, J. (1998). The influence of HLB on the encapsulation of oils by complex coacervation. *Journal of Microencapsulation*, *15*(6), 747–751.

Reis, P., Holmberg, K., Miller, R., Krägel, J., Grigoriev, D. O., Leser, M. E., & Watzke, H. J. (2008a). Competition between lipases and monoglycerides at interfaces. *Langmuir*, *24*(14), 7400–7407.

Reis, P., Miller, R., Leser, M., Watzke, H., Fainerman, V. B., & Holmberg, K. (2008b). Adsorption of polar lipids at the water-oil interface. *Langmuir*, *24*(11), 5781–5786.

Richardson, J. D., & Vasko, M. R. (2002). Cellular mechanisms of neurogenic inflammation. *Journal of Pharmacology and Experimental Therapeutics*, *302*(3), 839–845.

Ring, S. G., & Stainsby, G. (1985). A simple method for determining the shear modulus of food dispersions and gels. *Journal of the Science of Food and Agriculture*, *36*(7), 607–613.

Robbins, W. (2000). Clinical applications of capsaicinoids. *Clinical Journal of Pain*, *16*(2 SUPPL.), S86–S89.

Romier-Crouzet, B., van de Walle, J., During, A., Joly, A., Rousseau, C., Henry, O., Larondelle, Y., & Schneider, Y. J. (2009). Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food and Chemical Toxicology, 47*(6), 1221–1230.

Rosa, P., Sala, G., van Vliet, T., & van de Velde, F. (2006). Cold gelation of whey protein emulsions. *Journal of Texture Studies, 37*(5), 516–537.

Sakurai, K., & Goto, Y. (2002). Manipulating monomer-dimer equilibrium of bovine β-lactoglobulin by amino acid substitution. *Journal of Biological Chemistry, 277*(28), 25735–25740.

Sala, G., de Wijk, R., van de Velde, F., & van Aken, G. A. (2008). Matrix properties affect the sensory perception of emulsion-filled gels. *Food Hydrocolloids, 22*(3), 353–363.

Sala, G., Van Aken, G. A., Stuart, M. A. C., & Van De Velde, F. (2007a). Effect of droplet-matrix interactions on large deformation properties of emulsion-filled gels. *Journal of Texture Studies, 38*(4), 511–535.

Sala, G., van de Velde, F., Cohen Stuart, M. A., & van Aken, G. A. (2007b). Oil droplet release from emulsion-filled gels in relation to sensory perception. *Food Hydrocolloids, 21*(5–6), 977–985.

Sala, G., van Vliet, T., Cohen Stuart, M., van de Velde, F., & van Aken, G. A. (2009). Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. *Food Hydrocolloids, 23*(7), 1853–1863.

Salvia-Trujillo, L., Qian, C., Martín-Belloso, O., & McClements, D. J. (2013). Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry, 141*(2), 1472–1480.

Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M. L., Stammati, A., & Zucco, F. (2005). The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*, *21*(1), 1–26.

Sarkar, A., Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. *Food Hydrocolloids*, *23*(5), 1270–1278.

Sarkar, A., Horne, D. S., & Singh, H. (2010). Interactions of milk protein-stabilized oil-in-water emulsions with bile salts in a simulated upper intestinal model. *Food Hydrocolloids*, *24*(2–3), 142–151.

Sawyer, L. (2013). β-Lactoglobulin. In *Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, 4th Edition* (pp. 211–259).

Sawyer, L., & Kontopidis, G. (2000). The core lipocalin, bovine β-lactoglobulin. *Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology*, *1482*(1–2), 136–148.

Schmidt, R. H. (1981). *Gelation and coagulation.* Paper presented at the ACS Symposium Series.

Schreiber, G., & Urban, J. (1978). The synthesis and secretion of albumin. Reviews of Physiology Biochemistry and Pharmacology, 82, 27–95.

Schulze, K. (2006). Imaging and modelling of digestion in the stomach and the duodenum. *Neurogastroenterology and Motility*, *18*(3), 172–183.

Schwartz, G., Enomoto, S., Valiquette, C., & Lund, J. P. (1989). Mastication in the rabbit: A description of movement and muscle activity. *Journal of Neurophysiology*, *62*(1), 273–287.

Scoville, W. L. (1912). Note on capsicums. *Journal of the American Pharmaceutical Association, 1*(5), 453–454.

Shafer, R., Levine, A., Marlette, J., & Morley, J. (1985). Do calories, osmolality, or calcium determine gastric emptying? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *248*(4), R479–R483.

Shah, R. B., Palamakula, A., & Khan, M. A. (2004). Cytotoxicity evaluation of enzyme inhibitors and absorption enhancers in Caco-2 cells for oral delivery of salmon calcitonin. *Journal of Pharmaceutical Sciences*, *93*(4), 1070–1082.

Shani Levi, C., Goldstein, N., Portmann, R., & Lesmes, U. (2017). Emulsion and protein degradation in the elderly: Qualitative insights from a study coupling a dynamic *in vitro* digestion model with proteomic analyses. *Food Hydrocolloids*, *69*, 393–401.

Shani-Levi, C., Levi-Tal, S., & Lesmes, U. (2013). Comparative performance of milk proteins and their emulsions under dynamic *in vitro* adult and infant gastric digestion. *Food Hydrocolloids*, *32*(2), 349–357.

Siegel, J. A., Urbain, J. L., Adler, L. P., Charkes, N. D., Maurer, A. H., Krevsky, B., Knight, L. C., Fisher, R. S., & Malmud, L. S. (1988). Biphasic nature of gastric emptying. *Gut*, *29*(1), 85–89.

Simone, D. A., Nolano, M., Johnson, T., Wendelschafer-Crabb, G., & Kennedy, W. R. (1998). Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: Correlation with sensory function. *Journal of Neuroscience*, *18*(21), 8947–8959.

Singh, H., & Ye, A. (2013). Structural and biochemical factors affecting the digestion of protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, *18*(4), 360–370.

Sliwinski, E. L., Roubos, P. J., Zoet, F. D., Van Boekel, M. A. J. S., & Wouters, J. T. M. (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. *Colloids and Surfaces B: Biointerfaces*, *31*(1-4), 231–242.

Small, D. M. (1991) The effects of glyceride structure on absorption and metabolism. In*: Vol. 11. Annual Review of Nutrition* (pp. 413–434).

Smith, J., Wood, E., & Dornish, M. (2004). Effect of chitosan on epithelial cell tight junctions. *Pharmaceutical Research*, *21*(1), 43–49.

Snow, P., & O'Dea, K. (1981). Factors affecting the rate of hydrolysis of starch in food. *American Journal of Clinical Nutrition*, *34*(12), 2721–2727.

Sok Line, V. L., Remondetto, G. E., & Subirade, M. (2005). Cold gelation of β-lactoglobulin oil-in-water emulsions. *Food Hydrocolloids*, *19*(2), 269–278.

Spence, C. (2012). Multi-sensory integration and the psychophysics of flavour perception. In *Food Oral Processing: Fundamentals of Eating and Sensory Perception* (pp. 203–223).

Srinivasan, K. (2016). Biological activities of red pepper (Capsicum annuum) and its pungent principle capsaicin: A review. *Critical Reviews in Food Science and Nutrition*, *56*(9), 1488–1500.

Stevens, S. S. (1957). On the psychophysical law. *Psychological Review*, *64*(3), 153–181.

Stieger, M., & van de Velde, F. (2013). Microstructure, texture and oral processing: New ways to reduce sugar and salt in foods. *Current Opinion in Colloid and Interface Science*, *18*(4), 334–348.

Stone, H. (2012). *Sensory Evaluation Practices*.

Stull, A. J., Apolzan, J. W., Thalacker-Mercer, A. E., Iglay, H. B., & Campbell, W. W. (2008). Liquid and solid meal replacement products differentially affect

postprandial appetite and food intake in older adults. *Journal of the American Dietetic Association, 108*(7), 1226–1230.

Suresh, D., & Srinivasan, K. (2010). Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian Journal of Medical Research, 131*(5), 682–691.

Swaisgood, H. E. (1982). Chemistry of milk protein. *In 'Developments in dairy chemistry. I. Proteins' G [see FSTA (1983) 15 G8P1156]*. 1–59.

Synge, R. L. (1945). The kinetics of low temperature acid hydrolysis of gramicidin and of some related dipeptides. *The Biochemical journal, 39*(4), 351–355.

Szallasi, A., & Blumberg, P. M. (1999). Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacological Reviews, 51*(2), 159–211.

Szczesniak, A. S. (1963). Classification of textural characteristics. *Journal of Food Science, 28*(4), 385–389.

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*(4), 215–225.

Szolcsányi, J., & Barthó, L. (2001). Capsaicin-sensitive afferents and their role in gastroprotection: An update. *Journal of Physiology Paris, 95*(1–6), 181–188.

Tadros, T. F. (1980). Physical stability of suspension concentrates. *Advances in Colloid and Interface Science, 12*(2-3), 141–261.

Tadros, T. F. (2013). *Emulsion Formation and Stability*.

Tanaka, H., & Sigehuzi, T. (1994). Surface-pattern evolution in a swelling gel under a geometrical constraint: Direct observation of fold structure and its coarsening dynamics. *Physical Review E, 49*(1), R39–R42.

Tanaka, H., Tomita, H., Takasu, A., Hayashi, T., & Nishi, T. (1992). Morphological and kinetic evolution of surface patterns in gels during the swelling process: Evidence of dynamic pattern ordering. *Physical Review Letters, 68*(18), 2794–2797.

Tanaka, T., Sun, S. T., Hirokawa, Y., Katayama, S., Kucera, J., Hirose, Y., & Amiya, T. (1987). Mechanical instability of gels at the phase transition. *Nature, 325*(6107), 796–798.

Tang, J., Wolf, S., Caputto, R., & Trucco, R. E. (1959). Isolation and crystallization of gastricsin from human gastric juice. *The Journal of biological chemistry, 234*(5), 1174–1178.

Tekin, E., Sahin, S., & Sumnu, G. (2017). Physicochemical, rheological, and sensory properties of low-fat ice cream designed by double emulsions. *European Journal of Lipid Science and Technology, 119*(9).

Thiele, R., Mueller-Seitz, E., & Petz, M. (2008). Chili pepper fruits: Presumed precursors of fatty acids characteristic for capsaicinoids. *Journal of Agricultural and Food Chemistry, 56*(11), 4219–4224.

Thoennissen, N. H., O'Kelly, J., Lu, D., Iwanski, G. B., La, D. T., Abbassi, S., Leiter, A., Karlan, B., Mehta, R., & Koeffler, H. P. (2010). Capsaicin causes cell-cycle arrest and apoptosis in ER-positive and-negative breast cancer cells by modulating the EGFR/HER-2 pathway. *Oncogene, 29*(2), 285–296.

Todd, P. H., Bensinger, M. G., & Biftu, T. (1977). Determination of pungency due to capsicum by gas-liquid chromatography. *Journal of Food Science, 42*(3), 660–665.

Tolstoguzov, V. B., & Braudo, E. E. (1983). Fabricated foodstuffs as multicomponent gels. *Journal of Texture Studies, 14*(3), 183–212.

Tortora, G. J., & Derrickson, B. H. (2018). *Principles of anatomy and physiology*: John Wiley & Sons.

Tsukura, Y., Mori, M., Hirotani, Y., Ikeda, K., Amano, F., Kato, R., Ijiri, Y., Tanaka, K. (2007). Effects of capsaicin on cellular damage and monolayer permeability in human intestinal Caco-2 cells. *Biological and Pharmaceutical Bulletin, 30*(10), 1982–1986.

Turgut, C., Newby, B. M., & Cutright, T. J. (2004). Determination of optimal water solubility of capsaicin for its usage as a non-toxic antifoulant. *Environmental Science and Pollution Research, 11*(1), 7–10.

Tydeman, E. A., Parker, M. L., Faulks, R. M., Cross, K. L., Fillery-Travis, A., Gidley, M. J., Rich, G. T., & Waldron, K. W. (2010a). Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibility in the upper gastrointestinal tract. 2. *In vivo* digestions. *Journal of Agricultural and Food Chemistry, 58*(17), 9855–9860.

Tydeman, E. A., Parker, M. L., Wickham, M. S. J., Rich, G. T., Faulks, R. M., Gidley, M. J., Fillery-Travis, A., & Waldron, K. W. (2010b). Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibilty in the upper gastrointestinal tract. 1. *In vitro* simulations of carrot digestion. *Journal of Agricultural and Food Chemistry, 58*(17), 9847–9854.

Ubbink, J., Burbidge, A., & Mezzenga, R. (2008). Food structure and functionality: A soft matter perspective. *Soft Matter, 4*(8), 1569-1581.

Uhrínová, S., Smith, M. H., Jameson, G. B., Uhrín, D., Sawyer, L., & Barlow, P. N. (2000). Structural changes accompanying pH-induced dissociation of the β-lactoglobulin dimer. *Biochemistry, 39*(13), 3565–3574.

Ulleberg, E. K., Comi, I., Holm, H., Herud, E. B., Jacobsen, M., & Vegarud, G. E. (2011). Human gastrointestinal juices intended for use in *in vitro* digestion models. *Food Digestion, 2*(1–3), 52–61.

Urbain, J. L. C., Siegel, J. A., Charkes, N. D., Maurer, A. H., Malmud, L. S., & Fisher, R. S. (1989). The two-component stomach: Effects of meal particle size on fundal and antral emptying. *European Journal of Nuclear Medicine, 15*(5), 254–259.

Vachon, P. H., & Beaulieu, J. F. (1992). Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. *Gastroenterology, 103*(2), 414–423.

van Aken, G. A., Bomhof, E., Zoet, F. D., Verbeek, M., & Oosterveld, A. (2011). Differences in *in vitro* gastric behaviour between homogenized milk and emulsions stabilised by Tween 80, whey protein, or whey protein and caseinate. *Food Hydrocolloids, 25*(4), 781–788.

van Aken, G. A., Vingerhoeds, M. H., & de Hoog, E. H. A. (2007). Food colloids under oral conditions. *Current Opinion in Colloid and Interface Science, 12*(4-5), 251–262.

van der Bilt, A. (2012). Oral management of food. In *Food Oral Processing: Fundamentals of Eating and Sensory Perception* (pp. 63–93).

van der Bilt, A., Engelen, L., Abbink, J., & Pereira, L. J. (2007). Effects of adding fluids to solid foods on muscle activity and number of chewing cycles. *European Journal of Oral Sciences, 115*(3), 198–205.

van Eck, A., Fogliano, V., Galindo-Cuspinera, V., Scholten, E., & Stieger, M. (2019a). Adding condiments to foods: how does static and dynamic sensory perception change when bread and carrots are consumed with mayonnaise? *Food Quality and Preference, 73*, 154–170.

van Eck, A., Hardeman, N., Karatza, N., Fogliano, V., Scholten, E., & Stieger, M. (2019b). Oral processing behavior and dynamic sensory perception of composite foods: toppings assist saliva in bolus formation. *Food Quality and Preference, 71*, 497–509.

van Nieuw Amerongen, A., Bolscher, J. G. M., & Veerman, E. C. I. (2004). Salivary proteins: Protective and diagnostic value in cariology? *Caries Research, 38*(3), 247–253.

van Vliet, T. (1988). Rheological properties of filled gels. Influence of filler matrix interaction. *Colloid & Polymer Science, 266*(6), 518–524.

van Vliet, T., van Aken, G. A., de Jongh, H. H. J., & Hamer, R. J. (2009). Colloidal aspects of texture perception. *Advances in Colloid and Interface Science, 150*(1), 27–40.

van Vliet, T., & Walstra, P. (1995). Large deformation and fracture behaviour of gels. *Faraday Discussions, 101*, 359–370.

Vardhanabhuti, B., Foegeding, E. A., McGuffey, M. K., Daubert, C. R., & Swaisgood, H. E. (2001). Gelation properties of dispersions containing polymerized and native whey protein isolate. *Food Hydrocolloids, 15*(2), 165–175.

Vardhanabhuti, B., Khayankan, W., & Foegeding, E. A. (2010). Formation of elastic whey protein gels at low pH by acid equilibration. *Journal of Food Science, 75*(5), E305–E313.

Velchik, M. G., Reynolds, J. C., & Alavi, A. (1989). The effect of meal energy content on gastric emptying. *Journal of Nuclear Medicine, 30*(6), 1106–1110.

Verde, A. V., & Frenkel, D. (2010). Simulation study of micelle formation by bile salts. *Soft Matter, 6*(16), 3815–3825.

Verheul, M., & Roefs, S. P. F. M. (1998). Structure of particulate whey protein gels: Effect of NaCl concentration, pH, heating temperature, and protein composition. *Journal of Agricultural and Food Chemistry, 46*(12), 4909–4916.

Vij, R., Reddi, S., Kapila, S., & Kapila, R. (2016). Transepithelial transport of milk derived bioactive peptide VLPVPQK. *Food Chemistry, 190*, 681–688.

Walstra, P. (2002). *Physical chemistry of foods*: CRC Press.

Wan, L. S. C., & Lee, P. F. S. (1974). CMC of polysorbates. *Journal of Pharmaceutical Sciences, 63*(1), 136–137.

Wang, H. M., Chuang, S. M., Su, Y. C., Li, Y. H., & Chueh, P. J. (2011). Down-regulation of tumor-associated NADH oxidase, tNOX (ENOX2), enhances capsaicin-induced inhibition of gastric cancer cell growth. *Cell Biochemistry and Biophysics, 61*(2), 355–366.

Wang, H. M., Chueh, P. J., Chang, S. P., Yang, C. L., & Shao, K. N. (2009). Effect of Ccapsaicin on tNOX (ENOX2) protein expression in stomach cancer cells. *BioFactors, 34*(3), 209–217.

Wang, L., Hu, C. P., Deng, P. Y., Shen, S. S., Zhu, H. Q., Ding, J. S., Tan, G. S., & Li, Y. J. (2005). The protective effects of rutaecarpine on gastric mucosa injury in rats. *Planta Medica, 71*(5), 416–419.

Wang, X., Lin, Q., Ye, A., Han, J., & Singh, H. (2019). Flocculation of oil-in-water emulsions stabilised by milk protein ingredients under gastric conditions: Impact on *in vitro* intestinal lipid digestion. *Food Hydrocolloids, 88*, 272–282.

Wang, Y. J., Truong, V. D., & Wang, L. (2003). Structures and rheological properties of corn starch as affected by acid hydrolysis. *Carbohydrate Polymers, 52*(3), 327–333.

Ward, S. M., Bayguinov, J., Won, K.-J., Grundy, D., & Berthoud, H. R. (2003). Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. *The Journal of Comparative Neurology*, *465*(1), 121–135.

Watson, C. J., Rowland, M., & Warhurst, G. (2001). Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers. *American Journal of Physiology - Cell Physiology*, *281*(2 50–2), C388–C397.

Weiss, E. A. (2002). *Spice crops*.

Weurding, R. E., Veldman, A., Veen, W. A. G., van der Aar, P. J., & Verstegen, M. W. A. (2001). *In vitro* starch digestion correlates well with rate and extent of starch digestion in broiler chickens. *Journal of Nutrition*, *131*(9), 2336–2342.

Whitney, R. (1977). Milk proteins. In *Food colloids* (pp. 66–151): Avi Publ. Co, Westport, CT.

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Wikimedia Commons (2020c). Stomach. In Wikimedia Commons, the free media repository. OpenStax College, CC BY 3.0 <https://creativecommons.org/licenses/by/3.0>.

Wilde, P. J., & Chu, B. S. (2011). Interfacial & colloidal aspects of lipid digestion. *Advances in Colloid and Interface Science*, *165*(1), 14–22.

Wilde, P. J., & Clark, D. C. (1993). The competitive displacement of β-lactoglobulin by Tween 20 from oil-water and air-water interfaces. *Journal of Colloid and Interface Science, 155*(1), 48–54.

Wilson, C. E., & Brown, W. E. (1997). Influence of food matrix structure and oral breakdown during mastication on temporal perception of flavor. *Journal of Sensory Studies, 12*(1), 69–86.

Woda, A., Mishellany, A., & Peyron, M. A. (2006). The regulation of masticatory function and food bolus formation. *Journal of Oral Rehabilitation, 33*(11), 840–849.

Wolff, M., & Kleinberg, I. (1998). Oral mucosal wetness in hypo- and normosalivators. *Archives of Oral Biology, 43*(6), 455–462.

Wood, R. (2005). Bioavailability: definition, general aspects and fortificants. *Encyclopedia of Human Nutrition, 2nd ed. Oxford: Elsevier Ltd.*

Xu, Q., Hong, H., Wu, J., & Yan, X. (2019). Bioavailability of bioactive peptides derived from food proteins across the intestinal epithelial membrane: A review. *Trends in Food Science and Technology, 86,* 399–411.

Xu, W., & Bronlund, J. E. (2010) Mastication robots: Biological inspiration to implementation. In*: Vol. 290. Studies in Computational Intelligence* (pp. V–VIII).

Yamamoto, Y., & Dickinson, E. (2004). Rheology of protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. In *Food Colloids* (pp. 326–334): Woodhead Publishing.

Yang, Z. H., Wang, X. H., Wang, H. P., Hu, L. Q., Zheng, X. M., & Li, S. W. (2010). Capsaicin mediates cell death in bladder cancer T24 cells through reactive oxygen species production and mitochondrial depolarization. *Urology, 75*(3), 735–741.

Ye, A., Cui, J., Dalgleish, D., & Singh, H. (2016). Formation of a structured clot during the gastric digestion of milk: Impact on the rate of protein hydrolysis. *Food Hydrocolloids, 52,* 478–486.

Ye, A., & Taylor, S. (2009). Characterization of cold-set gels produced from heated emulsions stabilized by whey protein. *International dairy journal, 19,* 721–727.

Yiangou, Y., Facer, P., Dyer, N. H. C., Chan, C. L. H., Knowles, C., Williams, N. S., & Anand, P. (2001). Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet, 357*(9265), 1338–1339.

Yildirim, M., Sumnu, G., & Sahin, S. (2016). Rheology, particle-size distribution, and stability of low-fat mayonnaise produced via double emulsions. *Food Science and Biotechnology, 25*(6), 1613–1618.

Yoshitani, S. I., Tanaka, T., Kohno, H., & Takashima, S. (2001). Chemoprevention of azoxymethane-induced rat colon carcinogenesis by dietary capsaicin and rotenone. *International Journal of Oncology, 19*(5), 929–939.

Yost, R. A., & Kinsella, J. E. (1992). Microstructure of whey protein isolate gels containing emulsified butterfat droplets. *Journal of Food Science, 57*(4), 892–897.

Yost, R. A., & Kinsella, J. E. (1993). Properties of acidic whey protein gels containing emulsified butterfat. *Journal of Food Science, 58*(1), 158–163.

Yu, H., & Huang, Q. (2011). Investigation of the absorption mechanism of solubilized curcumin using caco-2 cell monolayers. *Journal of Agricultural and Food Chemistry, 59*(17), 9120–9126.

Zaman, A. A. (1998). *Techniques in rheological measurements: fundamentals and applications*: University of Florida, Gainesville, FL: NSF Engineering Resource Center for Particle Science & Technology.

Zhang, S., & Vardhanabhuti, B. (2014). Effect of initial protein concentration and pH on *in vitro* gastric digestion of heated whey proteins. *Food Chemistry, 145*, 473–480.

Zhang, Y., Huang, Z., Omari-Siaw, E., Lu, S., Zhu, Y., Jiang, D., Wang, M., Yu, J., Xu, X., Zhang, W. (2016). Preparation and *in vitro–in vivo* evaluation of sustained-release matrix pellets of capsaicin to enhance the oral bioavailability. *AAPS PharmSciTech, 17*(2), 339-349.

Zhong, H., Marcus, S. L., & Li, L. (2005). Microwave-assisted acid hydrolysis of proteins combined with liquid chromatography MALDI MS/MS for protein identification. *Journal of the American Society for Mass Spectrometry, 16*(4), 471–481.

Zhu, Y., Hsu, W. H., & Hollis, J. H. (2013). The impact of food viscosity on eating rate, subjective appetite, glycemic response and gastric emptying rate. *PLoS ONE, 8*(6):e67482.

Zhu, Y., Peng, W., Zhang, J., Wang, M., Firempong, C. K., Feng, C., Liu, H., Xu, X., Yu, J. (2014). Enhanced oral bioavailability of capsaicin in mixed polymeric micelles: Preparation, *in vitro* and *in vivo* evaluation. *Journal of Functional Foods, 8*(1), 358–366.

Zhu, Y., Wang, M., Zhang, J., Peng, W., Firempong, C. K., Deng, W., Wang, Q., Wang, S., Shi, F., Yu, J., Xu, X., & Zhang, W. (2015a). Improved oral bioavailability of capsaicin via liposomal nanoformulation: Preparation, *in vitro* drug release and pharmacokinetics in rats. *Archives of Pharmacal Research, 38*(4), 512–521.

Zhu, Y., Zhang, J., Zheng, Q., Wang, M., Deng, W., Li, Q., Firempong, C. K., Wang, S., Tong, S., Xu, X., & Yu, J. (2015b). *In vitro* and *in vivo* evaluation of capsaicin-loaded microemulsion for enhanced oral bioavailability. *Journal of the Science of Food and Agriculture, 95*(13), 2678–2685.

Appendices

Appendix A: Screening questionnaire for frequency and fondness of spicy foods consumption

(Spicy foods include Indian, Chinese, Thai, Mexican and any other foods that contain chili peppers and cause a burning or tingling sensation)

1. What is your frequency of ingestion of spicy foods: _____?
   1 = once a year or less; 2 = less than once a month; 3 = 1-3 times per month; 4 = once a week; 5 = 3-4 times per week; 6 = every day; 7 = more than once a day.

2. How much do you like the taste of chili pepper in your food: _____?

1	2	3	4	5	6	7	8	9
Dislike extremely								Like extremely

3. How much do you like the burn of chili pepper in your food: _____?

1	2	3	4	5	6	7	8	9
Dislike extremely								Like extremely

4. I think chili pepper makes food taste better. True / False

5. Without hot spices, I find that food tastes too bland. True / False

6. I find it hard to appreciate the flavours of food when the food contains hot spices.
   True / False

Appendix B: Dental health screening questionnaire

I am / am not a regular smoker.

I have / have not significant tooth crowning.

I have / have not obvious tooth decay.

I have / have not pain or clicking while mastication.

I have / have not other known oral or general health problems that could influence chewing.

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