Current Research in Food Science 6 (2023) 100473

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

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

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ARTICLE INFO

Handling Editor: Dr. Quancai Sun

Keywords:
Emulsion gel
Capsaicinoid
Whey protein
Tween 80
Digestion
Bioaccessibility

ABSTRACT

In this study, the effect of emulsifier type, i.e. whey protein versus Tween 80, on the digestion behaviour of emulsion gels containing capsaicinoids (CAPs) was examined. The results indicate that the CAP-loaded Tween 80 emulsion gel was emptied out significantly faster during gastric digestion than the CAP-loaded whey protein emulsion gel. The Tween-80-coated oil droplets appeared to be in a flocculated state in the emulsion gel, had no interactions with the protein matrix and were easily released from the protein matrix during gastric digestion. The whey-protein-coated oil droplets showed strong interactions with the protein matrix, and the presence of thick protein layer around the oil droplets protected their liberation during gastric digestion. During intestinal digestion, the CAP-loaded Tween 80 emulsion gel had a lower extent of lipolysis than the CAP-loaded whey protein emulsion gel, probably because the interfacial layer formed by Tween 80 was resistance to displacement by bile salts, and/or because Tween 80 formed interfacial complexes with bile salts/lipolytic enzymes. Because of the softer structure of the CAP-loaded Tween 80 emulsion gel, the gel particles were broken down much faster and the oil droplets were liberated from the protein matrix more readily than for the CAP-loaded whey protein emulsion gel during intestinal digestion; this promoted the release of CAP molecules from the gel. In addition, the Tween 80 molecules displaced from the interface would participate in the formation of mixed micelles and would help to solubilize the released CAP molecules, leading to improved bioaccessibility of CAP. Information obtained from this study could be useful in designing functional foods for the delivery of lipophilic bioactive compounds.

1. Introduction

Liquid emulsion-based systems have been widely studied for their use in delivering lipophilic bioactive compounds; the bioactive compounds are dissolved in the emulsion droplets to improve their stability (Ahmed et al., 2012; Humberstone and Charman, 1997; Lin et al., 2018; Lu et al., 2016; Salvia-Trujillo et al., 2013). However, less information on the use of emulsion gels as the delivery system is available. The solid/semi-solid emulsion gel possesses very different digestion behaviour from the liquid emulsion systems, because it requires mastication in the mouth and further disintegration in the subsequent gastrointestinal 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 systems and 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 the rheological properties of the emulsion gel. For instance, the effect of using whey protein versus Tween 20 as the emulsifier on the structure and rheological properties of heat-set whey protein emulsion gels has been investigated by Chen et al. (2000). The whey-protein-coated oil droplets interact strongly with the surrounding whey protein matrix, and thus contribute to the gel strength; the Tween-20-coated oil droplets are not bound to the protein matrix (weak or no interaction), and thus act as defects in the network and lead to a monotonic decrease in the gel strength. Emulsion gels formed using protein or a non-ionic small-molecule surfactant as the emulsifier possess distinct gel structure and rheological properties; however, their breakdown and digestion behaviour in the mouth and the subsequent gastrointestinal tract have not been studied.

The effects of using different types of emulsifier on the *in vitro* digestion behaviour of liquid oil-in-water emulsions have been widely studied in recent decades. van Aken et al. (2011) studied the use of

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https://doi.org/10.1016/j.crfs.2023.100473
Received 27 November 2022; Received in revised form 31 January 2023; Accepted 27 February 2023
Available online 2 March 2023

different emulsifiers on the *in vitro* gastric digestion behaviour of emulsions and reported that a whey-protein-stablized emulsion with additional Tween 80 was more resistant against flocculation, but had more oil droplet coalesence during gastric digestion, than an emulsion stabilized with whey protein alone. Li and McClements (2010) investigated the effect of additional non-ionic, anionic and cationic low-molecular-weight surfactants on the rate and the extent of lipolysis of an emulsion stabilized with β-lactoglobulin under simulated gastrointestinal conditions. They found that there was a lag phase during intestinal digestion with the presence of surfactants; when the surfactants were added at a sufficient concentration, the extent of lipolysis was significantly reduced. The effect of surfactant type on the bioaccessiblity of β-carotene after the *in vitro* digestion of an emulsion was studied by Mun et al. (2015). They reported that the extent of lipolysis was similar between emulsions stabilized with whey protein or Tween 20; however, the emulsion stabilized with Tween 20 had significantly higher bioaccessibility of β-carotene.

Capsaicinoids are the pungent lipophilic bioactive compounds in various peppers from the genus *Capsicum*. They have several biological activities such as anti-carcinogenic, anti-inflammatory, analgesic and beneficial influences on gastrointestinal systems (Srinivasan, 2016). However, their irritation effect on skin and mucosa through the digestion pathways and their low water solubility have limited their applications in functional foods. 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 and increased bioaccessibility (Lu et al., 2016; Popescu et al., 2013; Zhu et al., 2015a; Zhu et al., 2015b).

The effects of gel structure, i.e. soft gel versus hard gel and using whey protein versus Tween 80 as the emulsifying agent, on the *in vivo* oral processing behaviour and sensory perception of emulsion gels containing capsaicinoids (CAPs) have been investigated (Luo et al., 2019, 2020). It was found that the 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 the current work, we aimed to investigate the simulated gastrointestinal digestion behaviour of emulsion gels using either whey protein or Tween 80 as the emulsifier and the impact on the bioaccessibility of CAP. *In vitro* dynamic gastric digestion was carried out for 240 min using a human gastric simulator (HGS), and *in vitro* intestinal digestion was carried out for 120 min using a pH-stat. The hypothesis was that, because of its weak gel structure, the CAP-loaded Tween 80 emulsion gel would break down much faster and empty out much faster during gastric digestion.

2. Materials and methods

2.1. Materials

Powdered CAP (61% capsaicin, 32% dihydrocapsaicin and 2.5% other CAPs) was purchased from Wuxi AccoBio Biotech Inc., Wuxi, Jiangsu, China. Whey protein isolate 895 (WPI), instantized and with 93% protein content, was purchased from Fonterra Co-operative Group Limited, Auckland, New Zealand. Food-grade polysorbate 80 (or Tween 80) was purchased from Hawkins Watts Ltd, Auckland, New Zealand. Soybean oil was purchased from Davis Trading Company, Palmerston North, New Zealand, and was used without further purification. Milli-Q water (Millipore Corp., Bedford, MA, USA) was used for all experiments. 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^{-1}), bile bovine (#B3883) and Pefabloc® SC (#76307) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemical reagents used in this study were of analytical grade and were used without further modification, unless otherwise specified.

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 the simulated digestion fluids are referred to as stock simulated digestion fluids. The stock simulated digestion fluids were magnetically stirred at room temperature for 2 h to allow complete dissolution. The pH values of the stock SSF (1.25 ×), stock SGF (1.25 ×) and stock SIF (1.25 ×) were adjusted to 7.0, 1.5 and 7.0 respectively using 6 M HCl/10 M NaOH. They were stored at 4 °C, warmed to room temperature in a water bath before use and used within 1 month after preparation.

2.2. Methods

2.2.1. 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. A CAP-loaded whey protein coarse emulsion containing 0.02 wt% CAP, 19.98 wt% soybean oil and 10 wt% WPI was prepared using a high shear mixer (L4RT, Silverson, East Longmeadow, MA, USA) at 8000 rev min^{-1} for 5 min. The coarse emulsion was then homogenized by passing it four times through a two-stage valve homogenizer (Homolab 2, FBF ITALIA SRL, Sala Baganza, Parma, Italy) at first-stage/second-stage pressures of 250/50 bar to generate an average oil droplet size ($d_{4,3}$) of ~0.5 ± 0.05 μm.

2.2.2. Preparation of CAP-loaded tween 80 emulsions

Powdered CAP was added to soybean oil; Tween 80 and WPI were added to water. The solutions were magnetically stirred for 4 h at room temperature to allow complete dissolution. A CAP-loaded Tween 80 stock coarse emulsion containing 0.03 wt% CAP, 29.97 wt% soybean oil and 3 wt% Tween 80 was prepared using a high shear mixer (L4RT) at 8000 rev min^{-1} for 5 min. A CAP-loaded Tween 80 stock emulsion with an average oil droplet size ($d_{4,3}$) of ~0.5 ± 0.05 μm was generated under the same conditions as described in Section 2.2.1. The CAP-loaded Tween 80 stock emulsion was then mixed with stock WPI solution (30 wt% WPI) at a mass ratio of 2:1 to achieve a CAP-loaded Tween 80 emulsion 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 (Chen and Dickinson, 1995; Gomes et al., 2018) and that the whey proteins were present as a gelling agent only.

2.2.3. Formation of CAP-loaded emulsion gels

The required quantities of NaCl were added to the CAP-loaded emulsions to give a final concentration of 100 mM NaCl. They 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.

2.2.4. Preparation of simulated masticated gel bolus

A food processer (The Mini Wizz food chopper, BFP100, Breville Group Ltd, Australia) was used to mimic oral breakdown of the CAP-loaded emulsion gels and to produce *in vitro* masticated gel boluses that had similar bolus particle sizes to the *in vivo* masticated gel boluses (Luo et al., 2020). The CAP-loaded emulsion gels were cut into cylinders of 12 mm in height and 25 mm in diameter; then seven cylindrical gel samples were added into the processer. The CAP-loaded whey protein emulsion gel was initially ground for 3 s, and then a portion of ~10 g was taken out; the rest was ground for another 5 s, and then ~10 g was taken out; the remaining gel particles were ground for another 9 s; all three portions were then mixed together. The CAP-loaded Tween 80 emulsion gel was initially ground for 2 s, and then a portion of ~15 g was taken out; the rest was ground for another 2 s, and then ~15 g was

taken out; the remaining gel particles were ground for another 3 s; all three portions were then mixed together. Experiments were carried out at room temperature. Simulated masticated gel boluses were prepared by mixing 160 g of ground gel with 50 mL of SSF [consisting of 40 mL of stock SSF, 0.5 mL of $CaCl_2$ (0.3 M) and 9.5 mL of water].

2.2.5. Human gastric simulator

A dynamic gastric digestion model—the HGS designed by Kong and Singh (2010)—was used for the *in vitro* gastric digestion. A mesh bag with a pore size of 1 mm was placed inside the latex stomach chamber to mimic human gastric sieving. An enzyme solution containing 1.7% (w/v) pepsin, 0.275% (w/v) amano lipase A and 0.54 mmol L^{-1} of $CaCl_2$ was prepared to obtain pepsin and lipase activities of 1000 U mL^{-1} and 50 U mL^{-1} respectively and a calcium ion concentration of 0.075 mmol L^{-1} in the final gastric digestion mixture. The simulated masticated gel bolus (consisting of 160 g of ground gel and 50 mL of SSF) was warmed at 37 °C in a water bath for 2 min and then added into the latex stomach chamber. A 70 mL aliquot of SGF (consisting of 56 mL of stock SGF and 14 mL of enzyme solution) was also added into the stomach chamber to mimic the condition during the fasting state when the stomach contains a certain amount of gastric juice (Camilleri, 2006). The gastric digestion time was 240 min. The temperature of the HGS was set and maintained at 37 °C by a heater and a thermostat. The gastric secretion rate was set at 2.5 mL min^{-1} (Hoebler et al., 2002). The stock SGF (1.25 ×) and the enzyme solution were added in separately by two pumps; the secretion rates were 2 mL min^{-1} for the stock SGF and 0.5 mL min^{-1} for the enzyme solution. Gastric emptying started after 30 min because of the lag phase of solid foods (Siegel et al., 1988; Urbain et al., 1989). Every 15 min, 45 mL of gastric digesta was emptied from the bottom, corresponding to an emptying rate of 3 mL min^{-1} (Kong and Singh, 2010).

2.2.6. pH measurement

The initial pH was defined as the pH of the simulated masticated gel bolus after mixing with the fasting state SGF. As access into the HGS was prevented by the simulated gastric contractions, the pH in the HGS at different digestion times was represented by the pH of the emptied gastric digesta.

2.2.7. Measurement of solids content of emptied gastric digesta

The emptied gastric digesta collected at 15-min intervals was dried in an oven at 105 °C for 24 h to determine the dry matter content (A). In addition, a control experiment using 160 g of water instead of ground gel was carried out to determine the dry matter content of the simulated digestion fluids (i.e. SSF and SGF) retained in the emptied gastric digesta at different time points (B). The actual dry weight of the gel particles in the digesta emptied at different digestion times was determined by subtracting B from A.

2.2.8. Gastric emptying

The gel retention fraction at time t, denoted as y(t), was calculated as:

$$y(t) = \frac{W_t - W_0}{W_0} \quad (1)$$

where W_0 was the total dry weight of gel entering the HGS; W_t was the cumulative dry weight of emptied gastric digesta at time t.

The gel retention data was fitted to a modified power exponential function established by Siegel et al. (1988) for describing the gastric emptying behaviour of solid foods:

$$y(t) = 1 - \left(1 - e^{-kt}\right)^{\beta} \quad (2)$$

where $y(t)$ is the fractional gel retention at time t, k is the gastric emptying rate (min^{-1}) and β is the extrapolated y-intercept from the terminal portion of the curve.

2.2.9. Determination of particle size distribution of emptied gastric digesta

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 emptied gastric digesta. The refractive index for the gel particles was set at 1.47. The samples were measured immediately after collection. All measurements were conducted at room temperature with three replicates. The weight-to-volume diameter $d_{4,3}$ (μm) was used to denote the average particle size.

2.2.10. Determination of oil droplet size distribution

A MasterSizer 2000 was used to measure the average diameters and the particle size distributions of the oil droplets in the emptied gastric digesta. 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. Aliquots of 3 mL of 5 wt% sodium dodecyl sulphate (SDS) solution and 20 μL of β-mercaptoethanol were added to 2 mL of emptied gastric digesta. 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 with three replicates.

2.2.11. Protein hydrolysis

SDS 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 being emptied from the HGS, a 20 μL digesta sample was taken and mixed with 180 μL of 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 a boiling water bath for 10 min. After cooling to room temperature, the samples were centrifuged at 4200 *g* for 20 min and then 10 μL of the subnatant from each sample was loaded on to tricine gels previously prepared on a Mini PROTEAN 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. 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.

2.2.12. Confocal laser scanning microscopy (CLSM)

A confocal laser scanning microscope (Leica, Heidelberg, Germany) was used to observe the 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). A 200 μL aliquot of the emptied gastric digesta was put into a 1.5 mL Eppendorf tube and stained with 20 μL of Nile Red and 20 μL of Fast Green immediately after sample collection. The stained digesta samples were placed in an ice bath before CLSM. All stained samples were placed on concave microscope slides and covered with cover slips for CLSM.

2.2.13. In vitro intestinal digestion

A pH-stat (TitraLab 856; Radiometer Analytical, Villeurbanne, France) was used for the *in vitro* intestinal digestion and to measure the release of free fatty acids (FFAs) during the *in vitro* intestinal digestion. The gastric digesta emptied at 60, 120 and 240 min were used for the *in vitro* intestinal digestion. The pH of the gastric digesta was adjusted to 7.0 using 6 M HCl/10 M NaOH immediately after emptying from the HGS. Then, 23 mL of the gastric digesta was mixed with 16.4 mL of stock SIF (1.25 ×) and added into the temperature-controlled chamber of the

pH-stat. A 46 mg aliquot of pancreatin from porcine pancreas (8 × USP) was dissolved in 2 mL of stock SIF and then 46 μL of $CaCl_2$ (0.3 M) was added. A total of 0.18795 g of bile bovine was dissolved in 4.6 mL of Milli-Q water. The pancreatin solution and the bile bovine solution were added into the chamber at the same time and the intestinal digestion was started. The initial volume of the digestion mixture was 46 mL. The concentrations of bile bovine and $CaCl_2$ in the initial digestion mixture were 10 mM and 0.3 mM respectively. The concentration of pancreatin in the digestion mixture was 1 mg mL^{-1}, based on a trypsin activity of 100 U mL^{-1}. The intestinal digestion was carried out at 37.0 °C for 120 min with a constant stirring rate of 100 rev min^{-1}. The titration was performed with 0.05 M NaOH and the endpoint pH was set at 7.0. The quantity of FFAs released per millilitre of digestion mixture (Q, μmol mL^{-1}) was calculated as follows:

$$Q = \frac{Volume_{NaOH} \times C_{NaOH}}{Volume_{reaction\ mixture}} \quad (3)$$

where C_{NaOH} is 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 FFAs released per millilitre of reaction mixture per minute during the initial 2 min of reaction.

The fat content of the gastric digesta was measured by the Mojonnier method [Association of Official Analytical Chemists (AOAC) method 989.05].

2.2.14. Determination of bioaccessibility of CAP after in vitro intestinal digestion

The bioaccessibility of CAP was defined as the fraction of CAP that was released from the emulsion gel in the gastrointestinal tract after digestion and became available for intestinal absorption (Heaney, 2001). At the end of the intestinal digestion, part of the intestinal digesta was collected and mixed with 1 mM Pefabloc solution at a volume ratio of 8:1 to terminate 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 a 0.22 μm filter. After filtration, the filtrate was considered to be the bioaccessible fraction. The concentration of CAP in the bioaccessible fraction as well as the concentration in the intestinal digestion mixture were determined by high-performance liquid chromatography (HPLC). The bioaccessibility (%) of CAP was calculated as follows:

$$Bioaccessibility\ (\%) = \frac{CAP\ in\ bioaccessible\ fraction}{Total\ CAP\ in\ digestion\ mixture} \times 100\% \quad (4)$$

2.2.15. Quantification of CAPs by HPLC

The CAP contents in the mixed micelle phase (i.e. the bioaccessible fraction) and the intestinal digesta after *in vitro* gastrointestinal digestion of the CAP-loaded emulsion gels were quantified by reversed-phase HPLC. The chromatograph was equipped with a UV–VIS photodiode array detector (SPD-20AV; Shimadzu Corporation, Kyoto, Japan). The column was a Synergi™ 4 μm Hydro-RP 80 Å liquid chromatography column with dimensions of 150 mm × 4.6 mm (Phenomenex Inc., Torrance, CA, USA). The mobile phase was composed of acetonitrile and Milli-Q water at a volume ratio of 50:50. The running temperature was set at 30 °C, with a flow rate of 1 mL min^{-1} and a sample injection volume of 5 μL. Two main compounds from CAP were detected: capsaicin and dihydrocapsaicin. The detection wavelength was set at 280 nm. CAP molecules were extracted from the digesta samples by mixing samples and absolute ethanol at a volume ratio of 1:1. The mixtures were vortexed for 5 min and then stored overnight at 4 °C. The next day, the mixtures were centrifuged at 10,000 *g* at 4 °C for 15 min and then the supernatants were filtered through a 0.22 μm filter before being injected on to the chromatograph column. The quantification of capsaicin and dihydrocapsaicin was determined from a calibration curve of standard solutions of powdered CAP (capsaicin: 61.23%; dihydrocapsaicin: 31.96%; other CAPs: 2.51%) in methanol.

2.3. Statistical analysis

Each experiment was performed in triplicate using freshly prepared samples. The results are presented as the calculated means and standard deviations. The data were analysed by one-way analysis of variance using IBM SPSS Statistics 24 software. Means were compared by Tukey tests, $P < 0.05$ was considered as significant difference. Power-law fitting was performed using Origin 2017 64Bit software (OriginLab Corporation, Northampton, MA, USA). 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.

3. Results and discussion

3.1. Gastric behaviour of CAP-loaded emulsion gels

Fig. 1(A) shows the pH profiles of the CAP-loaded emulsion gels during 240 min of *in vitro* gastric digestion in the HGS. The pH of the emptied gastric digesta gradually decreased from 5.88 ± 0.27 at 0 min to 2.40 ± 0.23 at 240 min for the CAP-loaded whey protein emulsion gel and from 5.70 ± 0.12 at 0 min to 2.27 ± 0.06 at 240 min for the 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.

The solids content of the emptied gastric digesta as a function of the digestion time is presented in Fig. 1(B). Overall, with increasing digestion time, the solids content of the gastric digesta decreased gradually for both gels, because of constant addition of gastric fluid and gastric emptying. Gastric emptying occurred only after 30 min because of the lag phase during the digestion of solid/semi-solid foods (Siegel et al., 1988).

At 30–165 min, the gastric digesta emptied from the CAP-loaded Tween 80 emulsion gel had significantly higher solids content than those emptied from the CAP-loaded whey protein emulsion gel; no significant difference between the two gels was found for the gastric digesta emptied at 180–240 min. The results suggested that the CAP-loaded Tween 80 emulsion gel was emptied out faster than the CAP-loaded whey protein emulsion gel. This was probably due to the smaller gel particles, generated after mastication of the CAP-loaded Tween 80 emulsion gel, entering the stomach. Gastric sieving allows only particles smaller than 1 mm to pass through; after oral processing, the CAP-loaded Tween 80 emulsion gel had about 73% of the solids smaller than 1 mm whereas the CAP-loaded whey protein emulsion gel had about 47% (Luo et al., 2020). The greater proportion of small gel particles from the CAP-loaded Tween 80 emulsion gel facilitated its emptying from the stomach.

Fig. 1(C) presents the gel retention in the HGS during 240 min of gastric digestion. The CAP-loaded Tween 80 emulsion gel was emptied out faster than the CAP-loaded whey protein emulsion gel ($P < 0.05$). The curves were fitted to Equation (2) established by Siegel et al. (1988) for describing the gastric emptying behaviour of solid foods, the parameters of the fitted curves are presented in Table 1.

The CAP-loaded Tween 80 emulsion gel had a significantly higher gastric emptying rate (k) and needed significantly less time to empty out 50% of the total solids (T_{half}) than the CAP-loaded whey protein emulsion gel, which can be attributed mainly to the significantly smaller masticated gel bolus particle size from the CAP-loaded Tween 80 emulsion gel. Also, the particle size of the gastric digesta from the CAP-loaded Tween 80 emulsion gel was much smaller than that from the CAP-loaded whey protein emulsion gel during gastric digestion [Fig. 2 (A)]. As gastric emptying allows only particles smaller than 1 mm to pass through to the intestine, a smaller particle size of the gastric digesta would generally lead to a faster emptying rate. Similar results were reported by Koike et al. (2013) and Pera et al. (2002), who investigated the effect of mastication efficiency on the gastric emptying rate. They reported that insufficient mastication (leading to a larger masticated bolus

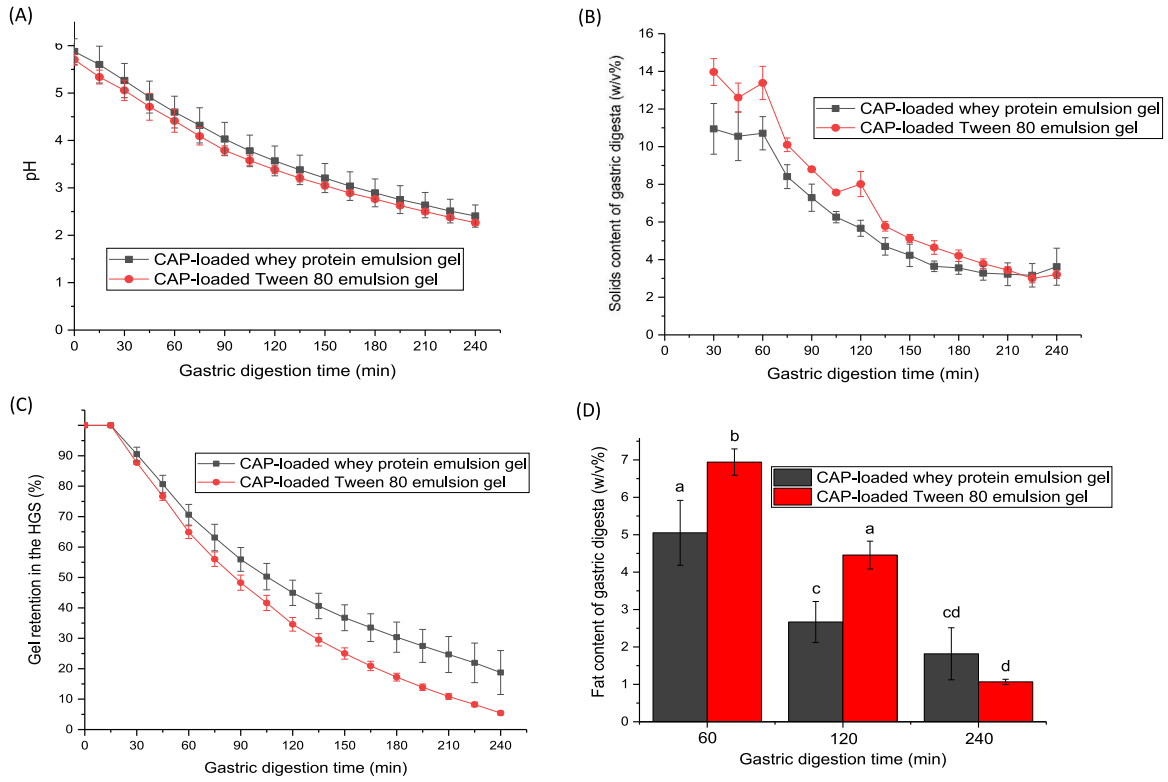

Fig. 1. Gastric behaviour of the CAP-loaded whey protein emulsion gel (whey protein as emulsifier, $d_{4,3}$–0.5 μm, 100 mM NaCl) and the CAP-loaded Tween 80 emulsion gel (Tween 80 as emulsifier, $d_{4,3}$–0.5 μm, 100 mM NaCl) during *in vitro* gastric digestion in the HGS: (A) changes in pH; (B) solids content of the emptied gastric digesta as a function of the digestion time; (C) gel retention in the HGS; (D) oil content of the gastric digesta emptied at 60, 120 and 240 min of digestion. Error bars represent standard deviations obtained from three replicates.

Table 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
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} [a] (min)	$111.7^{a} \pm 13.9$	$87.6^{b} \pm 3.9$
T_{lag} [b] (min)	$37.0^{a} \pm 5.2$	$46.1^{b} \pm 2.6$

[a, b] Values with different superscripts differ significantly ($P < 0.05$).
[a] T_{half} is defined as the time needed to empty out half of the solids [i.e. $y(t) = 0.5$].
[b] T_{lag} was calculated as $ln\,\beta/k$ (Siegel et al., 1988).

particle size) would result in a reduced rate of gastric emptying. In their studies, they used the same food and asked the participants to chew the food either sufficiently or not; as the masticated bolus particle size was controlled by the mastication efficiency, these studies did not consider the effect of food structure on mastication or gastric emptying. In our study, both gels were sufficiently masticated; the difference in the masticated gel bolus particle size was caused by the structural difference because of the difference in the type of emulsifier used.

For both gels, with increasing digestion time, the oil content of the gastric digesta gradually decreased [Fig. 1(D)]. For the gastric digesta emptied at 60 and 120 min, the CAP-loaded Tween 80 emulsion gel had significantly higher oil content than the CAP-loaded whey protein emulsion gel; no significant difference between the two gels in the gastric digesta emptied at 240 min was found. This trend is consistent with the trend of the solids content of the gastric digesta [Fig. 1(B)].

3.2. Particle size of emptied gastric digesta

With increasing digestion time, the average particle size ($d_{4,3}$) of the emptied gastric digesta decreased gradually for both gels [Fig. 2(A)], showing the gradual breakdown of gel particles during gastric digestion. During 30–180 min of gastric digestion, the emptied digesta from the CAP-loaded whey protein emulsion gel had larger particle size than the digesta from the CAP-loaded Tween 80 emulsion gel; this could be attributed to the smaller masticated gel bolus particle size and the release of oil droplets from the protein matrix during gastric digestion of the CAP-loaded Tween 80 emulsion gel. No significant difference between the two gels in the particle size of the gastric digesta emptied at 210 and 240 min was found. At 210 min of digestion, the pH had decreased to ~2.5 [Fig. 1(A)] for both gels. As pepsin has higher activity at this pH value, it is more effective in cleaving peptide bonds and facilitating gel disintegration. This effect was more evident in the CAP-loaded whey protein emulsion gel, which may explain the large decrease in particle size of the gastric digesta emptied from 180 to 240 min.

Fig. 2(C) and (D) present the particle size distributions of the CAP-loaded liquid emulsions and the emptied gastric digesta as a function of the digestion time. For the CAP-loaded whey protein emulsion gel [Fig. 2(C)], the gastric digesta emptied at 30, 60, 90 and 120 min had similar particle size distributions that resembled 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 whereas the peak near 10 μm showed a slight increase in volume; the peaks near 100 μm were similar. This indicates that the disintegration of the gel particles during the first 2 h of digestion was not significant. At 150 and 180 min of digestion, the particle size distributions of the gastric digesta also showed trimodal distributions with three peaks near 10, 100 and 1000 μm; the peak near 1000 μm decreased

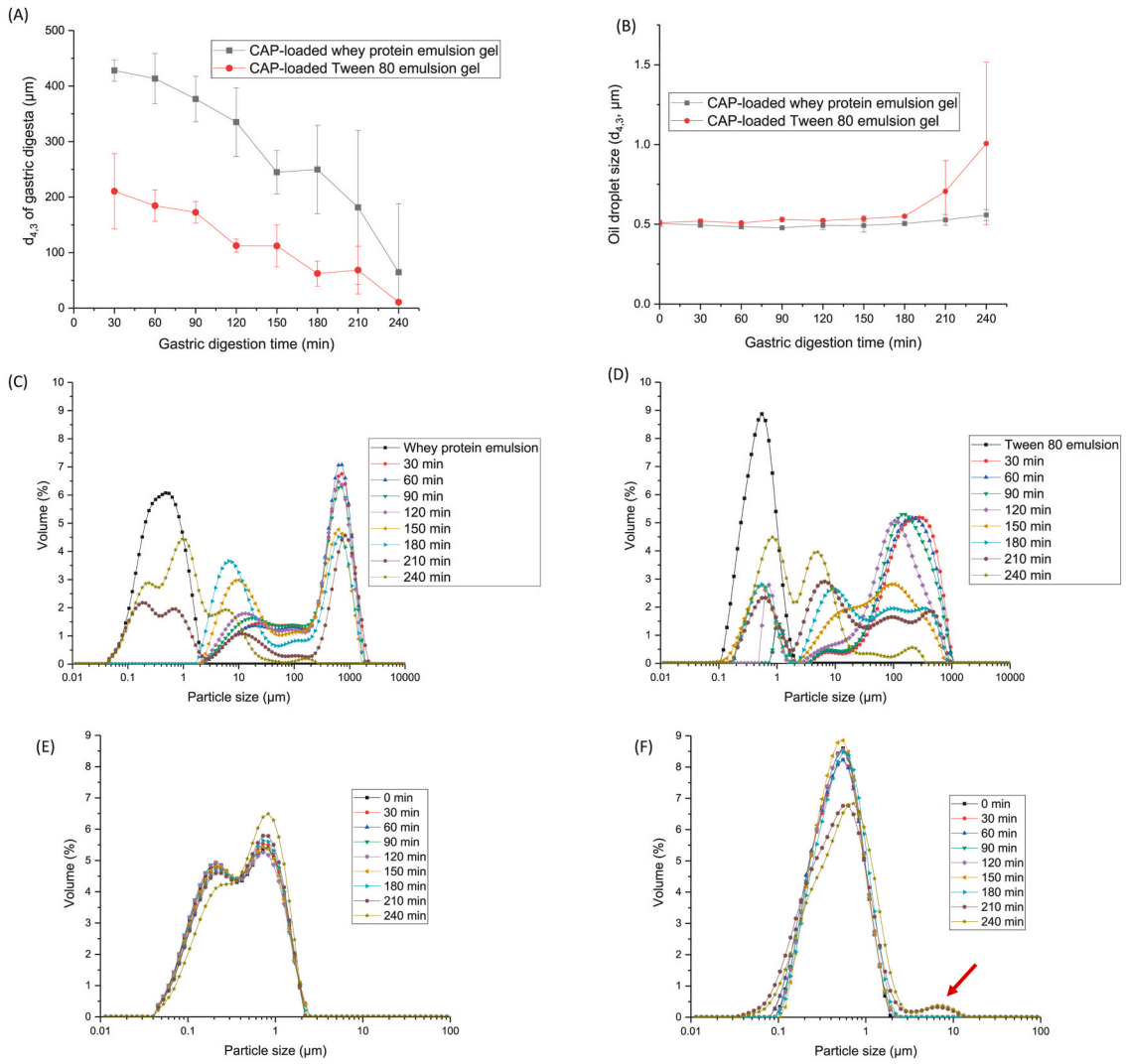

Fig. 2. (A) Average particle size ($d_{4,3}$, μm) of emptied gastric digesta and (B) average particle size ($d_{4,3}$, μm) of oil droplets of emptied gastric digesta; error bars represent standard deviations obtained from three replicates. Particle size distributions of the emptied gastric digesta from (C) the CAP-loaded whey protein emulsion gel and (D) the CAP-loaded Tween 80 emulsion gel. Particle size distributions of the oil droplets of the emptied gastric digesta from (E) the CAP-loaded whey protein emulsion gel and (F) the CAP-loaded Tween 80 emulsion gel, as a function of the digestion time. The red arrow in (F) indicates the coalesced oil droplets in the gastric digesta emptied at 210 and 240 min from the CAP-loaded Tween 80 emulsion gel. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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

These results from the CAP-loaded whey protein emulsion gel suggested that gel disintegration was not significant during 0–120 min of digestion. During 0–120 min of digestion, the pH of the gastric digesta decreased from 5.9 to 3.6; the pH decreased to 4.0 at 90 min of digestion [(Fig. 1(A)]. Clearly, after 120 min of digestion, because of the low pH, pepsin became more active in hydrolysing proteins and facilitating the breakdown of gel particles by disrupting the protein network structure.

The diffusion of pepsin through the gel particles was another factor affecting gel disintegration during gastric digestion. During the first 2 h of digestion, the particle size of the gastric digesta was relatively large, compared with 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 the constant addition of SGF, the concentration of pepsin in the stomach gradually increased while the solids content of the gastric digesta gradually decreased [Fig. 1(B)]. This gradual increase in the enzyme-to-substrate ratio was another contributing factor to the disintegration of the gel particles being more evident during the later stages of gastric digestion.

For the CAP-loaded Tween 80 emulsion gel [Fig. 2(D)], the gastric digesta emptied at 30, 60 and 90 min of digestion had similar trimodal particle size distributions with three peaks near 1, 6 and 200 μm. The small peak near 1 μm may have been small amounts of released oil droplets, because of 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 the protein matrix. At 150, 180 and 210 min of digestion, the peak near 100 μm gradually decreased in volume whereas the peak in the range 2–30 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 greater release of oil droplets from the 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 increased in volume compared with those for the gastric digesta collected at earlier digestion times. The peak near 0.6 μm was probably released oil droplets and the peak near 200 μm was probably solid gel particles; the peak near 4 μm may have been small solid gel particles and/or flocculated/coalesced oil droplets. Similar to the CAP-loaded whey protein emulsion gel, gel disintegration was not significant during 0–90 min of digestion of the 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, the pepsin activity gradually increased to its optimum and promoted gel disintegration.

The CAP-loaded whey protein emulsion gel had minor oil droplet release during the last 30 min of gastric digestion, mainly because of 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 the surrounding protein matrix through disulphide binding and hydrophobic interaction. Emulsion gels formed at relatively high NaCl concentrations had compact particulate structures; the thick protein layer around the oil droplets could prevent them from liberation during gastric digestion. In contrast, the 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 mechanical compression, shearing and cutting (Luo et al., 2020).

3.3. Particle size of oil droplets in the emptied gastric digesta

Fig. 2(B), (E) and 2(F) present the average particle size ($d_{4,3}$) and the particle size distributions of the oil droplets of the emptied gastric digesta as a function of the digestion time respectively. The oil droplet size of the gastric digesta from the 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 the gastric digesta from the 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 have represented some oil droplet coalescence at the end of digestion.

For the 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 the oil droplets of the 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 in the range 3–11 μm appeared, representing the coalesced oil droplets formed at the end of digestion. Oil droplet coalescence was much more evident in the CAP-loaded Tween 80 emulsion gel. This indicates that the whey-protein-coated oil droplets were more stable against coalescence during gastric digestion than the Tween-80-coated oil droplets.

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^{-1} of digestion mixture). For the 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 the 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, coalescence may occur, resulting from a combined effect of protein hydrolysis (leading to a smaller gel particle size and the release of oil droplets from the protein matrix) and the displacement of proteins/peptides at the interface by the lipolytic products (van Aken et al., 2011).

For the CAP-loaded Tween 80 emulsion gel, oil droplet release was observed from the beginning of gastric digestion, because of the low degree of interaction between the Tween-80-coated oil droplets and the protein matrix. The released oil droplets were more susceptible to being digested by gastric lipase, resulting in an earlier and greater extent of lipolysis. One possible mechanism for oil droplet coalescence in the 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. FFAs, monoglycerides and diglycerides) are non-ionic at the gastric pH, and 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 the gastric digestion (Plou et al., 1998, 1998van Aken et al., 2011). Nevertheless, oil droplet coalescence occurred much more slowly and was much less apparent for the CAP-loaded whey protein emulsion gel, compared with the CAP-loaded Tween 80 emulsion gel. Similar observations were reported by van Aken et al. (2011); 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 the full fat milk showed apparent oil droplet coalascence during gastric digestion, and that the Tween-80-stabilized emulsion had a greater extent of lipolysis than the 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 the whey-protein-stabilized emulsion could have stabilized the emulsion against coalescence.

3.4. Microstructure of emptied gastric digesta

CLSM images of the gastric digesta emptied at 30, 120 and 240 min of gastric digestion are presented in Fig. 3. For both gels, with increasing digestion time, the size of the gel particles decreased, indicating gel disintegration during gastric digestion. The gel disintegration is an effect of both mechanical breakdown and enzymatic hydrolysis of whey proteins into peptides (Guo et al., 2014; Kong and Singh, 2009).

At 240 min of digestion, gel particles of ~500 μm in size were still observed in the digesta from the CAP-loaded whey protein emulsion gel, whereas those in the CAP-loaded Tween 80 emulsion gel were mainly broken down into smaller particles, qualitatively indicating that, during gastric digestion, the CAP-loaded Tween 80 emulsion gel was more easily broken down than the CAP-loaded whey protein emulsion gel.

Oil droplet release was observed in the gastric digesta emptied at 30 min from the CAP-loaded Tween 80 emulsion gel. Tween 80 coated oil droplets were flocculated and had no interactions with surrounding protein matrix, therefore more susceptible to be released from protein matrix during digestion. No obvious oil droplet release was observed in the CLSM images of the gastric digesta emptied before 240 min from the CAP-loaded whey protein emulsion gel, indicating that most of the oil droplets were retained in the solid gel particles during gastric digestion. This is because the protein/peptide-coated oil droplets had strong interactions with surrounding protein matrix through disulphide binding and hydrophobic interaction, therefore were protected from being released.

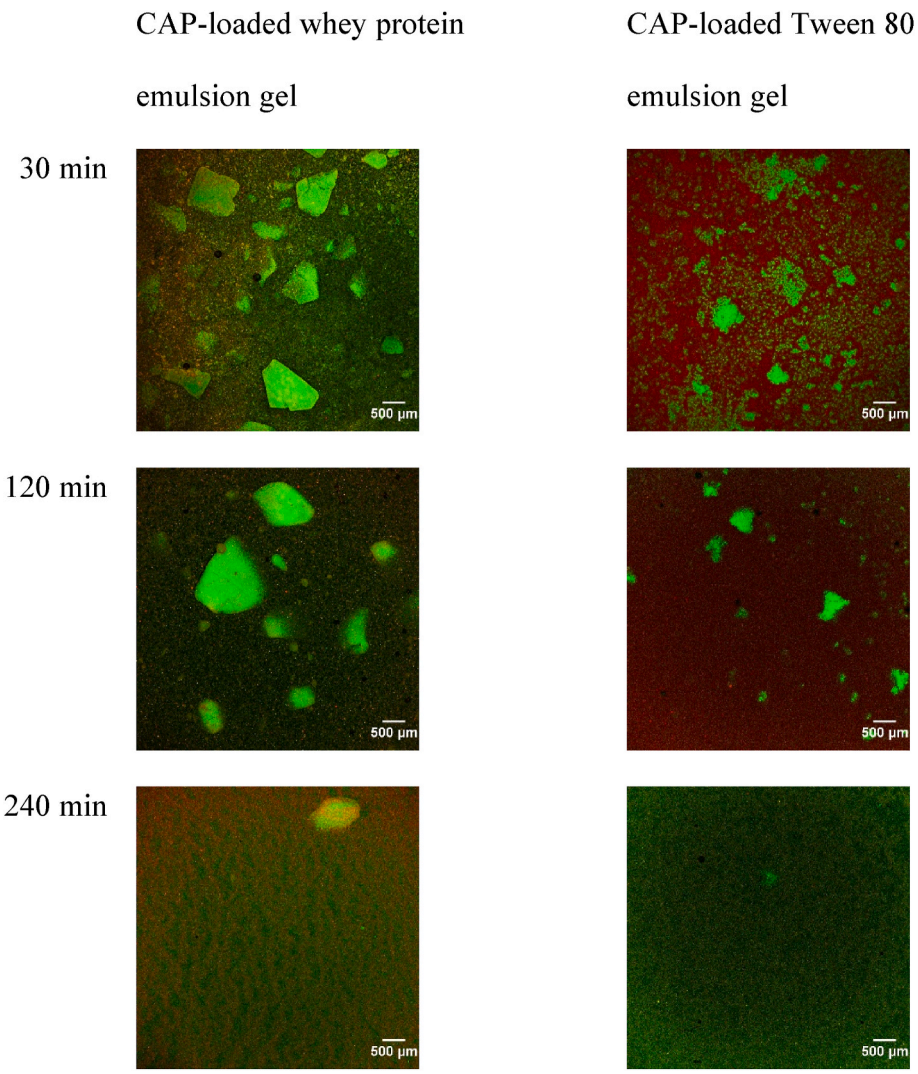

Fig. 3. Representative CLSM images of the emptied gastric digesta at 30, 120 and 240 min of gastric digestion. Green colour represents proteins; red colour represents oil; black colour represents water or air. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. SDS-PAGE patterns of emptied gastric digesta

The Tricine SDS-PAGE patterns under reducing conditions of the emptied gastric digesta as a function of the digestion time are presented in Fig. 4. 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 emulsion gels, with increasing digestion time, the band intensities of β-lg and α-la gradually decreased, which was caused by protein hydrolysis by pepsin and gradual dilution of the gastric content during gastric digestion. The bands from the gastric digesta of the CAP-loaded Tween 80 emulsion gel were more intense than those of the CAP-loaded whey protein emulsion gel emptied at the same digestion time, indicating a higher protein concentration in the gastric digesta from the CAP-loaded Tween 80 emulsion gel.

At 0 min, the bands for both gels were composed mainly of BSA, β-lg and α-la. Starting from 30 min of digestion, peptide bands appeared in the gastric digesta for both gels, indicating the occurrence of protein hydrolysis at pH values above 5.0. At 240 min of digestion, no intact whey proteins were found in the gastric digesta for both gels. Overall, the SDS-PAGE patterns were similar between the two gels, indicating that the proteins were hydrolysed at similar rates.

3.6. In vitro intestinal digestion

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

3.6.1. Initial lipolysis rate

The initial lipolysis rates (i.e. the μmoles of FFA released per millilitre of digestion mixture per minute during the initial 2 min of intestinal digestion) calculated from Fig. 5 are presented in Table 3. For the CAP-loaded whey protein emulsion gel, no significant difference in the initial lipolysis rate for the gastric digesta emptied at different times was found. For the CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 120 min had the highest initial lipolysis rate, followed by the gastric digesta emptied at 240 min; the gastric digesta emptied at 60 min had a significantly lower initial lipolysis rate. The gastric digesta emptied at 60 and 240 min from the CAP-loaded Tween 80 emulsion gel had

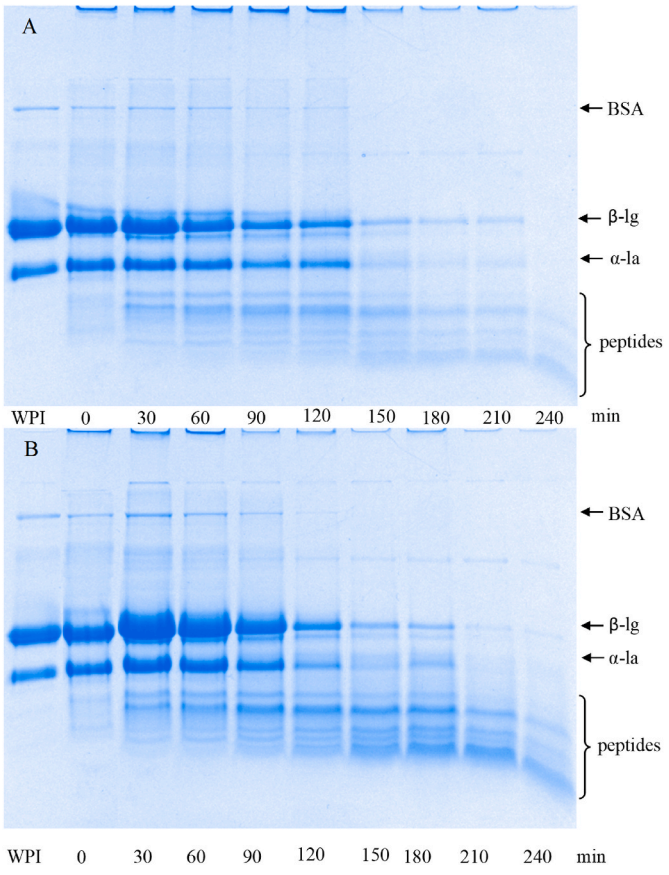

Fig. 4. Tricine SDS-PAGE patterns under reducing conditions of emptied gastric digesta from (A) the CAP-loaded whey protein emulsion gel and (B) the CAP-loaded Tween 80 emulsion gel, as a function of the digestion time.

Table 2
pH, solids 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.

Gastric Digestion Time	CAP-loaded Whey Protein Emulsion Gel			CAP-loaded Tween 80 Emulsion Gel		
	60 min	120 min	240 min	60 min	120 min	240 min
pH	$4.60^{a,x} \pm 0.34$	$3.57^{b,x} \pm 0.31$	$2.40^{c,x} \pm 0.23$	$4.41^{a,x} \pm 0.24$	$3.38^{b,x} \pm 0.08$	$2.27^{c,x} \pm 0.06$
Solids 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–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 ($P < 0.05$).

significantly lower initial lipolysis rates than those from the CAP-loaded whey protein emulsion gel; no significant difference between the two gels for the gastric digesta emptied at 120 min was found.

As discussed by Luo et al. (2021), the initial lipolysis rate is influenced by multiple factors including the substrate-to-enzyme ratio, the gel particle size, the gel structure etc. For the 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 the gastric digesta emptied at 60 min. For the CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 60 min had the lowest initial lipolysis rate probably because of its larger gel particle size and lower extent of oil droplet release; the gastric digesta emptied at 120 min had a higher initial lipolysis rate than the gastric digesta emptied at 240 min probably because of its higher oil content, even though the gastric digesta emptied at 240 min had a smaller gel particle size.

For the gastric digesta emptied at 60 min, the CAP-loaded Tween 80 emulsion gel had significantly higher oil content, smaller gel particle size and lower release of oil droplets, compared with the CAP-loaded whey protein emulsion gel. However, the gastric digesta from the CAP-loaded Tween 80 emulsion gel had a 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 (2010); they reported the effect of the presence of small-molecule surfactants on the *in vitro* gastrointestinal digestion of protein-stabilized emulsions, and found that there was a lag phase of FFA release during the intestinal digestion of a protein-stabilized emulsion with added Tween 80; the length of the lag phase increased with increasing Tween 80 concentration.

For the gastric digesta emptied at 120 min, the effect of Tween 80 on lipid digestion appeared to be less obvious, as no significant difference between the two gels was found. This could have been because the concentration of Tween 80 was lower in the gastric digesta emptied at 120 min than at 60 min, leading to a higher bile salts-to-Tween 80 ratio. In addition, the bile salts-to-lipids/interface ratio 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 the gastric digesta emptied at 120 min.

For the gastric digesta emptied at 240 min, the CAP-loaded Tween 80 emulsion gel had a significantly smaller gel particle size and a greater extent of oil droplet release, but the initial lipolysis rate was lower, compared with the CAP-loaded whey protein emulsion gel. In Fig. 2(B), oil droplet coalescence was seen in the CAP-loaded Tween 80 emulsion gel, leading to an increased oil droplet size and a decreased interfacial area, whereas the CAP-loaded whey protein emulsion gel was more resistant against oil droplet coalescence at the end of the gastric digestion. An increased oil droplet size would generally lead to a reduced lipolysis rate because less interfacial area would be available for the lipolytic reaction, which is in agreement with the report by Borreani et al. (2019).

3.6.2. FFA release profile

Fig. 5(A) and (B) show the FFA release profiles per millilitre of digestion mixture during *in vitro* intestinal digestion of the gastric digesta emptied at 60, 120 and 240 min from both gels. For the CAP-loaded whey protein emulsion gel, the gastric digesta emptied at 60 min had significantly more FFA release per millilitre of digestion mixture than the gastric digesta emptied at 120 and 240 min [Fig. 5(A)], probably because of its significantly higher oil content (Table 2). For the CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 240 min had significantly lower FFA release per millilitre of digestion mixture because of its lower oil content; no significant difference between the gastric digesta emptied at 60 and 120 min was found [Fig. 5 (B)]. For both gels, the FFA release did not reach a plateau at the end of the intestinal digestion for the gastric digesta emptied at 60 min; it took approximately 50 and 15 min respectively to reach a plateau for the gastric digesta emptied at 120 and 240 min.

For the gastric digesta emptied at 60 min, the lipolysis rate for the CAP-loaded whey protein emulsion gel gradually decreased with increasing digestion time during the first 45 min. After 45 min, the

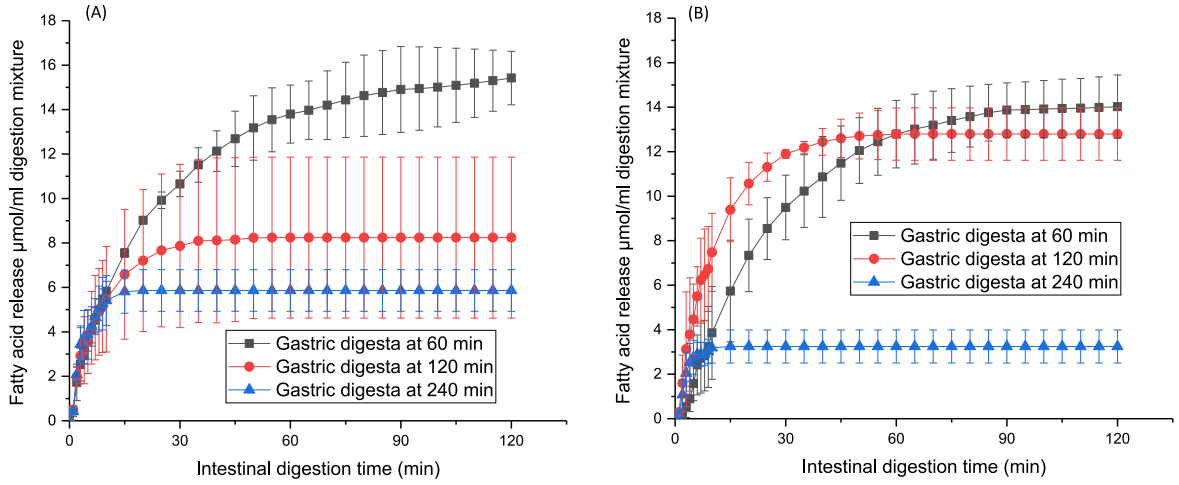

Fig. 5. FFA release profile per millilitre of digestion mixture of the gastric digesta emptied at 60, 120 and 240 min from (A) the CAP-loaded whey protein emulsion gel and (B) the CAP-loaded Tween 80 emulsion gel. Error bars represent standard deviations obtained from three replicates.

Table 3
Initial lipolysis rate (μmol·mL^{-1}·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 (μmol·ml^{-1}·min^{-1})	$0.99^{a,x} \pm 0.36$	$0.98^{a,x} \pm 0.32$	$0.97^{a,x} \pm 0.17$	$0.14^{a,y} \pm 0.01$	$1.15^{b,x} \pm 0.17$	$0.73^{c,y} \pm 0.03$

[a–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 ($P < 0.05$).

lipolysis rate was relatively constant until 120 min of intestinal digestion. This was presumably because of the gradual disintegration of solid gel particles and the gradual release of oil droplets so that the substrate-to-enzyme ratio remained relatively constant. For the CAP-loaded Tween 80 emulsion gel, there was a lag phase of about 3 min, followed by a rapid increase in the lipolysis rate. The lipolysis rate was relatively constant during the initial 5–25 min of digestion, i.e. the concentration of the substrate was relatively constant, because of the fast disintegration of gel particles and the release of oil droplets. After 25 min, the lipolysis rate gradually decreased because of the decreasing substrate concentration.

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

Lipid digestion is considered to be 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 and Wooster, 2010). The displacement of emulsifier from the interface by bile salts is essential for lipid digestion. Tween 80 appeared to exert some resistance to its displacement by bile salts at the interface. Koukoura et al. (2019) studied the effect of using caseinate versus Tween 20 as an emulsifier on the *in vitro* digestion behaviour of the emulsion and reported the gradual decrease of the zeta-potential of the emulsified 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 some 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 the extent of lipid digestion (Li and McClements, 2010).

As well as the effect of Tween 80, the rate and the extent of lipid digestion also depend on the ratio of bile salts and calcium to the substrate/lipolytic products or the interfacial area. The higher is the ratio, the more effective are the calcium and bile salts at precipitating and displacing the lipolytic products (i.e. FFAs and mono- and diglycerides) from the interface, which would free the interface for further lipolysis and, therefore, increase the rate and the extent of lipid digestion. This explains that, because of the higher oil content of the gastric digesta emptied at 60 min from the CAP-loaded Tween 80 emulsion gel, the ratio of bile salts/calcium to substrate was lower, leading to a lower rate and extent of lipid digestion, compared with the gastric digesta emptied at 120 and 240 min from the CAP-loaded Tween 80 emulsion gel. Similar results were reported by Li et al. (2011), i.e. a lower oil content would generally lead to a greater extent of lipid digestion. However, this effect was less evident in the CAP-loaded whey protein emulsion gel, for which there was no significant difference between gastric digesta emptied at different times, even though the ratio was lower in the gastric digesta emptied at 60 min.

3.7. Bioaccessibility of CAP

The bioaccessibility of CAP after *in vitro* gastrointestinal digestion is shown in Fig. 6. For both gels, the gastric digesta emptied at 60 min had significantly lower bioaccessibility of CAP than the gastric digesta emptied at 120 and 240 min; there was no significant difference in the bioaccessibility of CAP between the gastric digesta emptied at 120 and 240 min. For the gastric digesta emptied at 60 min, the CAP-loaded Tween 80 emulsion gel had higher bioaccessibility of CAP than the CAP-loaded whey protein emulsion gel ($P < 0.05$); there was no significant difference between the two gels for the gastric digesta emptied at 120 and 240 min.

The bioaccessibility of CAP depends on two steps: the release of CAP from the food matrix during gastrointestinal digestion and the

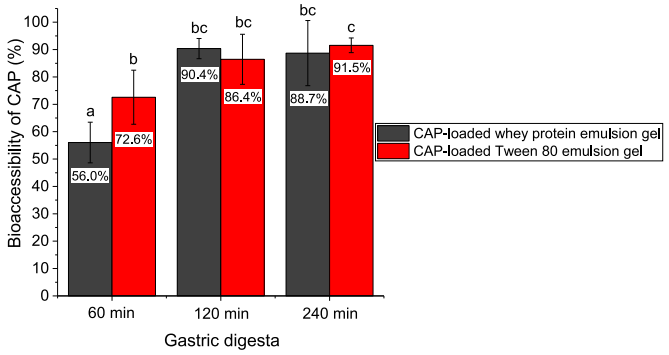

Fig. 6. Bioaccessibility of CAP after *in vitro* gastrointestinal digestion. Error bars represent standard deviations obtained from three replicates. Different lowercase letters indicate significant differences between samples ($P < 0.05$).

solubilization of CAP in the aqueous phase (in mixed micelles and vesicles). As 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 the protein matrix; however, the release of oil droplets would promote the release of CAP molecules. Moreover, they could be released because of lipid digestion, which relies largely 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 the 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 the gel particle size), the rate and the extent of oil droplet release, and the rate and the extent of lipid digestion. In general, smaller gel particles, faster oil droplet release and greater extent of lipid digestion would lead to more CAP release from the lipid phase.

For the CAP-loaded whey protein emulsion gel, the bioaccessibility of CAP was lower in the gastric digesta emptied at 60 min ($P < 0.05$). A possible reason could be that the gastric digesta emptied at 60 min had a significantly higher oil content and a larger interfacial area; the lipid digestion did not reach a plateau at 120 min of intestinal digestion, which means that undigested oil droplets remained. 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 can anchor on to the interface and hydrolyse the lipids; they also displace the lipolytic products from the interface and help to solubilize them in the aqueous phase by forming mixed micelles (Maldonado-Valderrama et al., 2011). Therefore, a higher bile salts-to-interface ratio would generally lead to a greater extent of lipid digestion, and a higher bile salts-to-CAP ratio would generally promote the solubilization of CAP molecules. For the gastric digesta emptied at 60 min, a substantial portion of the bile salts was probably still at the interface of the undigested oil droplets; therefore, the released CAP molecules were not well solubilized, leading to lower bioaccessibility of CAP. The results indicate that the release of CAP molecules from the food matrix was not the limiting step determining the bioaccessibility of CAP; rather, it was the solubilization of the CAP molecules.

For the CAP-loaded Tween 80 emulsion gel, the gastric digesta emptied at 60 min had significantly higher oil content, larger gel particle size, lower extent of oil droplet release and lower extent of lipid digestion than the gastric digesta emptied at 120 and 240 min, all of which probably contributed to the lower bioaccessibility of CAP. However, the higher oil content and the 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 the gastric digesta emptied at different times; therefore, the effects of the gel particle size and the rate and extent of oil droplet release were not evident. A greater extent of lipid digestion would lead to greater release of CAP molecules and more lipolytic products being generated; the lipolytic products would also participate in the formation of mixed micelles, which would help to solubilize the CAP molecules being released.

For the gastric digesta emptied at 60 min, the CAP-loaded Tween 80 emulsion gel had significantly higher bioaccessibility of CAP than the CAP-loaded whey protein emulsion gel. This means that more CAP molecules were released and solubilized during intestinal digestion of the gastric digesta from the CAP-loaded Tween 80 emulsion gel. The solid particles were broken down much more rapidly and the oil droplets were released much more rapidly from the gel with inactive filler particles (the CAP-loaded Tween 80 emulsion gel) than from the gel with active filler particles (the CAP-loaded whey protein emulsion gel), 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 to solubilize the released CAP molecules in the aqueous phase and, therefore, lead to higher bioaccessibility of CAP. Similar effects were observed for the gastric digesta emptied at 120 and 240 min. The results are in agreement with the publication by Mun et al. (2015); they studied the bioaccessibility of β-carotene after *in vitro* digestion of emulsions stabilized by whey protein or Tween 20 and found that the Tween-20-stabilized emulsion had significantly higher bioaccessibility of β-carotene.

4. Conclusions

The CAP-loaded Tween 80 emulsion gel was emptied out significantly faster during gastric digestion than the CAP-loaded whey protein emulsion gel. The Tween-80-coated oil droplets were flocculated in the emulsion gel, disrupting the protein network structure because they had no interactions with the surrounding protein matrix. The Tween-80-coated oil droplets started to be released from the protein matrix from the beginning of the gastric digestion. In contrast, the whey-protein-coated oil droplets had strong interactions with the surrounding protein matrix, mainly through hydrophobic interactions and disulphide binding; the thick protein layer around the oil droplets protected them from liberation during gastric digestion, which had an impact on the release from the gel matrix and the coalescence of oil droplets.

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

CRediT authorship contribution statement

Nan Luo: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Aiqian Ye:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing. **Frances M. Wolber:** Supervision, Writing –

review & editing. **Harjinder Singh:** Supervision, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The research was supported by the Riddet Institute, a New Zealand Centre of Research Excellence (CoRE), funded by the Tertiary Education Commission, New Zealand.

References

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

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 Biophys. 14 (3), 313–325.

Camilleri, M., 2006. Integrated upper gastrointestinal response to food intake. Gastroenterology 131 (2), 640–658.

Chen, J., Dickinson, E., 1995. Protein/surfactant interfacial interactions Part 3. Competitive adsorption of protein + surfactant in emulsions. Colloids Surf., A 101 (1), 77–85.

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 Sci. Technol. 33 (4), 299–307.

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 Funct. 10 (1), 112–124.

Dickinson, E., 2012. Emulsion gels: the structuring of soft solids with protein-stabilized oil droplets. Food Hydrocolloids 28 (1), 224–241.

Golding, M., Wooster, T.J., 2010. The influence of emulsion structure and stability on lipid digestion. Curr. Opin. Colloid Interface Sci. 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 Surf., B 164, 272–280.

Guo, Q., Ye, A., Lad, M., Dalgleish, D., Singh, H., 2014. Effect of gel structure on the gastric digestion of whey protein emulsion gels. Soft Matter 10 (8), 1214–1223.

Heaney, R.P., 2001. Factors influencing the measurement of bioavailability, taking calcium as a model. J. Nutr. 131 (4 Suppl. l), 1344S–1348S.

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. Int. J. Food Sci. Nutr. 53 (5), 389–402.

Humberstone, A.J., Charman, W.N., 1997. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv. Drug Deliv. 25 (1), 103–128.

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. J. Oral Rehabil. 40 (8), 574–581.

Kong, F., Singh, R.P., 2009. Modes of disintegration of solid foods in simulated gastric environment. Food Biophys. 4 (3), 180–190.

Kong, F., Singh, R.P., 2010. A human gastric simulator (HGS) to study food digestion in human stomach. J. Food Sci. 75 (9), E627–E635.

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 Biophys. 14 (1), 60–68.

Li, Y., McClements, D.J., 2010. New mathematical model for interpreting pH-stat digestion profiles: impact of lipid droplet characteristics on *in vitro* digestibility. J. Agric. Food Chem. 58 (13), 8085–8092.

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 Chem. 126 (2), 498–505.

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.

Lu, M., Cao, Y., Ho, C.T., Huang, Q., 2016. Development of organogel-derived capsaicin nanoemulsion with improved bioaccessibility and reduced gastric mucosa irritation. J. Agric. Food Chem. 64 (23), 4735–4741.

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, 106076.

Luo, N., Ye, A., Wolber, F.M., Singh, H., 2021. Effect of gel structure on the *in vitro* gastrointestinal digestion behaviour of whey protein emulsion gels and the bioaccessibility of capsaicinoids. Molecules 26 (5), 1379.

Maldonado-Valderrama, J., Wilde, P., Macierzanka, A., MacKie, A.R., 2011. The role of bile salts in digestion. Adv. Colloid Interface Sci. 165 (1), 36–46.

Mao, L., Roos, Y.H., Biliaderis, C.G., Miao, S., 2017. Food emulsions as delivery systems for flavor compounds: a review. Crit. Rev. Food Sci. Nutr. 57, 3173–3187.

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 Funct. 5 (6), 1113–1124.

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.

Pera, P., Bucca, C., Borro, P., Bernocco, C., De Lillo, A., Carossa, S., 2002. Influence of mastication on gastric emptying. J. Dent. Res. 81 (3), 179–181.

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. Biotechnol. Tech. 12 (3), 183–186.

Popescu, M., Chiutu, L., Mircioiu, C., Dima, Ş., 2013. Capsaicin microemulsions: Preparation, characterization and *in vitro* release study. Farmacia 62 (1), 58–68.

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 Chem. 141 (2), 1472–1480.

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.

Srinivasan, K., 2016. Biological activities of red pepper (Capsicum annuum) and its pungent principle capsaicin: a review. Crit. Rev. Food Sci. Nutr. 56 (9), 1488–1500.

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. Eur. J. Nucl. Med. 15 (5), 254–259.

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.

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. Arch Pharm. Res. (Seoul) 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. J. Sci. Food Agric. 95 (13), 2678–2685.