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Partition and digestive stability of α-tocopherol and resveratrol/naringenin in whey protein isolate emulsions

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ABSTRACT

Co-encapsulation of multiple bioactive components is an emerging field that shows promise as an approach to develop functional foods. Hydrophobic components are generally dissolved in the inner oil phase of protein-stabilised emulsions. Some components may co-adsorb to oil droplet surfaces, due to the ligand-binding properties of proteins. In this study, α-tocopherol and resveratrol/naringenin were co-encapsulated in emulsions stabilised by whey protein isolate (WPI). α-Tocopherol was totally encapsulated and its partitioning inside oil droplets was about 3.3 times that bound by free WPI in the aqueous phase. The total encapsulation efficiency for resveratrol or naringenin was 52% and 58%, respectively. Addition of resveratrol improved digestive stability of α-tocopherol, but naringenin did not. Co-encapsulation with α-tocopherol had no significant influence on the digestive stability of resveratrol/naringenin. The data gathered here should be useful for the delivery of bioactive components with different solubilities.

1. Introduction

Bioactive components, such as vitamins and polyphenols, have received increasing attention because of health benefits (Braithwaite et al., 2014). α-Tocopherol, as a lipophilic antioxidant, can delay or prevent chronic diseases associated with oxidative stress. Polyphenolic components including resveratrol (3,5,4′-trihydroxystibene) and naringenin (4′,5,7-trihydroxyflavanone) also possess antioxidant activity (Filip, Plockova, Smidrkal, Spickova, & Melzoch, 2003; Gülçin, 2010; Kimura et al., 1983; Zhang et al., 2015). Resveratrol could effectively reduce α-tocopheroxyl radical to regenerate α-tocopherol, providing synergistic activity (Fang & Zhou, 2008). However, low solubility, stability and permeability limit their applications in food and pharmaceutical industries. Stability and bioavailability of bioactive components have been improved through the encapsulation of a single bioactive component or the co-encapsulation of multiple bioactive components based on colloidal delivery systems, including emulsions, solid lipid nanoparticles, liposomes, biopolymer nanoparticles, and microgels (Chen et al., 2014; Chen, Remondetto, & Subirade, 2006; McClements, 2018).

Protein-stabilised oil-in-water (O/W) emulsions are considered to be one of the most effective carrier systems, since proteins possess high nutritional values, emulsifying properties and the ability to bind and transport ligands (Krissansen, 2007; Ye & Taylor, 2009). In general, proteins are used as emulsifiers and/or stabilisers to adsorb at the oil/water interface and to protect hydrophobic components dissolved in the inner oil phase (Kuhn & Cunha, 2012; Salminen, Herrmann, & Weiss, 2013). The hydro-solubility and stability of α-tocopherol could be improved by O/W emulsions stabilised by whey proteins (Somchue, Sermsri, Shiowatana, & Siripinyanond, 2009; Ubbink & Krüger, 2006). However, some bioactive components are not soluble in the oil phase. It has been reported that resveratrol could bind to soy protein to form an effective complex emulsifier, improving oxidative stability of corn oil-in-water emulsions (Wan, Wang, Wang, Yuan, & Yang, 2014). Resveratrol could improve storage stability of α-tocopherol in the oil phase, where resveratrol bound to whey protein isolate (WPI) at the interface provided a greater protective effect than that dispersed in the aqueous phase (Wang et al., 2016). Based on

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emulsifying and ligand-binding properties of proteins, O/W emulsions were proposed to be potential carriers for the co-encapsulation of bioactive components with different solubilities (Wang et al., 2016).

The protection for bioactive components during digestion is of utmost important for the development of an effective carrier. Flocculation, coalescence, aggregation and droplet disruption of protein-stabilised emulsions might result from physical and biochemical changes under gastrointestinal (GI) conditions. GI digestion could breakdown the mechanical structure of O/W emulsions, reducing emulsion stability and changing the physical-chemical properties of encapsulated bioactive components (Ting, Jiang, Ho, & Huang, 2014). In this study, partition and digestive stability of α-tocopherol, resveratrol and naringenin in WPI-stabilised emulsions were analysed to determine the potential of O/W emulsions for the co-encapsulation of bioactive components. Moreover, emulsion structure and stability were also investigated to clarify the protective mechanism. The data gathered here should be useful for the co-delivery of bioactive components with different solubilities.

2. Materials and methods

2.1. Materials

WPI (BiPRO, 92% protein) was obtained from Davisco International Inc. (Minnesota, USA). Sunflower oil (Brand Duoli), with a peroxide value of 4.22 ± 0.08 meq kg^{-1} estimated according to iodometric titration method (Wang et al., 2016), was purchased from a local retailer. (+)α-Tocopherol (1000 IU g^{-1}), α-tocopherol acetate (HPLC grade, > 96%), polydatin (HPLC grade, > 95%), naringenin (≥95%), naringin (HPLC grade, ≥ 95%), pepsin (porcine stomach mucose, ≥ 500 U mg^{-1}), pancreatin (porcine pancreas, 4×USP) were purchased from Sigma–Aldrich Co (St. Louis, MO, USA). Resveratrol (*trans*-isomer, > 98%), sodium dodecyl sulphate (SDS) solution 10% (w/w), ammonium persulfate (APS, >99%), N,N,N′,N′-tetramethylethylenediamine (TEMED, >99%), dithiothreitol (DTT, > 99%), Tris-HCl (pH = 8.8, 1.5 M) and Bio-Safe™ Coomassie G-250 Stain were purchased from Sango Biotech Co (Shanghai, China). Methanol (HPLC grade) was purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd (Huai'an, Jiangsu, China). Other reagents of analytical grade were purchased from Sino-Pharm CNCM Ltd (Shanghai, China).

2.2. Preparation of WPI emulsions containing α-tocopherol and resveratrol/naringenin

Oil-in-water emulsions stabilised by WPI containing α-tocopherol plus resveratrol or naringenin (Table 1) were prepared according to the literature method (Wang et al., 2016). α-Tocopherol was dissolved in sunflower oil to a content of 5% (w/w). WPI at 1% (w/v) was prepared by dispersing the powder in distilled water under stirring to allow complete dissolution. Exactly 6.4% (w/v) resveratrol or 7.6% (w/v) naringenin in 70% ethanol was diluted into the aqueous solution of WPI and incubated for 30 min. Oil-in-water emulsions were prepared by homogenising 5% (w/w) oil phase with 95% (w/w) aqueous phase using a high-speed blender (ATS Engineering Ltd., Brampton, ON, Canada) operating at 10,000 rpm followed by passing through an ATSAH2100 high-pressure homogeniser (ATS Engineering Ltd.) at 50 MPa. The pH of the emulsions was adjusted to 7 with 1 mol L^{-1} HCl or NaOH. The final concentrations of WPI, α-tocopherol, resveratrol and naringenin in emulsions were 0.5, 0.25, 0.0064 and 0.0076% (w/w), respectively. The molar concentration of resveratrol or naringenin was equal to that of WPI in the aqueous phase.

Table 1
Composition of WPI-stabilised emulsions without (control) and with α-tocopherol (α-Toc), resveratrol (Res) and/or naringenin (Nar).

Component	Emulsion					
	Control	α-Toc	Res	Nar	α-Toc + Res	α-Toc + Nar
Sunflower oil	5	4.75	5	5	4.75	4.75
α-Toc	–	0.25	–	–	0.25	0.25
WPI	0.5	0.5	0.5	0.5	0.5	0.5
Res	–	–	0.0064	–	0.0064	–
Nar	–	–	–	0.0076	–	0.0076
Ethanol	–	–	0.0518	0.0511	0.0518	0.0511

2.3. Size and ζ-potential measurements

Size distribution and ζ-potential were measured on a Zetasizer Nano ZS90 particle analyser (Malvern Instruments Ltd, Malvern, UK) with a He/Ne laser (λ = 633 nm). Particle size was analysed by the intensity-weighted distribution. All measurements were conducted at 25 °C and at a scattering angle of 173°. Samples were diluted by 100 times before measurement.

2.4. Quantitation of bioactive components

2.4.1. α-Tocopherol

α-Tocopherol was determined using liquid–liquid extraction followed by HPLC measurement (Liang, Tremblay-Hebert, & Subirade, 2011). In brief, samples were mixed with 8 mmol L^{-1} α-tocopherol acetate (internal standard) in ethanol at an equal volume and then with hexane at 4-times volume under vortexing followed by shearing at 10,000 rpm using a high-speed shearing mixer (ATS Engineering Ltd.). After centrifugation at 3500 ×*g* at 4 °C, hexane supernatant was evaporated under nitrogen and re-dissolved in methanol for HPLC measurement using a system comprising a 1525 Binary Pump, a 2489 UV/Visible Detector and a C18 column (5 μm, 4.6 mm × 250 mm, Waters, USA). UV detection was used at 292 nm. The mobile phase was 100% methanol, the flow rate was 1 mL min^{-1}.

2.4.2. Resveratrol and naringenin

Resveratrol was determined according to our previous method (Wang et al., 2016). Naringenin was determined with naringin as internal standard. Exactly 10 mmol L^{-1} naringin in methanol was added into sample and then diluted with methanol. After centrifugation at 3500 ×*g*, the supernatant was injected into the HPLC system. UV detection was used at 280 nm. A gradient of mobile phase consisting of methanol (solvent A) and water (solvent B) was developed and used according to the following program: starting with 40% A and 60% B until 1 min, changing to 60% A until 5 min and to 70% A until 11 min, then returning to 40% A until 16 min and keeping the initial condition within the next 4 min. The flow rate was 1 mL min^{-1} and the column temperature was 35 °C.

2.4.3. Partition of bioactive components

Partition of bioactive components in emulsions was analysed according to our previous method (Fan et al., 2017; see diagram, Fig. 1). WPI emulsion was centrifuged at 14,000 ×*g* at 4 °C for 30 min. The aqueous phase was further clarified at 150,000 ×*g* for 1 h using a CP70ME ultra-centrifuge (Hitachi Co. Ltd, Tokyo, Japan). The percentage of bioactive components bound to free WPI (P_b) was calculated from the difference between the total amount in the aqueous phase (A_{aq}) and the amount in the clarified aqueous phase (A_c) divided by the amount in the whole emulsion (A_w) using the expression $P_b = (A_{aq} - A_c)/A_w \times 100\%$. The percentage of free

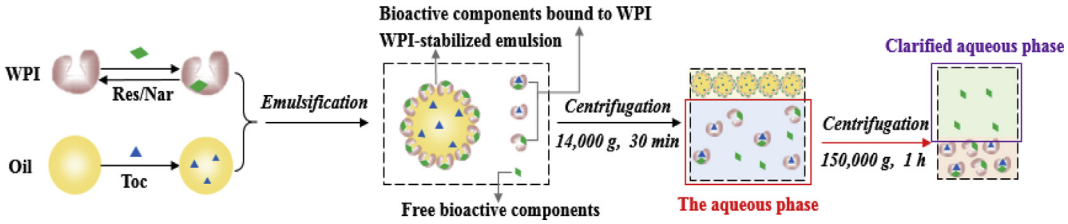

Fig. 1. Schematic diagram of sample treatment for partition analysis of bioactive components [α-tocopherol (Toc), resveratrol (Res) and naringenin (Nar)] in whey protein isolate (WPI)-stabilised emulsions.

bioactive components (P_f) in the aqueous phase was calculated as $P_f = A_c/A_w \times 100\%$. Bioactive components encapsulated inside the oil droplets or at the oil-water interface (P_e) was calculated as $P_e = (A_w - A_{aq})/A_w \times 100\%$.

2.5. In vitro digestion of WPI emulsions

The procedure of in vitro digestion was performed at 37 °C under agitation at 100 rpm using a shaking water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.) according to the literature methods (Nik, Wright, & Corredig, 2011; Sarkar, Horne, & Singh, 2010). Briefly, 15 mL WPI emulsions were mixed with 25 mL simulated gastric fluid (SGF; 3.2 mg mL^{-1} pepsin, pH 1.2) or simulated intestinal fluid (SIF, 5 mg mL^{-1} pancreatin, 20 mmol L^{-1} phosphate buffer, pH 6.8) for 6.5 h. Under simulated GI conditions, WPI emulsions were digested in SGF for 0.5 h and in SIF for 6.0 h. Digesta withdrawn at predetermined time point was twice centrifuged at 4 °C and at 14,000 ×*g* for 25 min to separate the aqueous phase. Retention of bioactive components in the whole digestion fluid or in the aqueous phase of digesta was calculated from the amount of corresponding bioactive components during digestion divided by the amount before digestion.

2.6. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis

Proteolysis of WPI was determined using SDS-PAGE under reducing conditions. WPI emulsions before and after digestion were centrifuged at 14,000 ×*g*. The collected subnatant and the whole emulsions were mixed with 0.1 mmol L^{-1} DTT solution and heated at 90 °C for 3 min. The resulting mixture at 10 μL was loaded onto a 10% resolving gel and 6% stacking gel using a Bio-Rad Mini-Protean Tetra System (Bio-Rad Laboratories, Hercules, CA, USA). The gels were stained using Bio-Safe™ Coomassie G-250 Stain and destained with a solution of 10% methanol and 10% acetic acid. The gels were scanned by using Image Lab Software 4.0.1 (Bio-Rad Laboratories).

2.7. Laser scanning confocal microscopy

A laser scanning confocal microscope (LSCM 710, Carl Zeiss AG, Germany) was used to view the microstructure of oil droplets during simulated GI digestion, according to the literature method (Zhang, Wu, Yang, He, & Wang, 2012). Nile red (10 μL, 0.1%, w/v) was added into 1 mL of sample under vortex-mixing for 1 min and kept in dark for 30 min. Subsequently, 10 μL of stained samples were placed on a concave confocal microscope slide, covered with a cover slip and examined with a 63× magnification lens and an argon/krypton laser having an excitation wavelength of 633 nm.

2.8. Statistical analysis

For all experiments, samples were prepared and tested at least in duplicate. Data for each sample were presented as mean values ± standard deviations. Statistical analysis was performed using the software package SPSS 20.0 (SPSS Inc., Chicago, USA). A *p*-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Characterisation of WPI emulsions

WPI-stabilised emulsions had a uniform size of 183.2 nm with a PDI value of 0.189 and a ζ-potential of −55.2 mV at pH 7.0 (Table 2). Size of WPI emulsions was not significantly different in the absence and presence of α-tocopherol, resveratrol, naringenin, α-tocopherol/resveratrol or α-tocopherol/naringenin. The PDI of the emulsions varied from 0.189 to 0.230. The ζ-potential of WPI emulsions was similar in the absence and presence of α-tocopherol, resveratrol, or α-tocopherol/resveratrol. The ζ-potential of WPI emulsions in the presence of naringenin or α-tocopherol/naringenin was lower than in its/their absence. All emulsions had absolute values of ζ-potential larger than 30 mV, indicating that they were stable due to electric repulsion between particles (Honary & Zahir, 2013).

3.2. Partition of bioactive components

Free α-tocopherol was not detected in the aqueous phase in the presence of resveratrol or naringenin (Table 3), suggesting that the vitamin was totally encapsulated in WPI emulsions. About 23% α-tocopherol was bound to free WPI in the aqueous phase, since α-tocopherol could interact with β-lactoglobulin (β-LG), a major whey protein, to form complexes (Liang et al., 2011). The percentage of α-tocopherol encapsulated in the oil droplets was about 77%, which was greater than that (~72%) in the absence of the polyphenols. It has been reported that resveratrol is not soluble in sunflower oil (Filip et al., 2003) and can bind to WPI at the oil-water interface (Wang et al., 2016). Moreover, resveratrol could improve the affinity of α-tocopherol to β-LG (Zhang, Liu,

Table 2
Characterisation of WPI emulsions without and with α-tocopherol, resveratrol and/or naringenin.[a]

Sample	Diameter (nm)	PDI	ζ-potential (mV)
Blank	183.2 ± 1.9^{a,b}	0.189 ± 0.005^{a}	−55.2 ± 1.2^{a}
α-Tocopherol	182.3 ± 1.3^{a,b}	0.203 ± 0.022^{a}	−53.3 ± 1.0^{a,b}
Reseratrol	180.4 ± 2.0^{a,b}	0.191 ± 0.017^{a}	−52.0 ± 0.3^{a,b}
Naringenin	187.4 ± 0.5^{b}	0.230 ± 0.009^{a}	−48.2 ± 0.6^{b}
α-Tocopherol/reseratrol	178.4 ± 2.3^{a}	0.194 ± 0.019^{a}	−51.5 ± 2.0^{a,b}
α-Tocopherol/naringenin	189.1 ± 0.5^{b}	0.221 ± 0.009^{a}	−47.2 ± 0.7^{b}

[a] Values within a column with different superscript letters are significantly different ($P < 0.05$).

Table 3
Percentage (%) of α-tocopherol (α-Toc) in the oil phase and polyphenol [resveratrol (Res) or naringenin (Nar)] at the oil-water interface, bound and free in the continuous phase of WPI emulsions.[a]

Sample	Oil phase/oil-water interface		Bound in the continuous phase		Free in the continuous phase	
	α-Toc	Polyphenol	α-Toc	Polyphenol	α-Toc	Polyphenol
WPI-α-Toc/Res	76.66 ± 0.32[a]	39.00 ± 0.50[a]	23.34 ± 0.32[a]	12.66 ± 0.22[a]	nd	48.34 ± 0.28[a]
WPI-α-Toc/Nar	76.60 ± 0.50[a]	47.31 ± 0.42[b]	23.40 ± 0.50[a]	10.72 ± 0.91[b]	nd	41.97 ± 0.49[b]

[a] Values within a column with different superscript letters are significantly different ($P < 0.05$); nd, not detected.

Subirade, Zhou, & Liang, 2014). Therefore, polyphenol-increased partitioning of α-tocopherol in oil droplets was due to the vitamin bound to WPI at the oil-water interface. The percentage of resveratrol was about 39% at the oil-water interface, 13% bound to free WPI in the aqueous phase and 48% free in the aqueous phase. The percentage of naringenin was about 47% at the oil-water interface, 11% bound to free WPI in the aqueous phase and 42% free in the aqueous phase. The total encapsulation efficiency of resveratrol and naringenin was 52% and 58% in WPI emulsions, respectively.

3.3. *In vitro digestion of WPI emulsions*

WPI emulsions had a unimodal size distribution with mean diameter around 190 nm (Fig. 2A) and ζ-potential of about +70 mV (Fig. 2B) in SGF. Both size and ζ-potential distributions were independent on digestion time, which was consistent with that of 20% soya oil emulsions stabilised by 1% WPI (Li, Ye, Lee, & Singh, 2013).

WPI emulsions also had a unimodal size distribution with mean diameter around 190 nm in SIF (Fig. 2C). These results indicate that digestion in SGF or SIF had no significant influence on the size of

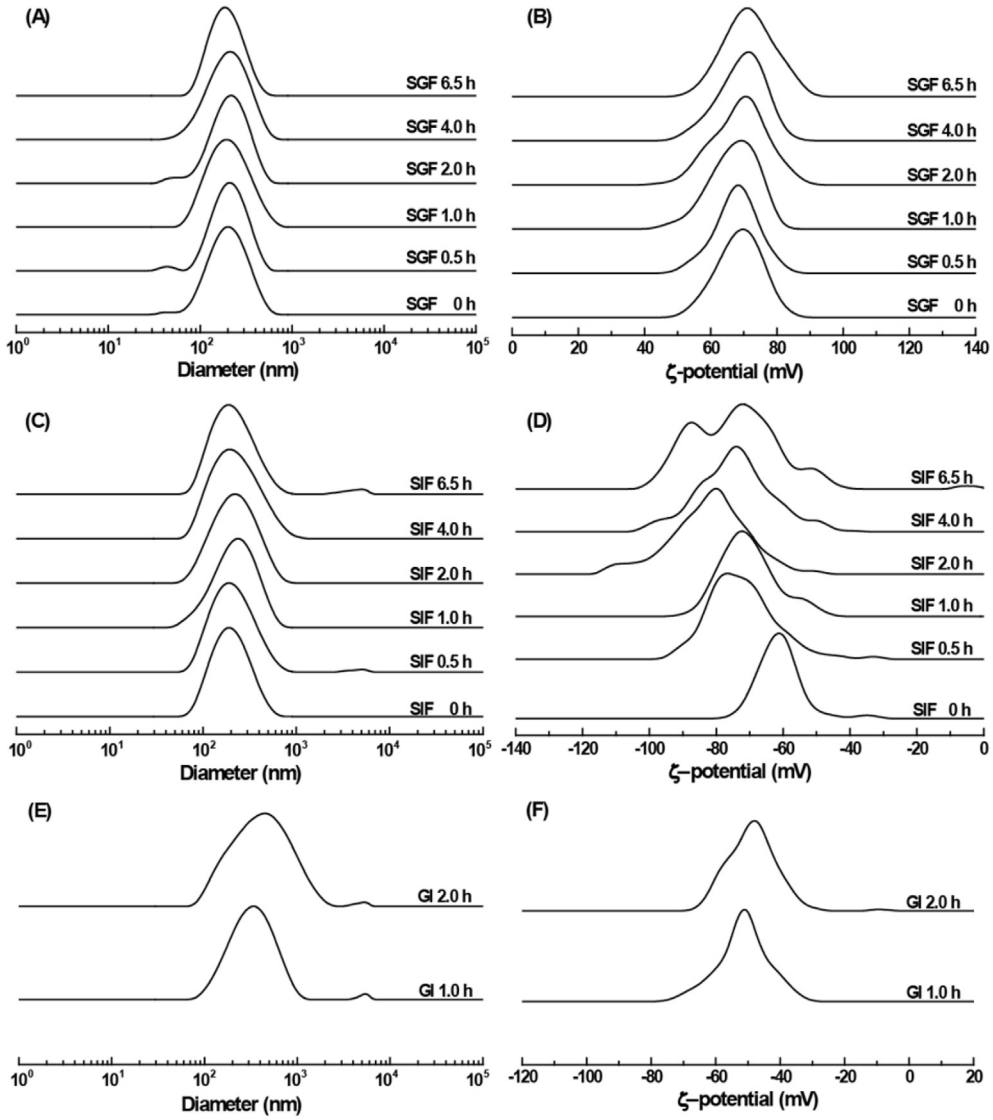

Fig. 2. Size (A, C, E) and ζ-potential (B, D, F) distributions of WPI emulsions in simulated gastric fluid (SGF, A and B), simulated intestinal fluid (SIF, C and D) and simulated gastrointestinal fluid (GI, E and F).

WPI emulsions. The ζ-potential of WPI emulsions was about −60 mV upon adding into SIF, which became more negative and multimodal with prolonging digestion time (Fig. 2D). The size of WPI emulsions in SIF under GI conditions (Fig. 2E) was larger than that in SIF alone (Fig. 2C), suggesting that gastric digestion accelerated the aggregation of WPI emulsions during the subsequent SIF digestion (Li et al., 2013). The ζ-potential of WPI emulsions in SIF under GI condition was −50 mV (Fig. 2F), whose absolute value was lower than that in SIF alone (Fig. 2D). Size distribution of the emulsions was beyond the measuring range under GI condition after 2.0 h. LSCM images indicate gradual coalescence of oil droplets with digestion time under GI conditions (Fig. 3).

Fig. 4A,B indicates that WPI coexisted at the oil-water interface and in the aqueous phase, with interfacial amount was greater. The bands of β-LG, α-LA and BSA reduced or disappeared with the appearance of <10 kDa bands, indicating that both WPI at the oil-water interface and in the aqueous phase hydrolysed into peptides in SGF (Fig. 4A). The intensity of peptides increased with digestion time. BSA hydrolysed completely into small peptides, whereas an appreciable amount of β-LG and α-LA still remained in the whole digestion fluid and the aqueous phase of digesta after 6.5 h. These results indicate that β-LG and α-LA were more resistant to pepsin than BSA, possibly due to the unfolding and rearrangement resulted from interfacial adsorption during pepsin digestion (Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009; Sarkar, Goh, & Singh, 2010). Fig. 4B shows that intensity of protein bands in the whole digestion fluid and the aqueous phase of digesta significantly decreased during the first 0.5 h of SIF digestion, and all protein bands disappeared by prolonging the digestion time up to 6.5 h. The results were similar after gastric digestion under GI conditions (Fig. 4C) and in SGF (Fig. 4A) for 0.5 h. However, all protein bands in the digesta disappeared after transferred into SIF for 0.5 h (Fig. 4C). These results demonstrated that the hydrolysis was more pronounced in SIF than in SGF, while the hydrolysis was more pronounced in GI fluid than in SGF and SIF.

It is noted that the main size distribution of WPI emulsions did not change (Fig. 2), although the protein was enzymatically hydrolysed by pepsin and/or pancreatin (Fig. 4) during digestion. Pancreatin is a mixture of trypsin, amylase, lipase and colipase, where amylase and lipase could form complexes and adsorb at the oil-water interface (Bauer, Jakob, & Mosenthin, 2005). Moreover, the peptides could also adsorb at the oil/water interface (Kenmogne-Domguia, Meynier, Viau, Llamas, & Genot, 2012; Persaud, Dalgleish, Nadeau, & Gauthier, 2000). The peptides produced from partial hydrolysis of whey protein possessed greater flexibility and higher emulsifying capacity than non-hydrolysed protein. However, the products of highly hydrolysis were too short to act as effective emulsifiers and of low hydrolysis had poorer hydro-solubility and emulsifying ability, leading to flocculation and coalescence (Euston, Finnigan, & Hirst, 2001), as seen in Figs. 2 and 3. The change in the droplet charge during digestion (Fig. 2) was mainly due to two aspects: firstly, proteolysis resulted in the exposure of more electrostatically-charged residues, stabilising the droplets by electrostatic repulsion (Chu, Ichikawa, Kanafusa, & Nakajima, 2007); secondly, the initial interfacial layers were displaced by some amphiphilic peptides, providing protection against complete coalescence (Singh & Sarkar, 2011).

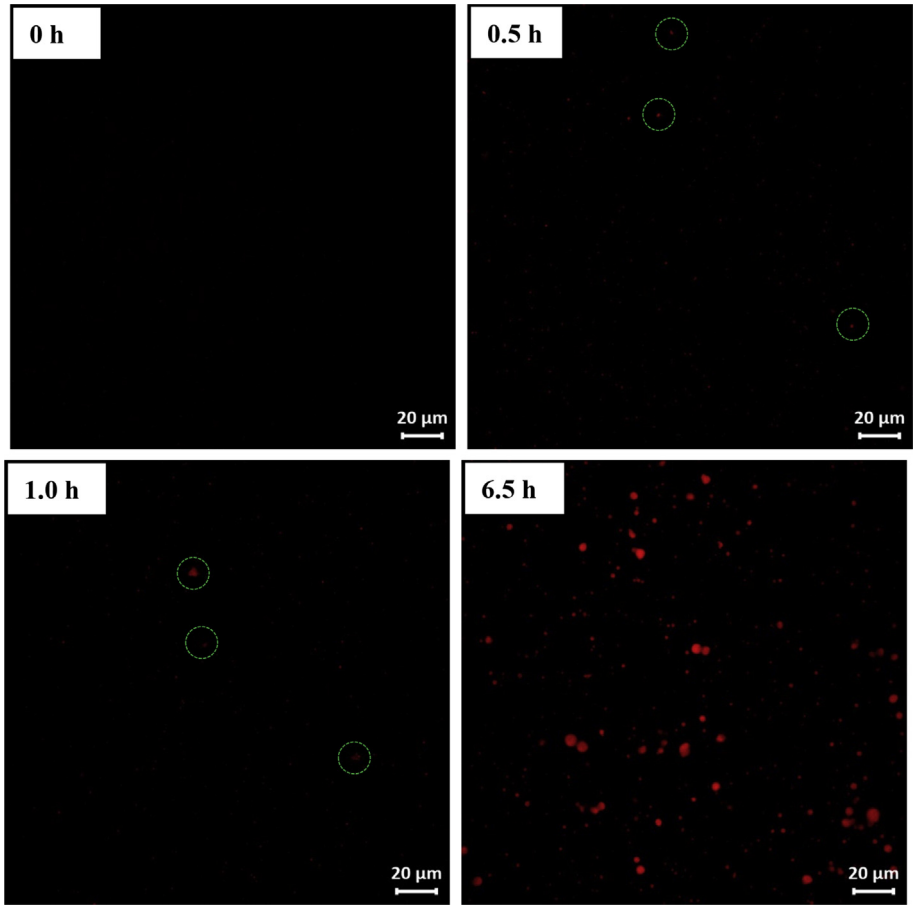

Fig. 3. LSCM images of WPI emulsions during in vitro gastric-intestinal digestion for 0, 0.5, 1.0 and 6.5 h.

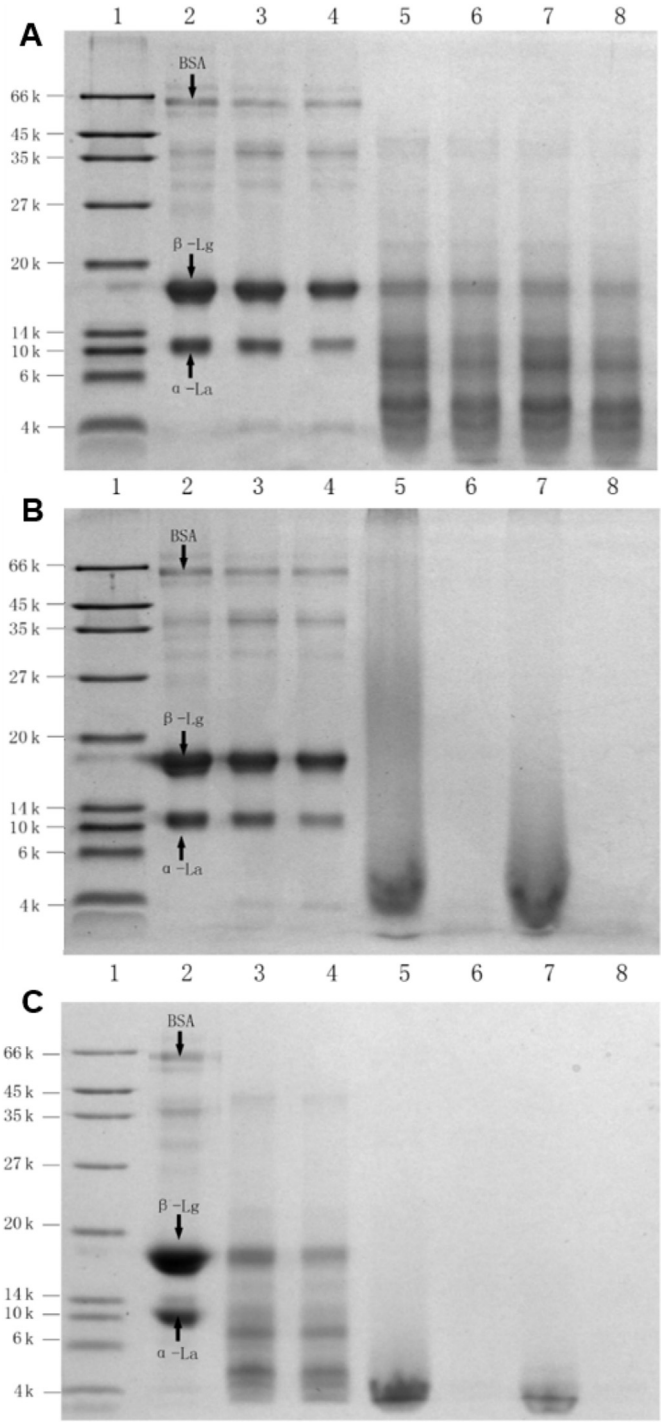

Fig. 4. SDS-PAGE of WPI emulsions in simulated gastric (A), intestinal (B), or gastrointestinal (C) fluids for 6.5 h. Panels A and B lanes are: 1, molecular mass markers; 2, WPI; 3, WPI emulsions; 4, the aqueous phase of WPI emulsions; 5 and 6, digestion fluid and its aqueous phase after 0.5 h; 7 and 8, digestion fluid and its aqueous phase after 6.5 h. Panel C lanes are: 1, molecular mass markers; 2, WPI emulsions; 3 and 4, digestion fluid and its aqueous phase after 0.5 h; 5 and 6, digestion fluid and its aqueous phase after 1.0 h; 7 and 8, digestion fluid and its aqueous phase after 6.5 h.

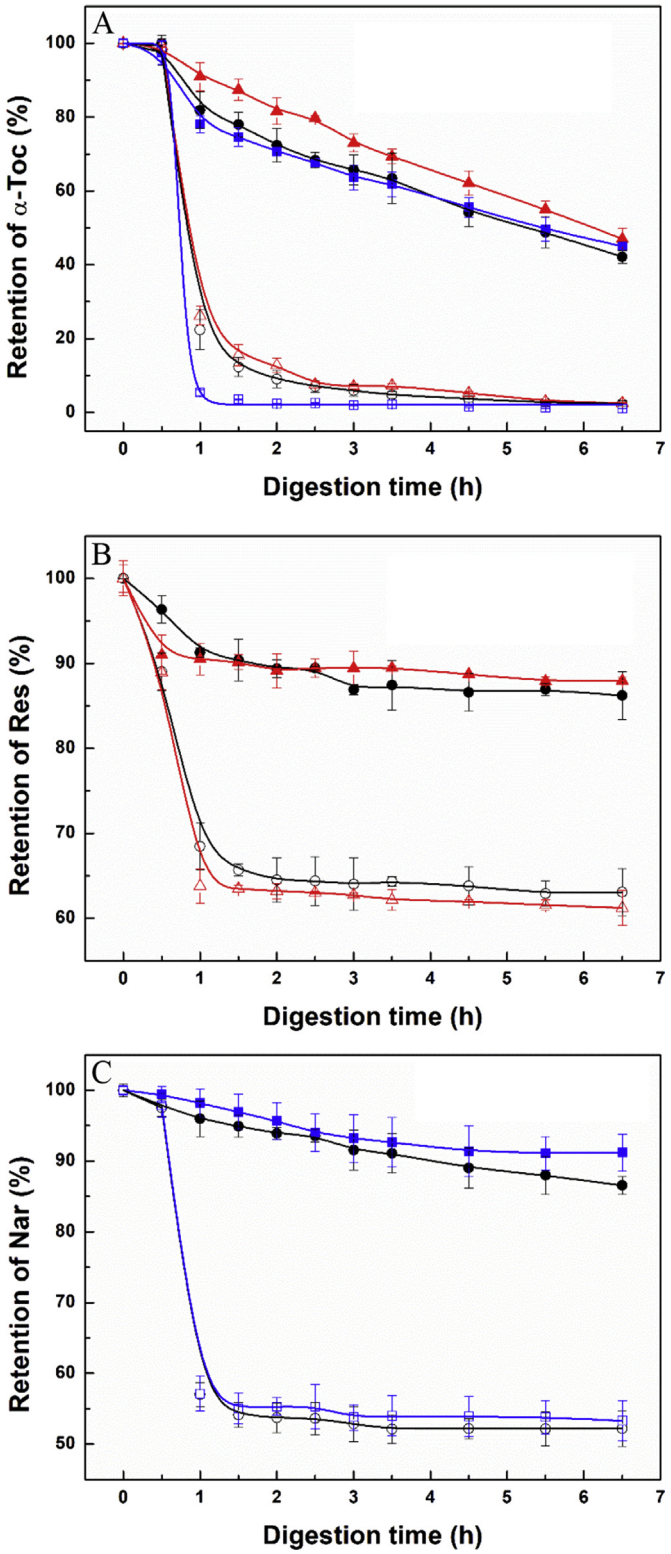

Fig. 5. Retention of (A) α-tocopherol (α-Toc) in the absence (●,○) and presence of resveratrol (Res; ▲,△) or naringenin (Nar; ■,□), (B) resveratrol in the absence (●,○) and presence (▲,△) of α-tocopherol, (C) naringenin in the absence (●,○) and presence (■,□) of α-tocopherol in WPI-stabilised emulsions during digestion in simulated gastrointestinal fluid (GIF, filled symbols) and in the aqueous phase of digesta (open symbols).

3.4. Retention of bioactive components during digestion

It has been reported that pure α-tocopherol remained about 45% in SGF after 0.5 h (Liang, LeungSok, Remondetto, & Subirade, 2010). α-Tocopherol encapsulated in WPI emulsions was stable in SGF and in the aqueous phase of the digesta after 0.5 h (Fig. 5A), suggesting that the encapsulation improved its stability in comparison with free α-tocopherol. α-Tocopherol began to

degrade upon transferring simulated digestion fluid from SGF to SIF. Its retention was similar in the absence and presence of naringenin but improved by resveratrol during digestion in the whole SIF. Protection of resveratrol against the loss of α-tocopherol in WPI emulsions has been reported at neutral pH during storage (Wang et al., 2016). The loss of α-tocopherol was faster in the aqueous phase of digesta than in the whole SIF (Fig. 5A). Its retention was similar in the absence and presence of resveratrol in the aqueous phase of digesta, consistent with that the binding of resveratrol to β-LG did not affect the protein's protection for α-tocopherol during storage (Zhang et al., 2014). The loss of α-tocopherol accelerated in the presence of naringenin in the aqueous phase of digesta.

The percentage of resveratrol remaining in digestion fluid and its aqueous phase was 96% and 89% in SGF after 0.5 h and 86% and 63% in GIF after 6.5 h, respectively (Fig. 5B), indicating that resveratrol bound at the oil-water interface was more stable than that in the continuous phase. Pure *trans*-resveratrol remained 47% by using a 3-phase digestion model including simulating oral, gastric and intestinal phases (Koga, Andrade, Ferruzzi, & Lee, 2016). Resveratrol in grapes degraded under pancreatic condition and remained only 30.5% after digestion in SGF for 2 h and in SIF for 2 h (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). In comparison, the stability of resveratrol in WPI emulsions was better during digestion (Fig. 5B). Stability of resveratrol could be improved by complexation with whey proteins (Hemar, Gerbeaud, Oliver, & Augustin, 2011; Liang, Tajmir-Riahi, & Subirade, 2008). Co-encapsulation with α-tocopherol had no significant influence on the stability of resveratrol in GIF and in the aqueous phase of the digesta (Fig. 5B).

Naringenin was stable in SGF and in the aqueous phase of digesta after 0.5 h (Fig. 5C). Naringenin degraded slowly and remained about 90% in the absence and presence of α-tocopherol in GIF after 6.5 h. However, its retention decreased rapidly in the aqueous phase upon transferring simulated digestion fluid from SGF into SIF and was about 55% after 1 h. The retention then kept constant during further digestion. Co-encapsulation with α-tocopherol had no significant influence on the stability of naringenin in GIF and in the aqueous phase of digesta (Fig. 5C). It has been reported that the bioaccessibility of free naringenin was about 20% after 5 min of saliva, 2 h of gastric then 2 h of intestinal digestion (Ban, Park, Lim, Choi, & Choi, 2015). Naringenin could bind to β-LG to form complexes (Shpigelman, Shoham, Israeli-Lev, & Livney, 2014). Therefore, complexation of naringenin with whey protein and encapsulation of naringenin at the oil-in-water interface increased its digestion stability (Fig. 5C).

The enhanced stability of bioactive components during digestion added as an emulsion may be due to a physical barrier of WPI and hydrolysed peptides adsorbed at the oil/water interface and their antioxidant activity (Adjonu, Doran, Torley, & Agboola, 2014; Embiriekah, Bulatovic, Boric, Zaric, & Rakin, 2018). It has been reported that the stability of bioactive components released from WPI emulsions was improved by binding to protein and/or hydrolysis products or by incorporating into the mixed micelles and sediment formed during digestion (Yang & McClements, 2013). Heat-denaturation of whey proteins caused exposure of more hydrophobic residues, which can strengthen hydrophobic interaction and hydrogen bonding of the protein with polyphenols (Liang & Subirade, 2012; Shpigelman, Israeli, & Livney, 2010). Similarly, proteolysis can expose more residues, which contribute not only to the binding with bioactive components but also to antioxidant activity (Adjonu et al., 2014; Embiriekah et al., 2018), thus keeping the stability of polyphenols during digestion after 1.5 h (Fig. 5B and C).

4. Conclusions

α-Tocopherol was totally encapsulated in WPI emulsions. The total encapsulation efficiency of resveratrol or naringenin was 52% and 58%, respectively. The percentages of α-tocopherol inside oil droplets and of resveratrol and naringenin at the oil-water interface were about 3.3, 3.1 and 4.4 times that bound by WPI in the aqueous phase, respectively. Addition of these bioactive components had no influence on size and ζ-potential of WPI emulsions, except that the presence of naringenin decreased ζ-potential. Enzymatic hydrolysis of WPI and coalescence of oil droplets occurred during gastrointestinal digestion. Addition of resveratrol improved digestive stability of α-tocopherol but naringenin did not. α-Tocopherol had no influence on digestive stability of resveratrol or naringenin. These results should be useful for the development of the carriers for simultaneous encapsulation and delivery of bioactive components with different solubilities.

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