Effect of resveratrol or ascorbic acid on the stability of α-tocopherol in O/W emulsions stabilized by whey protein isolate: Simultaneous encapsulation of the vitamin and the protective antioxidant

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ABSTRACT

Food proteins have been widely used as carrier materials due to their multiple functional properties. Hydrophobic bioactives are generally dissolved in the oil phase of O/W emulsions. Ligand-binding properties provide the possibility of binding bioactives to the protein membrane of oil droplets. In this study, the influence of whey protein isolate (WPI) concentration and amphiphilic resveratrol or hydrophilic ascorbic acid on the decomposition of α-tocopherol in the oil phase of WPI emulsions is considered. Impact of ascorbic acid, in the continuous phase, on the decomposition depended on the vitamin concentration. Resveratrol partitioned into the oil–water interface and the cis-isomer contributed most of the protective effect of this polyphenol. About 94% of α-tocopherol and 50% of resveratrol were found in the oil droplets stabilized by 0.01% WPI. These results suggest the feasibility of using the emulsifying and ligand-binding properties of WPI to produce carriers for simultaneous encapsulation of bioactives with different physicochemical properties.

1. Introduction

Since food proteins have high nutritional value and multiple functional properties, including emulsifying and gelling capacities, they have been used widely as carrier materials for the encapsulation and protection of bioactive molecules. Whey protein components, such as those of whey protein, partially unfold and aggregate to form continuous membranes around oil droplets, further increasing the stability of O/W emulsion against flocculation and/or coalescence. Whey protein isolate (WPI) is often used in food emulsion systems and could reportedly protect unsaturated-fatty-acid-riched flaxseed oil and fish oil against oxidation. Hydrophobic bioactive compounds, in general, may be protected in food or pharmaceutical products by dissolving them in an oil phase and then creating an O/W emulsion stabilized by one or more proteins.

Edible carrier systems can be classified into two groups: emulsion-based and lipid-free. In the latter type, entrapped bioactive nutrients generally interact with carrier materials to some extent by non-covalent interaction. Whey protein components, such as β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins, are all known ligand-binding proteins.β-Lactoglobulin constitutes about 50% of the protein in whey and can interact with a wide range of compounds, including fatty acids, vitamins, phospholipids, polyphenols and indole derivatives, to form protein–ligand complexes. This suggests the possibility of binding these compounds to the membrane of oil droplets in O/W emulsions stabilized by whey protein.

α-Tocopherol is the most abundant and biologically active form of vitamin E. As a lipophilic antioxidant, it can contribute to preventing chronic diseases associated with oxidative stress. However, its applications in food systems are limited, due to its sensitivity to heat, oxygen and light and also due to its hydrophobic nature. Its hydro-solubility and stability need to be improved, using an effective carrier system such as encapsulation. Various antioxidant molecules have been...
used in attempts to improve the oxidative stability of oil and protein in emulsions (Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009; Jayasinghe, Gotoh, & Wada, 2013; Vijanen, 2005). Their effectiveness depends on factors, such as polarity, location, mobility and micellization formation, as well as the type of emulsifiers in the system (Shahidi & Zhong, 2011).

Ascorbic acid, known as vitamin C, is a water-soluble vitamin that is a necessary component in human health. It is widely used as an additive in foods due to its strong antioxidant activity. Ascorbic acid has been added to bulk oil, buffer solution, and also oil-in-water (O/W) emulsion. In the O/W emulsions stabilized by Tween 60, decaglycerol monolaurate (SY-Glyster<sup>a</sup> ML-750) and medium-chain triacylglycerol, ascorbic acid could reportedly delay lipid oxidation of the emulsion systems (Ululata, Mc Clements, & Decher, 2015; Van Ruth, Roosen, Pothumus, & Jansen, 1999; Watanabe et al., 2010). However, ascorbic acid acted as a prooxidant in riboflavin-photosensitized O/W emulsions stabilized by Tween 20 (Kim, Decher, & Lee, 2012).

Resveratrol (3,5,4′-trihydroxystibine) possesses antioxidant activity, due to its polyphenolic structure. It exists as trans- and cis-isomers. The trans-isomer is the naturally occurring form produced by plants and it is converted to the cis-isomer under exposure to light (Lopez-Hernandez, Paseiro-Losada, Sanches-Silva, & Lage-Yusti, 2007; Trela & Waterhouse, 1996). Although most of the biological activities of resveratrol are attributed to the trans-isomer, it has been reported that cis-resveratrol inhibits collagen-induced platelet aggregation and kinase activity related to cancer (Bertelli et al., 1996; Jayatilake et al., 1993). Resveratrol could interact with various proteins, including soy protein, whey proteins, caseins and collagen, to form complexes (Bourassa, Bariyanga, & Tajmir-Riahi, 2013; Hemar, Gerbeaud, Oliver, & Augustin, 2011; Liang, Tajmir-Riahi, & Subirade, 2008; Wan, Wang, Wang, Yuan, & Yang, 2014; Zhang, Mi, & Shen, 2012). It has been reported that soy protein isolate-resveratrol complexes were efficient emulsifiers and improved the oxidative stability of corn oil/water emulsions (Wan et al., 2014).

In this study, a whey-protein-stabilized O/W emulsion was used as an encapsulation model in which α-tocopherol is protected against oxidative decomposition. The influence of whey protein concentration and of two additional antioxidants, namely resveratrol (amphiphilic) and ascorbic acid (hydrophilic), on the stability of α-tocopherol are investigated. The binding of resveratrol and ascorbic acid to whey protein and their stabilities were analyzed and are considered in terms of the possibility of simultaneous encapsulation of hydrophobic and amphiphilic/hydrophilic bioactive components, respectively, in the oil phase and at the oil–water interface.

2. Materials and methods

2.1. Materials

Whey protein isolate (Biopro, ~92%) was obtained from Davisco International Inc (Le Sueur, MN, USA). Sunflower oil (Brand Duoli), with a peroxide value of 4.22 ± 0.08 meq kg<sup>−1</sup> (estimated according to iodometric titration method), was purchased from a local retailer. (+)-α-Tocopherol (1000 IU/g), α-tocopherol acetate (HPLC grade, purity >96%), polydatin (HPLC grade, purity >95%) and nico- tinic acid (HPLC grade, purity >99.5%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Resveratrol (trans-isomer, purity >98%) and l-ascorbic acid (purity >99%) were purchased from Sango Biotech Co. (Shanghai, China). Methanol (HPLC grade) was purchased from Jiangsu Hanbon Sci. & Tech. Co. Ltd. (Huaian, Jiangsu, China). Absolute ethanol and n-hexane, sodium hydroxide (NaOH), hydrochloric acid (HCl), formic acid, metaphosphoric acid and Coomassie brilliant blue G250 were purchased from Sino-Pharm CNCM Ltd. (Shanghai, China).

2.2. Preparation of O/W emulsion

Oil-in-water emulsions, stabilized by WPI, were prepared according to the method described by Line, Remondetto, and Subirade (2005) with some modifications. Protein solution (2.0%, w/w) was prepared by dispersing WPI in distilled water and stirring for 1 h at ambient temperature to allow complete dissolution. The pH was adjusted to 7.0 using 1 M NaOH or 1 M HCl. Coarse emulsions, containing 4% sunflower oil, 10% α-tocopherol in the oil phase and 0.001–1% protein in the water phase (Appendix Table 1) were produced by mixing appropriate amounts of protein solution and sunflower oil with a high speed blender (ATS Engineering Ltd., Brampton, Ontario, Canada) operating at 10,000 rpm for 1 min. Oil droplet size was then reduced further by passing the coarse emulsion twice through an ATSAH2100 high-pressure homogenizer (ATS Engineering Ltd.) for about 1 min at a pressure of 50 MPa. Ascorbic acid or resveratrol was added to WPI solutions and incubated for 1 h before emulsification at 1, 2, 5, 10 and 50 times the molar concentration of WPI (Appendix Table 2). Sodium azide (0.02%, w/w) was added as an antimicrobial agent and samples were stored at ambient temperature (23 ± 3 °C). For all experiments, samples were prepared and tested at least in duplicate.

2.3. Size and ζ-potential measurements

Size distribution and ζ-potential of WPI emulsions were measured using a Zetasizer Nano ZS particle analyzer (Malvern Instruments Ltd, Malvern, Worcestershire, UK) with a He/Ne laser (λ = 633 nm). All measurements were conducted at 25 °C and at a scattering angle of 173°. The relative refractive index was 1.450 and the absorbance value was 0.001 for protein samples (Makra, Terejanszky, & Gyurcsanyi, 2015). Samples were diluted 100 times and incubated for 2 min before measurement.

2.4. Determination of WPI content at the oil/water interface of emulsions

Content of WPI adsorbed at the oil–water interface was determined according to the method described by Wan et al. (2014) with some modifications. WPI emulsions were centrifuged at 13,000g for 30 min at 4 °C, using a 5804 R centrifuge (Eppendorf Co. Ltd, Hamburg, Germany). The WPI content of the subnatants was determined, using the Coomassie brilliant blue method (Bradford, 1976). The interfacial protein proportion was calculated from the difference between the amount of protein used to prepare the emulsion and that measured in the subnatants after centrifugation.

2.5. Quantification of α-tocopherol using HPLC

The α-tocopherol content was determined by using our method published previously (Liang, Leung Sok Line, Remondetto, & Subirade, 2010; Liang, Tremblay-Hebert, & Subirade, 2011) with some modifications. Exactly 0.4 ml of WPI emulsion or subnatant, obtained by centrifuging at 13,000g for 30 min at 4 °C was vortex-mixed for 15 s with 0.4 ml of 10 mM α-tocopherol acetate (internal standard) in ethanol. Extraction of α-tocopherol was performed by adding 4 ml of hexane, then shaking for 1 min at 10,000 rpm, using a high speed shearing mixer (ATS Engineering Ltd.) and then centrifuging at 2500g at 4 °C for 5 min. Exactly 1.0 ml of hexane supernatant was collected and evaporated under nitrogen. The α-tocopherol residue was re-dissolved in 2 ml of methanol for about 45 min and passed through a 0.45 μm syringe filter.
filter into a 2 ml amber vial. The solution (10 µl) was injected into an HPLC system equipped with 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 and the column temperature was 45 °C. The retention times of α-tocopherol and α-tocopherol acetate were about 8.5 and 10.6 min, respectively. The efficiency of encapsulation of α-tocopherol in the oil phase was calculated from the difference between the vitamin content in the whole emulsion and that in the subnatant divided by the content in the whole emulsion.

2.6. Quantitation of ascorbic acid using HPLC

Ascorbic acid content was determined according to the method of Tarrago-Trani, Phillips, and Cotty (2012). Exactly 0.4 ml of WPI emulsion or subnatant was added to 0.4 ml of 15 µM or 150 µM nicotinic acid (internal standard) aqueous solution and followed by vortex-mixing for 15 s. Exactly 2 ml of metaphosphoric acid were added, followed by vortex mixing for 1 min and then centrifuging at 3500g at 4 °C for 5 min. Exactly 1.0 ml of supernatant was collected and passed through a 0.45 µm syringe filter into a 2 ml amber vial. All samples were protected from light and kept in an ice bath to avoid loss of ascorbic acid during the process. The solution (20 µl) was injected into an HPLC system equipped with a T3 column (5 µm, 4.6 mm × 250 mm, Waters, USA). The mobile phase consisted of a mixture of 0.3% (v/v) formic acid in a mixture of methanol and distilled water (90:10, v/v) at a flow rate of 1 ml min⁻¹ and the column temperature was 30 °C. UV detection at 245 nm was used. The retention times of ascorbic acid and nicotinic acid were, respectively, 4.8 and 5.6 min.

2.7. Quantitation of resveratrol using HPLC

The resveratrol contents of the emulsion and its continuous phase were determined by liquid–liquid extraction, followed by separation of the two resveratrol isomers, using HPLC (Camont et al., 2009). Exactly 0.4 ml emulsion or subnatant, obtained by centrifuging at 13,000g for 30 min at 4 °C, was mixed with 0.4 ml of polydextin methanol solution (internal standard, 15 µM or 150 µM) by vortexing for 15 s. Exactly 4 ml of methanol were added by vortex-mixing for 1 min and then centrifuging at 3500g at 4 °C for 5 min. Exactly 1.0 ml supernatants were collected and passed through a 0.45 µm syringe filter into 2 ml amber vials. The solution (20 µl) was injected into an HPLC system equipped with a C18 column (5 µm, 4.6 mm × 250 mm, Waters, USA). The mobile phase was a mixture of methanol and distilled water (50:50, v/v), the flow rate was 1 ml min⁻¹ and the column temperature was 30 °C. UV detections were used at 295 and 285 nm to quantitate α- and cis-resveratrol, respectively. Retention times were about 8.5 and 10.6 min, respectively, for polydatin, trans-resveratrol and cis-resveratrol.

Since trans-resveratrol and cis-resveratrol have different spectral characteristics, their molar absorptivities were analyzed according to a method described by Trela and Waterhouse (1996) with modifications. Trans-resveratrol was dissolved in a mixture of methanol and distilled water (90:10, v/v) at a concentration of 150 µM. The solution was exposed to UVA (λ = 365 nm), using a UVL-21 ultraviolet lamp (VWR International Inc., Radnor, PA, USA) and analyzed at intervals of 20 min (up to 60 min) by HPLC as described above. The decrease in the trans-isomer peak area and the increase in the cis-isomer peak area were used to calculate the ratio of molar absorptivities (~2.13) and then used to quantify isomer stability, which was expressed as a percentage relative to the initial concentration of trans-isomer.

2.8. Statistical analysis

Data are presented as mean values ± standard deviations and analyzed for significant differences, using the online GraphPad QuikCalcs Free t-test calculator (GraphPad Software Inc. San Diego, CA, USA).

3. Results and discussion

3.1. Characterization of WPI stabilized emulsions

The structure and physical stability of O/W emulsions stabilized by WPI were investigated by analyzing size distribution and ζ-potential. When the concentration of WPI was 0.001%, droplet size in fresh emulsions was distributed in two peaks, around 130 and 460 nm (Fig. 1). The size distribution remained unchanged for 11 days, after which two peaks were observed around 295 and 6440 nm, suggesting aggregation, resulting possibly from flocculation and coalescence (Kim, Decker, & McEvelyn, 2004). Droplet size distribution in fresh emulsions containing 0.01% WPI was similar and remained so until day 24. Further increases in the protein concentration resulted in monotonic droplet size distributions. At 0.1% and 1% WPI, the size distribution was broad, with a peak around 220 nm, and remained stable for 24 days. Zeta-potentials of emulsions were negative (Fig. 2), since the isolectric points of the principal whey proteins, namely β-lactoglobulin and α-lactalbumin, are around pH 5. Fresh emulsions had ζ-potentials of about −34 mV at 0.001% WPI and of about −39 mV at higher protein concentrations. The ζ-potentials increased gradually during storage and reached about −50 mV after 24 days. This may be due to an increase in pH, which reached about 7.7 after storage. Strongly negative ζ-potentials prevent aggregation of emulsion droplets and increase their stability through electrostatic repulsion.

Adding of resveratrol and ascorbic acid, respectively, at concentrations up to 50 and 10 times the molar concentration of WPI, had no significant influence on the droplet size, ζ-potential or pH of fresh emulsions stabilized with 0.01% WPI (data not shown). However, significant aggregation and creaming were observed when the ascorbic acid concentration was 50 times the concentration of WPI, which resulted in the pH dropping to 5.9. Ascorbic acid was therefore used at a lower concentration in subsequent experiments.

3.2. Encapsulation of bioactive molecules using WPI emulsions

α-Tocopherol in WPI-stabilized emulsions was dissolved in the oil phase. When the concentrations of WPI were 0.001%, 0.01%, 0.1% and 1%, encapsulation efficiencies of α-tocopherol were, respectively, 90.3(±1.74)%, 93.7(±0.85)%, 87.5(±1.13)%, 83.8(±2.76)% while the proportions of the protein found in the continuous phase were, respectively, 29.2(±0.29)%, 30.1(±0.11)%, 37.0(±0.67)% and 39.9(±0.36)% (Appendix Table 3). β-Lactoglobulin reportedly interacts with α-tocopherol to form complexes and improves its hydrosolubility (Lieng et al., 2011). Higher concentrations of WPI should therefore cause more α-tocopherol to dissolve in the continuous phase and encapsulation efficiency to decrease. A WPI concentration of 0.01% was thus selected for the study of the influence of ascorbic acid or resveratrol on the stability of α-tocopherol encapsulated in the oil phase of O/W emulsions.

After centrifuging the emulsion, ascorbic acid was not found bound in the droplet phase. Using equilibrium dialysis and gel filtration, it has been found that ascorbic acid does not interact with β-lactoglobulin or other bovine whey proteins (Puyol, Perez, Mata, & Calvo, 1994). Since ascorbic acid is very hydrophilic, it likely
remains freely dissolved in the aqueous phase of emulsions. This is consistent with the partition behavior of hydrophilic anthocyanins in emulsions stabilized by whey protein (Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005).

In contrast, about 50% of the resveratrol was bound to emulsion droplets (Appendix Table 4). The proportion of bound resveratrol remained essentially constant, being 49.4(±3.60)%, 49.8(±3.01)%, 50.2(±1.08)%, 48.8(±0.73)% and 51.0(±8.28)% when the resveratrol/WPI ratios were 1, 2, 5, 10 and 50, respectively. It has been reported that resveratrol interacts with whey protein or its fractions to form 1:1 complexes (Hemar et al., 2011; Liang et al., 2008). In this study, resveratrol was added to WPI solutions to form protein–ligand complexes before emulsion formation. In view of its very low solubility in both water and oil, it seems probable that the resveratrol molecules bound to WPI at the oil droplet interface.

Oil-in-water emulsions consist of three essential components: the dispersed oil phase (in the form of droplets), the continuous aqueous phase, and the oil–water interface (stabilized by whey protein in the present case). The partition of various molecules among the three different components depends on their solubility characteristics, surface activity and affinity for the emulsifier at the oil–water interface (Shahidi & Zhong, 2011; Wan et al., 2014). Being hydrophobic, α-tocopherol was found mostly in the oil phase, while ascorbic acid was dissolved in the aqueous phase. Due to its amphiphilic nature, resveratrol partitions between the aqueous phase and the oil–water interface by binding to whey proteins. These results suggest the feasibility of encapsulating α-tocopherol and resveratrol simultaneously in WPI-stabilized O/W emulsions.

3.3. Stability of bioactive molecules in WPI emulsions

3.3.1. Stability of α-tocopherol

α-Tocopherol is sensitive to environmental factors. Under storage conditions with exposure to air at room temperature, α-tocopherol decomposed quickly, only 52% remaining after two days, 37% after four days and only 6% after 25 days in emulsions stabilized with 0.001% WPI (Fig. 3). The decomposition was slower than that for α-tocopherol dispersed in aqueous solution (Liang et al., 2011). A significant decrease in decomposition in emulsions was observed as the concentration of WPI increased. In the case of 0.01% WPI, 81% and 30% of the α-tocopherol remained after, respectively, 4 and 25 days. At WPI concentrations of 0.1% and 1%, decomposition was delayed over the first two days and approximately 60% and 77%, respectively, remained after 25 days. In general, the higher the WPI concentration, the better was the protective effect. These results are consistent with lipid oxidation in emulsions stabilized by WPI above 0.5% (Ries, Ye, Haisman, & Singh, 2010). The physical barrier of WPI adsorbed to the oil/water interface and the antioxidant effect of the protein, both at the interface and in the continuous phase, provide protection against decomposition (Ries et al., 2010). As shown above, encapsulation efficiency of α-tocopherol decreased slightly as the protein concen-
The influence of ascorbic acid or resveratrol on the stability of α-tocopherol is shown in Fig. 4. Inclusion of ascorbic acid in WPI emulsions significantly delayed the decomposition of encapsulated α-tocopherol, about 45% of which remained after 25 days when the molar concentration of ascorbic acid was equal to that of WPI (Fig. 4A). However, this protection decreased as the ascorbic acid concentration increased. Decompositions of α-tocopherol were similar in the absence and presence of ascorbic acid at an ascorbic acid/WPI ratio of 5. Further increases in the ascorbic acid concentration resulted in faster decomposition of α-tocopherol than in the absence of ascorbic acid, with only about 5% of the α-tocopherol remaining after 25 days at an ascorbic acid/WPI ratio of 10. In the presence of resveratrol, the decomposition of α-tocopherol was significantly delayed, with about 44% α-tocopherol remaining after 25 days, essentially independent of the resveratrol/WPI molar ratio (Fig. 4B).

3.3.2. Stability of ascorbic acid
Ascorbic acid is sensitive to environmental factors, such as oxygen, light, temperature, pH and water activity, and is highly susceptible to oxidation. The stability of ascorbic acid dispersed in WPI emulsions is presented in Fig. 5. Loss of ascorbic acid during sample storage was very fast, and almost complete after 3, 7, 10 and 17 h, respectively, at ascorbic acid/WPI molar ratios of 1, 2, 5 and 10. These results agreed with those obtained for ascorbic acid dispersed in the continuous phase after centrifugation (data not shown).

The concentration increased above 0.01%. However, no initial quick decomposition was observed (Fig. 3). This might be due to binding of α-tocopherol molecules to WPI dispersed in the continuous phase (Liang et al., 2011).

Fig. 2. Zeta-potential of O/W emulsions stabilized by WPI at concentrations of 0.001% (A), 0.01% (B), 0.1% (C) and 1% (D) and stored for up to 24 days.

Fig. 3. Persistence of α-tocopherol in O/W emulsions made with various concentrations of WPI and stored for up to 25 days.
shown). As a strong antioxidant, ascorbic acid can regenerate α-tocopherol (Carr & Frei, 1999) and provide a protective effect against the decomposition of α-tocopherol (Fig. 4). Upon oxidation, ascorbic acid becomes a pro-oxidant, in turn causing peroxidation of lipid and other molecules (Chakraborty, Ramani, Sherlin, Premkumar, & Natesan, 2014; Jayasinghe et al., 2013). This may be a reason for the negative effect of higher concentrations of ascorbic acid on the stability of α-tocopherol (Fig. 4). On the other hand, as the emulsions ages, pro-oxidation allows WPI to anchor more firmly in the oil phase via carbonyl groups (Vijanen, 2005), thus providing a protective effect after complete decomposition of ascorbic acid.

3.3.3. Stability of resveratrol

Both trans- and cis-resveratrol are unstable and sensitive to oxidation/degradation (Silva et al., 2013). Their stability over time was therefore analyzed in both the continuous phase and the emulsion as a whole. A significant initial decrease in trans-resveratrol content in the whole emulsion was observed when the initial molar resveratrol concentrations were lower than and equal to 10 times that of WPI (Fig. 6A). Loss of trans-resveratrol was almost complete after about 12 days. At higher resveratrol concentration, the loss was slower but was almost complete after 20 days. The decrease in trans-resveratrol content was slightly slower in the continuous phase (Fig. 6B). These results suggest that loss of trans-resveratrol was faster at the oil/water interface than in the continuous phase of the emulsion.

Cis-resveratrol could not be detected in the continuous phase or the emulsions at the resveratrol/WPI ratio of 1. Starting at an initial ratio of 2, the content of cis-isomer in the whole emulsion increased gradually over time, reaching about 12% on day 6, and then began to decrease, with only 5% remaining after 24 days (Fig. 6C). At an initial ratio of 10, the increase in the cis-isomer content was faster, reaching about 24% after 12 days and decreasing to 17% after 24 days. From an initial ratio of 50, the cis-resveratrol content increased monotonically to 30% after 24 days. The change in the cis-isomer content displayed a similar pattern but was significantly greater in the continuous phase (Fig. 6D). The difference between the concentration in the continuous phase and in the emulsion appeared greater than that for trans-resveratrol.

Fig. 6E and F show the stability of total resveratrol in the emulsion as a whole and in the continuous phase. In the emulsion, resveratrol, at an initial resveratrol/WPI ratio of 1, decreased quickly and was almost completely lost after 8 days, similar to the kinetics of cis-isomer loss (Fig. 6A). The decrease in the total resveratrol content slowed as the initial ratio was increased (Fig. 6E), the residual contents after 25 days being 6%, 13%, 20% and 34%, respectively, for initial ratios of 2, 5, 10 and 50. Loss of total resveratrol was less than that of the trans-isomer (Fig. 6A) due to formation of cis-isomer by isomerization (Fig. 6C). The loss of total resveratrol in the continuous phase after 25 days, followed a similar pattern but was considerably smaller, with 12%, 27%, 52% and 68% remaining, respectively, for initial ratios of 2, 5, 10 and 50 (Fig. 6F). These results suggest that losses of total resveratrol were smaller in the continuous phase than those at the emulsion oil/water interface, due to a smaller loss of trans-isomer and more formation of cis-isomer in the continuous phase (Fig. 6A–D). Around 50% of the resveratrol was bound to emulsion droplets, regardless of the resveratrol/WPI ratios (Appendix Table 4). Therefore, with increasing resveratrol/WPI ratios, the interactions between WPI and resveratrol (Hemar et al., 2011; Liang et al., 2008) in the continuous phase may be an important factor leading to the slow decrease of total resveratrol.

According to the polar paradox, oil-in-water emulsions are better protected from oxidation by non-polar and amphiphilic antioxidants than by polar ones, which is attributed primarily to the greater affinity of the former for the oil–water interface (Shahidi & Zhong, 2011). Being amphiphilic, resveratrol was absorbed partially into WPI at the oil–water interface, where its degradation was faster than in the continuous phase (Fig. 6E and F), thus providing a greater protective effect against α-tocopherol decomposition than did ascorbic acid (Fig. 4). Loss of trans-resveratrol was fast due to isomerization to cis-isomer and degradation.
It is thus possible that the protection of \( \alpha \)-tocopherol during long-term storage was primarily due to cis-resveratrol (Fig. 4B).

It has been reported that lipid-soluble vitamin E and water-soluble vitamin B\(_2\) can be encapsulated together in water–oil–water double emulsions stabilized by WPI–polysaccharide complexes (Li et al., 2012). However, preparation of double emulsions is more complicated and their stability is not as good as that of single emulsions. Due to its amphiphilic nature and affinity for whey proteins (Hemar et al., 2011; Liang et al.,...
resveratrol partitions into the oil–water interface of O/W emulsions, suggesting the possibility of encapsulating α-tocopherol and resveratrol together in WPI-stabilized emulsions. Simultaneous encapsulation of bioactive components with different physicochemical properties will no doubt expand the applications of single-emulsion-based carrier systems to functional food. However, only about 50% of the resveratrol was associated with the oil/water interface and the loss of this portion was faster than the loss of the portion that remained in the continuous phase (Fig. 6). In addition to their emulsifying and ligand-binding properties, whey proteins can also form gels and interact with polysaccharides (Chen et al., 2006; De Wolf & Brett, 2000; Matalanis, Jones, & McClements, 2011). We shall continue to improve encapsulation efficiency of resveratrol and its stability at the oil–water interface by exploiting the multiple functional properties of whey proteins in the future.

4. Conclusions

WPI-stabilized oil-in-water emulsions were prepared at protein concentrations ranging from 0.001% to 1%. The efficiency of encapsulation of α-tocopherol in the oil phase peaked at ∼93.71% at 0.01% protein. The higher the WPI concentration, the better was the protection of α-tocopherol against decomposition. The stabilizing effect of dispersing ascorbic acid in the continuous aqueous phase depended on concentration. Ascorbic acid delayed α-tocopherol decomposition at ascorbic acid/WPI molar ratios below 5 but promoted decomposition at higher concentration. Resveratrol partitioned into the oil–water interface by binding to WPI and improved the overall stability of α-tocopherol, which was attributed mainly to the cis-isomer. The results suggest the feasibility of encapsulating α-tocopherol and resveratrol together in the oil phase and at the oil–water interface of O/W emulsions. It is thus possible to use assemblies based on emulsifying and ligand-binding properties of WPI for simultaneous encapsulation of bioactive components with different physicochemical properties. These experiments provide support for expanding the use of single emulsions as carrier systems in functional food applications.

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Appendix A. Supplementary data

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References


