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Vitamin D3 and phytosterols affect the properties of polyglycerol polyricinoleate (PGPR) and protein interfaces

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ABSTRACT

The present work tested the effect of addition of hydrophobic compounds at interfaces containing polyglycerol polyricinoleate (PGPR) and β-lactoglobulin or sodium caseinate, using drop tensiometry. Vitamin D3 or phytosterols were also tested on a model double emulsion stabilized with PGPR and sodium caseinate. Emulsions were prepared using a high pressure homogenizer, and changes in particle size were followed using light scattering. The encapsulation efficiency of the double emulsions was estimated by adding Mg^{2+} and measuring its release from the inner droplet. PGPR dominated the oil-water interfacial properties. In the presence of proteins there was a decrease of the interfacial tension, with little changes in the viscoelastic properties. The presence of vitamin D3 and phytosterols further affected the interfacial properties. Double emulsions were then prepared with 2% PGPR. While control emulsions showed limited stability with an increase in the particle size after one week of storage, emulsions containing 0.05% (w/w) of vitamin D3 or phytosterol resulted in better stability over the storage period. Results suggested that vitamin D_3 and phytosterol molecules may interact with the emulsifiers at the interface, affecting the physico-chemical properties, and possibly their release during digestion.

1. Introduction

Double emulsions have gained attention in the past years, because of their increased interest in cosmetic, pharmaceutical and food applications. A water-in-oil-in-water emulsion ($W_1/O/W_2$) consists of water-in-oil (W_1/O) droplets dispersed in a secondary aqueous phase (W_2). It is possible to carry within this system, both hydrophilic and hydrophobic substances. Different phases can protect bioactives or nutrients from unwanted reactions (Dickinson, 2011; McClements, 2011). Double emulsions continue to be focus of basic research, as the interactions occurring at the interface between the various components in the mixtures are yet to be fully understood.

A stable primary W_1/O emulsion is essential to obtain a double emulsion system applicable to the food industry. Efforts have been carried out to improve steric repulsion at the interface of the inner droplets (Bouyer et al., 2011; Garti, Aserin, Tiunova, & Binyamin, 1999; Lutz, Aserin, Wicker, & Garti, 2009; Muschiolik et al., 2006; Su, Flanagan, Hemar, & Singh, 2006). Polyglycerol polyricinoleate (PGPR), a synthetic emulsifier, is the most common molecule employed to stabilize primary W_1/O emulsion droplets. Albeit it has gained GRAS status (generally recognized as safe) from FDA (Food and Drug Administration, 2006), the facts of being synthetic and having labeling requirements make its reduction or removal from the formulations desirable (Márquez, Medrano, Panizzolo, & Wagner, 2010). Mixing emulsifiers has been shown as a good strategy to decrease PGPR in $W_1/O/W_2$ emulsions, and synergistic effects have been found, for example, between sodium caseinate and PGPR (Su et al., 2006). The interactions between PGPR with other emulsifiers may improve stability of the inner phase and enhance the entrapment efficiency of the emulsion (Gülseren & Corredig, 2012, 2014).

Other hydrophobic compounds present in the oil may also affect the interface. When bioactive compounds are added, their release during digestion may depend on their interaction with the interface. A recent *in-vitro* digestion study of oil-in-water emulsions loaded with hydrophobic molecules showed differences in the

Abbreviations: β-lg, β-lactoglobulin; β-sit, β-sitosterol; DWS, Diffusing wave spectroscopy; NaCas, sodium caseinate; PGPR, polyglycerol polyricinoleate; PS, phytosterols; Vit. D3, vitamin D3; W_1/O, water-in-oil; $W_1/O/W_2$, water-in-oil-in-water.

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transfer of the bioactives to the aqueous phase in the absence of lipolysis, suggesting partitioning of the molecules at the interface depending on their structure. For example, vitamin D3 and phytosterols were transferred into mixed micelles in absence of lipolysis to a higher extent (around 30%) compared to β-carotene and coenzyme Q10 (only about 2%) (Malaki Nik, Corredig, Wright, & Nik, 2011). The authors suggested that phytosterols and vitamin D3 would be preferentially closer to the interface of the oil droplet, while β-carotene and coenzyme Q10 would place towards the core.

Interactions between hydrophobic bioactives and emulsifiers may occur, and affect the properties of the oil-water interface. In this study, it was hypothesized that if interactions occur, interfacial properties will change, as well as the physico-chemical properties of W_1/O as well as $W_1/O/W_2$ emulsions. To test this hypothesis vitamin D3 and phytosterols were used as model bioactives, and interactions at the interfaces were tested using mixed interfaces containing PGPR and milk proteins. Furthermore, the physico-chemical properties of W_1/O and $W_1/O/W_2$ emulsions stabilized with PGPR and sodium caseinate, and containing vitamin D3 and phytosterols were studied.

2. Material and methods

2.1. Materials

Soybean oil (S7381, Sigma–Aldrich, St. Louis, MO, USA), was used as oil phase. In case of tensiometry measurements, the oil was pre-treated with Florisil® (46385, Sigma–Aldrich) (Gülseren & Corredig, 2012). In brief, oil (45 g) was mixed with 4.5 g Florisil using a shaking plate (60 rpm for 2 h). The adsorbent was then filtered (Whatman No:1, cellulose filter, Fisher Scientific, Fair Lawn, NY, USA). This process was carried out three times and during the final step, two filter papers were used. Emulsions were prepared using MilliQ water, while tensiometry experiments were carried out using HPLC grade water (Fisher Scientific). Synthetic emulsifier polyglycerol polyricinoleate (PGPR 4150) was obtained by Palsgaard (Juelsminde, Denmark) which stated the content of minimum 75% di-, tri- and tetraglycerols with a maximum of 10% of heptaglycerol or higher. Phytosterols (β-sitosterol (β-sit) as main compound – 85451) and vitamin D3 (VitD3) (Cholecalciferol – C9756) as well β-lactoglobulin (β-lg) (L0130), were all obtained by Sigma–Aldrich, while sodium caseinate (NaCas) (NaCas 180) was purchased from Fonterra Inc. (Rosemont, IL, USA). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$ – BP214) and sodium chloride (NaCl – S271) were obtained by Fisher Scientific.

2.2. Drop shape tensiometry

The interfacial tension and dilational elasticity modulus of a soybean oil-water interface was measured using drop shape tensiometry (Tracker, IT Concept, Longessaigne, France) at room temperature. The aqueous drop (6 μl), was delivered by a syringe into an optical glass cuvette containing the oil phase. A video image was obtained with a CCD camera and processed using the Young–Laplace equation, while monitoring its shape. The interfacial tension was recorded over time (Benjamins, Cagna, & Lucassen-Reynders, 1996; Fainerman, Lylyk, Makievski, & Miller, 2004; Wang & Narsimhan, 2005). After an equilibration period of at least 3 h, the equilibrium interfacial tension (γ) was extrapolated, with results not significantly different from overnight equilibration experiments (Dopierala et al., 2011). After 3 h oscillatory changes of volume/surface area of the drop were performed with the strain amplitude kept constant at 10% ($\Delta A/A = 0.1$, where A is the droplet surface area) and with harmonic expansion and dilation cycles ranging 5–100 mHz of frequency. The surface dilational modulus was obtained based on the following equation:

$$E^{SD} = d\gamma(d \ln A) \quad (1)$$

2.3. Emulsions preparation

Water-in-oil emulsions (primary emulsions for the $W_1/O/W_2$) were prepared by mixing 30% (w/w) of aqueous and 70% (w/w) oil phases. The internal aqueous phase consisted of 0.5% (w/w) sodium caseinate and 0.1 M NaCl. $MgCl_2$ (0.1 M) was also added to the internal aqueous phase in selected systems to follow its encapsulation efficiency in $W_1/O/W_2$. The oil phase contained 2% (w/w) PGPR with or without 0.05% (w/w) of bioactives (vitamin D3 or phytosterols) in soybean oil. The oil and the water were pre-mixed using an ultra-turrax (PowerGen 125, Fisher Scientific) for 1 min, and this emulsion was then circulated through a microfluidizer (Microfluidcs, Newton, MA, USA) at 65 MPa for 5 min to form the final W_1/O emulsion, time equivalent of 10 passes of the sample volume of 30 mL.

A dispersion of 10% (w/w) primary emulsion (W_1/O emulsion) was then mixed in 2% (w/w) sodium caseinate (NaCas) solution (prepared with MilliQ water) to obtain a $W_1/O/W_2$ emulsion with final ratio of 3:7:90. To avoid multimodal distribution, small oil globules and possible disruption of the inner aqueous droplets, which would impact on encapsulation of Mg^{2+} ions, a gentler homogenization step was necessary. After optimization, the second emulsification step was carried by passing the formulation once through the homogenizer (EmulsiFlex C5, Avestin, Ottawa, Canada) at approximately 3.5 MPa.

2.4. Emulsion characterization

Diffusing wave spectroscopy (DWS) measurements were carried out to obtain the average droplet radius of primary W_1/O emulsions. The sample was transferred to a 10 mm optical glass cuvette (Hellma Canada Limited, Concord, Canada) and placed in a 25 °C water bath, which was illuminated by a 350 mW solid-state laser (532 nm) (Coherent, Santa Clara, Ca, USA). Transmitted scattered light was collected by a single fiber optic that was then bifurcated and fed to two matched photomultipliers (HC120-03, Hamamatsu, Loveland, OH) and a correlator (FLEX2K- 12×2, Bridgewater, NJ). Correlation functions and transmitted light intensity was collected for 2 min. Data was analyzed using DWS-Fit (Mediavention Inc., Guelph, ON, Canada). The principles of DWS have been published elsewhere (Horne & Davidson, 1993; Weitz, Zhu, Durian, Gang, & Pine, 1993).

Droplet sizes distributions of the double emulsions were measured using integrated light scattering (Malvern Mastersizer 2000S, Malvern Instruments Inc, Westborough, MA). A small volume of sample was diluted in distilled water present in the measuring cell and kept under stirring at room temperature. Refractive indices used were 1.33 for the dispersant (water) and 1.473 for the soy oil phase. We assumed that the refractive index of the oil droplet was not affected by the presence of the internal aqueous droplets (Bonnet et al., 2010; Pawlik, Cox, & Norton, 2010).

The osmolalities of internal and external aqueous phases, as well the water used to disperse the samples, were measured using a vapor pressure osmometer (VAPRO 5520, Wescor Inc, Logan, Utah, USA) and standard calibration solutions of 100, 290 and 1000 mmol/kg, to estimate the osmotic pressure gradient between them.

2.5. W/O/W encapsulation efficiency

To determine the integrity of the primary emulsion in the