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Review

Acid induced destabilization of emulsions prepared with sodium caseinate–epigallocatechin-gallate complexes

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ABSTRACT

Complexes of epigallocatechin-gallate (EGCG) and sodium caseinate adsorbed at the interface of oil in water emulsions may affect the stability and processing functionality of the emulsion droplets. The objective of this work was to determine the effect of presence of these complexes at the oil–water interface on the colloidal behaviour of the emulsion during acidification. Emulsions were prepared with 7% soybean oil, 0.35% sodium caseinate, and 0, 2, or 6 mg/ml EGCG. The acid induced aggregation was studied in the presence of high methoxyl pectin at concentration ranging from 0 to 0.45%. *In situ* light scattering measurements demonstrated that the presence of EGCG at the interface lowered the pH of destabilization of sodium caseinate covered droplets, and caused changes in the processing properties of the emulsions. The complex formed at the interface also reduced the electroadsorption of high metoxyl pectin, and caused differences in the emulsions microstructure at acid pH. In spite of the widespread utilization of EGCG as a bioactive ingredients in emulsion dairy drinks, this work illustrates how the presence of protein-EGCG complexes at the interface affect the processing behaviour of emulsion droplets.

Contents

1. Introduction

For many years, a diverse range of food and pharmaceutical products with different nutritional and functional properties have been created using emulsion science and technology. Emulsions are also a common type of carrier for the delivery of bioactives, and

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structural design of emulsion-based delivery systems continues to gain attention.

A protein commonly used as emulsifier is sodium caseinate (Nacas). Nacas is derived from micellar casein and composed of a soluble mixture of four proteins (α_{s1}, α_{s2}, β and κ-caseins), excluding the phosphate and calcium components. In solution, Nacas is a mixture of monomeric or small aggregated particles (Lucey, Srinivasan, Singh, & Munro, 2000; Surh, Decker, & McClements, 2006). At neutral pH Nacas adsorbs on the surface of oil droplets and creates a polyelectrolyte layer at the interface leading to electrostatic and steric repulsion, which stabilizes the droplets (Dalgleish, 1997; Dickinson, 1989, 1992). The net charge of Nacas-emulsified droplets is neutral at pH values close to the proteins' isoelectric point (pH = 4.6), whereas at pH below and above the pI, the droplets hold a positive and negative charge, respectively. Therefore when the emulsion's pH is lowered towards the pI of protein, electrostatic and steric repulsions between the emulsion droplets are reduced. Therefore, close to the proteins' isoelectric point, droplets destabilize, come closer together, and finally aggregate into a gel (Dalgleish, 1997).

It is possible to stabilize Nacas-coated droplets during acidification by the addition of pectins (Bonnet, Corredig, & Alexander, 2005; Dalgleish & Hollocou, 1997; Dickinson, Semenova, & Antipova, 1998; Liu, Corredig, & Alexander, 2007). Pectins are polymers of 1, 4 D-galacturonic acid with different extent of esterification with methanol. Depending on their degree of esterification, pectins are categorized as either high or low methoxyl pectin. Owing to the ionization of the free carboxylic groups, pectin has a negative charge in mildly acidic conditions, and its pKa is around pH 3.5 (McClements, 2005; Sinha & Kumria, 2001). At pH values slightly above the isoelectric point of Nacas, pectin molecules interact with caseins through electrostatic interactions between negatively charged high methoxyl pectin molecules and positively charged patches of protein, aiding to colloidal stabilization (Bonnet et al., 2005; Dalgleish & Hollocou, 1997; Dickinson et al., 1998; Liu et al., 2007). The degree of esterification and the distribution of the charges on the pectin chain will affect the colloidal stabilization behaviour, as different portions of the molecules will be available to interact with the solvent and provide steric stabilization.

Tea polyphenols have been reported to easily interact with proline rich proteins such as caseins (Frazier et al., 2010; Haratifar & Corredig, 2014; Jöbstl, Howse, Fairclough, & Williamson, 2006; Papadopoulou & Frazier, 2004). In a recent study we showed that epigallocatechin-gallate (EGCG), the major and most bioactive tea catechin, has a high affinity for Nacas at the oil/water interface and that EGCG can be loaded at the Nacas-stabilized interface (Sabouri, Geng, & Corredig, 2015). Hence, Nacas-stabilized emulsions may be used as a delivery vehicle for polyphenols (Sabouri et al., 2015). However, the stability and processing properties of Nacas-stabilized emulsions containing tea polyphenols at acidic pH may need to be investigated. In addition, the colloidal and physicochemical behaviour of the emulsions containing EGCG and Nacas in the presence of high methoxyl pectin (HMP) is not known.

The objective of this work was to determine the effect of presence of EGCG on emulsions stabilized by Nacas during acidification in the presence and absence of HMP. In this study advanced spectroscopic techniques were employed to determine emulsion's colloidal and physicochemical changes during acidification *in situ* without dilution.

2. Materials and methods

2.1. Emulsion preparation

A Nacas solution was prepared by dispersing 1.25% w/w Nacas (New Zealand Milk Proteins, Mississauga, Ontario, Canada) in ultrapure water. Sodium azide (0.02% w/v) (Catalog No: S227I, Fisher Scientific, Fair Lawn, NJ, USA) was added to prevent bacterial growth. The mixture was continuously stirred for 2.5 h at room temperature. After an overnight storage at 4 °C, the solution was filtered through 0.8 μm filters (Millex-HV, Millipore Co., Billerica, MA, USA). 80% w/w filtered Nacas solution was added to 20% w/w soybean oil (purchased from Sigma Chemical Co. St. Louis, MO, USA) to prepare a final emulsion of 20% w/w oil and 1% w/w protein. The mixture was pre-homogenized for 2 min at 10000 rpm using an Ultra Turrax stand homogenizer (IKA T18 Basic, Germany) and were immediately processed thorough a microfluidizer (M-110EH, Microfluidics, MA, USA) with four passes and an operating pressure of 69 MPa. The particle size distribution of the oil droplets was measured by static light scattering (Mastersizer 2000S, Malvern Instruments Inc., Southborough, MA, USA), and the diameter ($D_{3,2}$) obtained after homogenization of the NaCas emulsion was 140 ± 5 nm.

A commercial green tea polyphenol extract containing a high concentration of EGCG (min 94%) was obtained from DSM Nutritional Products (Ayr, Ontario, Canada). EGCG solutions were made in ultrapure water fresh and added to Nacas-stabilized emulsion. The mixtures were then vortexed for 1 min and incubated at room temperature (22 °C) for 15 min before addition of pectin solution or ultrapure water for the treatments with no pectin. Pectin solutions were prepared by dispersing HMP (DE 71.4, unstandardized, CpKelco, San Diego, CA, USA) in 70 °C Milli-Q water followed by continuous stirring for 3 h and overnight storage at 4 °C. The pH of the pectin solutions was adjusted to 6.8 right before mixing with the emulsions with and without EGCG and the final mixtures were vortexed for 1 min. Final emulsion formulations contained 7% oil, 0.35% Nacas, 0, 2 and 6 mg EGCG/ml emulsion and 0, 0.15, 0.3 and 0.45% HMP. The two concentrations of EGCG (2 and 6 mg/ml) were chosen based on recent results, and corresponded to 90 and 66% of total added EGCG adsorbed to the o/w interface (Sabouri et al. 2015).

2.2. Dynamic light scattering (DLS)

The change in the overall surface charge of the emulsions was measured using a Zetasizer Nano ZS ZEN3600 (Malvern Instruments Ltd., Malvern, UK) equipped with a MPT-2 Autotitrator (Malvern Instruments Ltd., Malvern, UK) which enabled ζ-potential determination as a function of pH. Emulsions were diluted in 1:300 ratio using Milli-Q water to minimize multiple scattering effects. The measurements were conducted at 25 °C in disposable capillary cells (Malvern Instruments, UK). The autotitrator was programmed to lower the pH of samples in the capillary cell from their initial pH of preparation to pH 3.5 with 0.5 intervals using 0.01 and 0.001 M HCl.

2.3. Diffusing wave spectroscopy (DWS)

DWS is well suited to study turbid systems such as emulsions *in situ* (Alexander, Corredig, & Dalgleish, 2006; Weitz, Zhu, Durian, Gang, & Pine, 1993). By means of DWS, early stages of colloidal destabilization can be noted (Bonnet et al., 2005; Gancz, Alexander, & Corredig, 2005; Ruis, Venema, & Van der Linden, 2006). DWS was utilized to characterize the stability and *in situ* colloidal properties of the Nacas-stabilized emulsions during acidification in the presence of different concentrations of EGCG and/or pectin.

The experimental set up used for DWS experiments in this study has been previously described (Alexander & Dalgleish, 2004; Gancz et al., 2005). In brief, the samples were placed in a 5 mm optical glass cuvette (Hellma Canada Limited, Concord, Canada) immersed