



Influence of pH value and locust bean gum concentration on the stability of sodium caseinate-stabilized emulsions



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ABSTRACT

Sodium caseinate emulsions of 20% sunflower oil in water were prepared at various pH and Locust Bean Gum concentrations (LBG). The presence of LBG was examined to assess the stabilizing properties in relation to flocculation, creaming and coalescence, in the initial emulsions as well as freeze-thawed and freeze-dried/reconstituted samples. We found the initial emulsions to be stable at pH 6.0 and 6.5, both in the absence or presence of LBG, against creaming. However we found evidence for the aggregates of emulsion droplets in the presence of LBG. Strong shear thinning behavior, even at low shear rates, and micrographs indicated that the presence of LBG may promote flocculation by mediating depletion forces between the oil droplets. However, the absence or low concentrations of LBG resulted in creaming followed by the emulsion break-up for the freeze-dried/reconstituted emulsions, particularly at lower pH. We have also detected a proliferation in the number of very small sub-micron particles in the particle size distribution, for samples containing higher concentrations of LBG, following the freeze-thaw cycle. We believe these to be LBG aggregates nucleating during the freezing phase of the process as the polysaccharide solubility declines with temperature.

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1. Introduction

An emulsion is a heterogeneous system comprising of a dispersion of two immiscible liquids in which small droplets of one the liquids are homogeneously dispersed throughout the other one, i.e., the continuous phase. Emulsions often become destabilized through a variety of different mechanisms including creaming, aggregation, coalescence and Ostwald ripening. To slow down all or some of these processes emulsifiers and stabilizers are used in the formation and subsequent kinetic stabilization of food emulsions. Emulsifiers are referred to as surface-active agents and possess both hydrophilic and hydrophobic sites within their molecules. These surface active materials promote the formation of the emulsion by lowering the interfacial tension due to their adsorption at the interface and provide a protective film between the two immiscible liquids (Hill, Ledward, & Mitchell, 1998, pp. 302–303; Sikorski, 2001, pp. 126–129) that enhances the short term stability of the droplets through the so called Gibbs–Marangoni effect (Dickinson, 1992).

Like emulsifiers, many proteins are surface active molecules and possess emulsifying properties. Some proteins are capable of adsorbing to the interface forming a charged protective layer around the oil droplets, and consequently retarding the flocculation and creaming rate by providing steric and electrostatic repulsion (Friberg, Larsson, & Sjoblom, 2003, pp. 2–4; Sikorski, 2001, pp. 126–129). The determining factors affecting the emulsifying properties of proteins encompass the presence of hydrophobic fragments on the surface of protein (a prerequisite for adsorption of a protein to the surface of the oil droplets), its charge, size and steric properties, cross-linking ability, solubility in aqueous phase, chain flexibility and the rigidity of protecting layers formed by the protein molecules (Sikorski, 2001, pp. 126–129).

Milk proteins are widely used as emulsifying agents in both soluble and dispersed forms in the food industry, since these ingredients show excellent surface-active and emulsion stabilizing characteristics (Dickinson, 1999). Casein, a protein derivative of milk, is commonly used as an emulsifier in oil-in-water systems. Due to the lack of an ordered structure, casein has a considerable number of exposed non-polar residues which facilitates its rapid absorption to the surfaces of newly formed oil droplets during homogenization. Furthermore, caseins form a thick protective layer of up to 10 nm around the oil droplets as compared with other milk proteins, e.g., whey proteins with only a layer of thickness 1–2 nm

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(Surh, Decker, & McClements, 2006). The thick interfacial layer confers strong and long ranged steric repulsion on the freshly formed oil droplets and protects fine droplets against flocculation and hence coalescence (Dickinson, 1999; Surh et al., 2006). Caseinate (CAS) covered emulsions are more stable to elevated temperature treatments when compared with whey protein stabilized emulsions since in contrast to the globular proteins, e.g., whey proteins, casein molecules are relatively flexible and do not develop heat-induced structural changes (McClements, 2004; Surh et al., 2006). One drawback of CAS-stabilized emulsion is their susceptibility to droplet flocculation at pH close to the isoelectric point (pH 4.6) of the casein since this drastically reduces the electrostatic repulsion between the oil droplets (Perrechil & Cunha, 2010; Surh et al., 2006).

A variety of oil-in-water emulsions, e.g., frozen foods and pharmaceuticals, are preserved at low temperature to improve their shelf life against microbial, enzymatic, and chemical deterioration. Upon freezing and chilling, oil-in-water emulsions are significantly destabilized, and then readily broken down after thawing (McClements, 2004). Deep under-cooling of emulsions may induce crystallization, and consequently the formation of a fat crystal network in the oil droplets. Some of these crystals may protrude from the fat globules and disrupt the surrounding protective film which results in the formation of rigid granules. This phenomenon is called partial coalescence in which the granules cannot form a larger droplet due to the crystal network in the fat globules. However, upon melting of the fat crystals at sufficiently higher temperatures, i.e., thawing, rigid granules are gradually transformed into larger droplets with coalescence now progressing to completion (Walstra, Wouters, & Geurts, 2005).

Drying technologies are also used to enhance the shelf life of oil-in-water emulsions. The resultant dried emulsions can either be easily used as food ingredients, e.g., flavoring agents, or can be reconstituted with water by the consumer, e.g., dairy creamers (McClements, 2004). There is a growing interest in freeze drying of emulsions in the pharmaceutical industry since the dried emulsions are easy to handle and physically stable ingredients (Christensen, Pedersen, & Kristensen, 2002). During the dehydration step of freeze drying process, the removal of water from the protein may lead to a loss of protein functionality and stability (McClements, 2004).

In the context of food colloids, the term stabilizer is often defined as a component having sufficient capacity to stabilize emulsions (Hill et al., 1998, pp. 302–303). The addition of polysaccharide as a stabilizer in oil-in-water emulsions not only improves the textural properties but also increases the viscosity of the aqueous phase. This slows down the rate of creaming (McClements, 2000). It also arrests the Brownian motion of the droplets, thus preventing their collision and coalescence. Locust bean gum (LBG) is an important thickening polysaccharide composed of a main backbone of 1-4-linked β -D-mannopyranosyl units, with 1-6-linked α -D-galactopyranosyl units. When LBG suspension is heated to around 85 °C, most particles swell, producing a high viscous solution with some degree of thixotropic properties (Whistler & BeMiller, 1997, pp. 171–177). Locust bean gum is compatible with other food polymers, widely used in dairy and frozen dessert products, due to the neutral character of this hydrocolloid (Everett & McLeod, 2005; Whistler & BeMiller, 1997).

In addition, this neutral galactomannan gum has also been reported to possess emulsifying properties at a relatively low LBG/oil ratio (1:5) by lowering the interfacial tension due to a peptide fraction tightly bound to the polysaccharide (Dickinson, 2003). Wu, Cui, Eskin, and Goff (2009) studied and contrasted the emulsion capacity and stability for several different gums. It was found that the emulsifying property of LBG is lower than fenugreek gum, guar

gum and tara gum. There have also been some studies that have suggested that locust bean gum is not surface active and that the surface activity is the result of the presence of impurities in the LBG used (Gaonkar, 1991). Nevertheless, in the present study LBG has to compete with the significantly more surface active sodium caseinate for adsorption onto the surface of oil droplets. CAS is known to be able to displace many other proteins from the interface (Dalglish, 2011). In this respect the behavior of CAS is akin to that of smaller molecular weight emulsifiers (surfactants). Due to their much better packing on the surface, surfactant molecules are efficient displacers of larger amphiphilic macromolecules from the air–water and oil–water interfaces (Pugnali, Dickinson, Ettelaie, Mackie, & Wilde, 2004). Thus, in presence of a sufficient amount of CAS it is unlikely that the much larger molecule such as LBG, comprising largely of hydrophilic polysaccharide, can directly adsorb to the interface. If there is to be any adsorption of LBG this would have to be in the form of a secondary layer adsorbed on top of the primary caseinate layer. Adsorption of polysaccharides to hydrophobic–hydrophilic interfaces in this manner has been investigated both experimentally (Guzey & McClements, 2006, 2007; Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009) and through numerical theoretical calculations (Ettelaie, Akinshina, & Dickinson, 2008; Ettelaie, Akinshina, & Maurer, 2012). These studies have shown the need for strong favorable interactions between protein and polysaccharides before the formation of a secondary adsorbed layer by the latter can commence. The favorable strong interaction between protein and polysaccharide is required in order to compensate for the loss of configurational entropy incurred by the polysaccharide, upon its adsorption on the interface. Such interactions can arise from the opposite electrical charge of proteins and polysaccharide at low pH values, below pI of the protein. Locust bean gum lacks the necessary charge to form such complexes with the protein, even if one overlooks the fact that our studies are all performed at pH values above the isoelectric point of caseinate. In fact, there are even some studies that suggest that there might be some degree of incompatibility between LBG and CAS (Vega, Dalglish, & Goff, 2005). Therefore, we expect that a large fraction, if not all of the LBG to remain in the bulk solution in the emulsion systems investigated in this work. The point is highlighted here since it is a rather important consideration in the discussion that will follow when interpreting our experimental results.

To the best of our knowledge, only a few studies so far have been conducted to specifically investigate the influence of LBG ratio on the stability of CAS-stabilized emulsion. Perrechil and Cunha (2010) investigated the influence of LBG on the stability of neutral emulsions stabilized by sodium caseinate during the storage at room temperature. Furthermore, Perrechil, Braga, and Cunha (2009) studied the effect of LBG concentration on the microstructure and rheological properties at the protein isoelectric point of sodium caseinate.

The aim of this study was to investigate how pH and LBG concentration would influence the stability of CAS-stabilized emulsion during aging, freezing, and freeze drying.

2. Materials and methods

2.1. Materials

Sodium caseinate was purchased from Arla Foods Ingredients Group (Leeds, UK), and locust bean gum (LBG) was obtained from Agrisales (Agrisales Ltd, Bridgewater, UK). Refined sunflower oil was purchased from a local supermarket. The different buffer solutions were made with de-ionized water.

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