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Efficacy of whey protein–tragacanth on stabilization of oil-in-water emulsions: Comparison of mixed and layer by layer methods

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ABSTRACT

Layer by layer (LBL) and mixed emulsions are two well known techniques in which charged proteins and polysaccharides are linked to each other via electrostatic interactions. Therefore, in this study, the effect of these techniques on the stability of emulsions at different pHs (3, 5 and 7), and oil concentrations (1, 4, 8 and 16% w/w) was studied. Regarding LBL method, tragacanthin (1% w/w) was added to whey protein isolate-coated emulsion, following by ultrasound treatment for 0.5 min. However, in the mixed method, oil was added to the soluble mixture of whey protein isolate–tragacanthin (0.4:1% w/w) before being homogenized using ultrasound for 4 min. Then, the droplet size, zeta potential and rheological measurements were performed to investigate the stability mechanisms. According to our findings, in contrast to mixed emulsions, LBL ones were stable at pH 5 in the presence of different oil contents. Furthermore, LBL emulsions had lower particle size than mixed emulsions which illustrates that the presence of polysaccharide in the mixed method may decrease the efficiency of homogenization processing by using ultrasound. Based on rheological analyses, lower apparent viscosity of the mixed emulsions in comparison with LBL can be related to bridging flocculation due to applying high power sonication for long time.

1. Introduction

Since contact between oil and water molecules is thermodynamically unfavorable, emulsions are unstable systems. Creaming, flocculation, coalescence and Ostwald ripening are different instability mechanisms which are responsible for emulsion destabilization during storage (McClements, 2005). Notably, surface active ingredients (emulsifiers) with capability of decreasing the interfacial tension and/or texture modifiers which lead to enhancement of system viscosity are used to form kinetically stable emulsions. Proteins, as common food emulsifiers, form a protective layer around the freshly formed droplets during the homogenization which prevent contact of droplets. However, protein based emulsions are highly sensitive to destabilization by acidification, addition of ions, heat treatment and freezing-thawing (Dickinson, 2009; McClements, 2005; Yadav, Parris, Johnston, Onwulata, & Hicks, 2010). In fact, there is relationship between solubility of proteins and their emulsification properties. For example, whey protein based emulsions are sensitive to heat treatments (Day, Xu, Lundin, & Wooster, 2009; Dickinson & Parkinson, 2004). Furthermore, addition of calcium ions or acid that causes aggregation of caseins lead to a decrease in their emulsification properties (Dickinson, 2006). In other words, unstable emulsions are expected at pHs near to p*I* of protein. Using lactoferrin as a protein that is positively charged at pHs lower than 8 is suggested as a solution for increasing the stability of protein-based emulsions against environmental stresses, especially low pH (Tokle & McClements, 2011), even though, it may not be preferred if the cost of product increases extremely using this protein.

As another solution, it has been claimed that electrostatic interaction of proteins and oppositely charged polysaccharides can be used to increase the stability of protein-based emulsions against environmental stresses since presence of polysaccharide with side branches leads to formation of a thicker membrane at the oil–water interface (Dickinson, 2009). In this regard, layer by layer (multilayered) and mixed emulsions are two well known techniques in which charged proteins and polysaccharides are linked to each other via non-covalent interactions. In layer by layer (LBL) method, charged emulsifier (protein) adsorbed onto the oil–water

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interface during homogenization, forming primary emulsion. In the next step, a polyelectrolyte with opposite charge is added to protein-based emulsion to form interfacial complex, finally a second homogenization treatment is done and the obtained emulsion is called secondary emulsion (Gu, Decker, & McClements, 2007; Guzey & McClements, 2007; Hou, Zhang, Liu, Yan, YuanXu, & Gao, 2012). It is also possible to add other oppositely charged polyelectrolyte to secondary emulsion to generate tertiary emulsion containing oil droplets are covered with three layers of biopolymers (Guzey & McClements, 2006). In contrast, in mixed emulsion preparation method, oil is added to the previously prepared protein–polysaccharide mixture following by homogenization process (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008; Salminen & Weiss, 2014). Besides, it is even possible to combine these two methods by addition of biopolymers complexes to primary emulsion to stabilize emulsion at acidic pH (Moschakis, Murray, & Biliaderis, 2010). Interestingly, the oil–water interface of oil droplets in mixed and LBL techniques are structurally different from each other (Dickinson, 2009).

Gum tragacanth (GT), exudates of Asiatic species of *Astragalus*, is consisted of two fractions: water soluble (tragacanthin, T) and water-insoluble (bassorin, B) (Azarikia & Abbasi, 2010; Weiping & Branwell, 2000). In different varieties of GT, the ratio of water soluble and water-insoluble fractions changes (Ahmadi Gavlighi, Meyer, Zaidel, Mohammadifar, & Mikkelsen, 2013). The lowest ratio of tragacanthin: bassorin belongs to A. *gossypinus* (Balaghi, Mohammadifar, & Zargaraan, 2010) which interestingly contains the higher amount of galacturonic acid (37%) among the other species of Astragalus (Balaghi, Mohammadifar, Zargaraan, Ahmadi Gavlighi, & Mohammadi, 2011). As a heterogeneous anionic acid-resistant gum, GT, has been used in food, pharmaceutical and cosmetic industries for years. Since, GT has good stability in acidic conditions; it is suggested to be used in salad dressings and other low pH products (McClements, 2005). Interestingly, tragacanthin contains higher amounts of uronic acid and degree of *methoxylation* than bassorin (Weiping & Branwell, 2000). A group of researchers has studied the correlation between galacturonic acid content and methoxylation degree of GT on the stability of WPI-based emulsions (Ahmadi Gavlighi et al., 2013).

Whey proteins consist of β-lactoglobulin, α-lactalbumin, proteose peptone, serum albumin, Immunoglobulins and lactoferrin. Secondary, α-helical and β-sheet as well as tertiary structures have been observed in whey proteins. β-lactoglobulin is the main whey protein with 162 residues. In addition, 2 disulfide and 1 free sulfhydryl groups are found in this molecule (Kilara, 2004). The sulfhydryl group has significant role in interaction of β-lactoglobulin with β-lactoglobulin, α-lactalbumin and in particular κ-casein during heat treatment. Polymerization of β-lactoglobulin depends on pH. That is to say that, monomers of β-lactoglobulin are found at pHs lower than 3.0 and higher than 8; while, at acidic pH (3.1–5.1) 8 molecules attached to each other, forming octamer. In addition, dimers of β-lactoglobulin generate at normal pH of milk, they exist in this way because of hydrophobic linkages (Madadlou & Azarikia, 2013).

In a very recent report, we have investigated the phase behavior of WPI and T as a function of pH (Azarikia & Abbasi, 2014). According to our results, mixture of WPI–T exhibited no precipitation at the mixing ratio of 0.4:1% over a wide pH range (2–7). Thereupon, our objectives in the current research, in extension of our previous study, were: a) stabilization of emulsion at pH near and lower than protein p*I* using electrostatic interaction between WPI and T (at the concentration of 0.4:1%), b) to compare the efficiency of two emulsion preparation techniques (LBL and Mixed methods) in the case of using ultrasound for homogenization of emulsions, c) to investigate the effective mechanisms of stabilization of emulsions at different pHs, and d) to elucidate the effect of increasing the oil concentration in emulsion formulation at constant concentration of protein and polysaccharide.

2. Materials and methods

2.1. Materials

GT (A. *gossypinus*) was purchased from a local herbal store (Tehran, Iran). Then, the gum was ground, sieved and the collected gum powder (mesh size <60) stored in a sealed vessel. BiPRO® Whey protein isolate (WPI) was provided from Davisco (Food international Inc. Le Sueur, MN, USA) which composed of protein, fat, ash and lactose with typical range of 97.6 ± 0.3, <0.5, 2.0 ± 0.2, and 0.4 ± 0.2, respectively. Sunflower oil (Nina®, Iran) was purchased from local market. In addition, sodium azide, hydrochloric acid and sodium hydroxide supplied by Merck Chemicals Co. (Darmstadt, Germany). Deionized water was utilized to prepare the samples.

2.2. Preparation of dispersions

In order to prepare aqueous dispersion of tragacanthin (T), GT powder was gradually added to distilled water, mixed gently (2 h), and kept at 4 °C overnight to assure complete hydration. At the next step, the suspension was centrifuged at 20379 *g* (14,000 rpm) for 45 min to separate the soluble and insoluble fractions. Then, soluble fraction was collected to be used (Azarikia & Abbasi, 2010). For preparation of stock WPI solution, protein powder was separately added to the deionized water while stirred for 60 min at room temperature. We used stock solution to prepare samples in which final concentration of T and WPI were 1 and 0.4% w/w, respectively.

2.3. Emulsion preparation

For preparation of mixed emulsion, WPI solution and T aqueous dispersion were mixed gently for 120 min at room temperature (after adjusting the pH at 3, 5 and 7) using a magnetic stirrer to form WPI–T complex (final concentration in samples 0.4:1% w/w). Afterward, sunflower oil was added to protein–polysaccharide complex at different concentrations (1, 4, 8 and 16%) following by mixing at 400 rpm for 5 min by a magnet stirrer. Samples were pre-homogenized using a rotor-stator homogenizer (WiseTis®HG-15D, Germany) at 4000 rpm for 3 min and 10,000 rpm for 1 min before being homogenized using high power ultrasound (Sonicator 4000, 20 kHz, high gain cylindrical titanium sonotrode of 19.1 mm in diameter, Misonix, Inc, New York, USA) at amplitude of 100% for 4 min. Notably, the sample was poured inside a double-walled cylindrical glass container and chilled antifreeze (propylene glycol) liquid passed through the jacket and the embedded spiral coil to keep sonication process temperature constant (22 °C) and tip of the sonotrode was immersed 1 cm below the pre-emulsion (Mirmajidi Hashtjin & Abbasi, 2015). Finally, the pH of emulsions was measured and again adjusted at 3, 5 and 7, followed by addition of sodium azide (0.04% w/w). The emulsions were stored at 4 °C before next analysis.

For LBL emulsion preparation, a coarse primary emulsion was prepared by mixing sunflower oil (2, 8, 16, 32% w/w) and WPI solution (0.8% w/w) using magnetic stirrer (at 400 rpm for 5 min), pre-homogenized using a rotor-stator homogenizer (at 4000 rpm for 3 min and 10,000 rpm for 1 min) followed by sonication (at amplitude of 100% for 4 min). As a next step, 50 g of T dispersion (2% w/w) was added gradually to the 50 g of primary emulsion, mixed using magnet stirrer (at 300 rpm for 90 min); then, pre-homogenized using a rotor-stator homogenizer (3000 rpm for 0.5 min and 10,000 rpm for 0.5 min) before being homogenized by