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Physical and flow properties of D-limonene-in-water emulsions stabilized with whey protein concentrate and wild sage (*Salvia macrosiphon*) seed gum

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ABSTRACT

The effect of varying concentrations of whey protein concentrate (WPC, 5–15% w/v) and wild sage seed gum (SSG, 0–0.3% w/v) on interfacial tension, zeta potential, physical stability, droplet size, flow properties and viscosity of D-limonene-in-water emulsions at pH 7 was investigated. The results indicated that the addition of SSG had no significant effect on zeta potential while the interfacial tension decreased with increasing gum concentration. For freshly prepared emulsions, the mean diameter of droplets slightly decreased as gum concentration increased from 0.0% to 0.3%. In contrast, protein concentration in the range of 5–15% showed no particular effect on the size of droplets. Storage of samples for a period of 4 weeks resulted in an increase in the size of droplets. This was substantially noticeable for the emulsions containing no SSG and negligible for those prepared with 0.3% SSG and 15% WPC. The presence of SSG in these emulsions increased the emulsion stability indices, evidently because of the higher viscosity it imparted to the aqueous phase. The emulsions containing only WPC showed Newtonian behavior, while those consisting of both protein and gum exhibited shear thinning characteristics. Various time-independent rheological models were examined to fit the experimental data; of which Herschele–Bulkley model was found to be the best model to describe steady shear flow behavior of these emulsions.

1. Introduction

Flavor is an important sensory characteristic which plays a critical role in food preference. Natural food flavors often exist in the form of a well balanced combination of various volatile constituents. However, only one or a few compounds are responsible for the typical flavor. Limonene is the major flavor compound present in orange oil (Anker & Reineccius, 1988). It is widely used as a flavor additive in cosmetics, foods, and other consumer products. D-Limonene is insoluble in water at neutral pH and prone to chemical degradation when exposed to environmental conditions. Therefore, emulsification is a good method to solubilize, encapsulate and protect this compound. The degree of stability, however, is very much influenced by emulsion physical properties, including droplet size and viscosity (Benjamin, Silcock, Leus, & Everett, 2012).

Emulsions are thermodynamically unstable systems that tend to destabilize by flocculation, coalescence or creaming. The stability can be enhanced by using appropriate emulsifier and thickening agent. Proteins are the most popular emulsifiers that are used in food emulsions, individually or more commonly in combination with polysaccharides (Dickinson, 1992). When used together, they have the ability to control the texture, structure and stability of the emulsion system (Dickinson & McCalements, 1995). The use of polysaccharides to enhance the stability of emulsions that contain proteins has been previously reported by many researchers (Dickinson, 2011; Khalloufi, Alexander, Douglas Goff, & Corredig, 2008; Sun, Gunasekaran, & Richards, 2007; Ye, Hemar, & Singh, 2004).

Owing to the coexistence of hydrophilic and hydrophobic parts in their structure, proteins show amphiphilic nature which is essential for having emulsifying property. They reduce the oil–water interfacial tension and thus facilitate formation of emulsion. They also stabilize the oil droplets against coalescence or flocculation by spatial and electrostatic repulsion mechanisms (Calero, Munoz, Cox, Heuer, & Guerrero, 2013). Among food proteins, whey proteins are widely used as emulsifiers in food systems. They readily adsorb at the oil–water interface forming a thin film around the oil droplet (Dickinson, 1997; Soleimanpour, Koocheki, & Kadkhodaee, 2012, 2013).

In contrast, hydrophilic character of polysaccharides means that they generally exhibit no surface activity and therefore are not useful as emulsifying agent. Polysaccharides generally contribute to the emulsion stability via thickening the aqueous phase and interaction with other ingredients such as proteins. Our previous works demonstrated that seed gums are able to bind and immobilize a large amount of water thus increasing viscosity, modifying texture and stabilizing

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emulsion against phase separation (Koocheki, Kadkhodaee, Mortazavi, Shahidi, & Taherian, 2009; Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009; Koocheki, Taherian, & Bostan, 2011; Soleimanpour et al., 2012, 2013).

The genus *Salvia* (*Labiatae*) comprises more than 700 species, of which 200 grow in Iran. Wild sage seed (*Salvia macrosiphon*) is a small rounded seed, which readily swells in water to give mucilage. Gum extracted from these seeds has shown good thickening properties (Razavi, Taheri, & Quinchia, 2011). It is composed of 6.72% moisture, 0.85% lipid, 8.17% ash, 2.84% protein, 1.67% crude fiber and 79.75% carbohydrate (Razavi et al., 2011). The carbohydrate fraction of SSG consists mainly of mannose (69.31%) and galactose (37.42%), but traces of glucose (3.15%), arabinose (1.59%) and rhamnose (1.34%) also exist in the gum (Razavi, Cui, Guo, & Ding, submitted for publication). According to Razavi, Mohammadi Moghaddam, Emadzadeh, and Salehi (2012), the molecular weight of SSG is about 1.5×10^6 Da at 25 °C which is similar to that of guar and locust bean gums. Uronic acids have been found to be the main functional groups in the gum. It, therefore, carries negative charge in aqueous solution (Farahnaky, Shanesazzadeh, Mesbahi, & Majzoobi, 2013).

The emulsion stabilizing properties of SSG is not yet studied. Furthermore, there is lack of sufficient knowledge on the possible interactions that may take place between this gum and proteins in an emulsion. Therefore, the objective of the present study was to investigate the effect of SSG and WPC on the physical stability and flow properties of D-limonene-in-water emulsion.

2. Materials and methods

2.1. Materials

D-Limonene ($\rho = 840$ kg/m^3, $\eta = 8.8$ mPa s at 25 °C, refractive index = 1.487) was purchased from Merck (Darmstadt, Germany). Commercial WPC (code: A635, 82.2% protein, 4% moisture, less than 4% ash) was donated by Milk Powder-Multi Company (Mashhad, Iran). Wild sage seeds were purchased from local market in Mashhad, Iran. SSG was extracted, purified and dried based on the method described by Bostan, Razavi, and Farhoosh (2010).

2.2. Measurement of interfacial tension

The interfacial tension between aqueous solution and D-limonene was determined by Du Nouy ring method (Krüss K100 Tensiometer, Germany) at 20 °C. All measurements were carried out in triplicate.

2.3. Preparation of emulsion

Aqueous solution of WPC (5–15% w/w) containing 0–0.3% w/w SSG was used for preparation of emulsions. Appropriate amount of each biopolymer was separately dissolved in deionized water (Milli-Q, Millipore, Bedford, USA) under slow stirring at room temperature. The dispersions were kept overnight at 4 °C to fully hydrate and then mixed before emulsion preparation. While mixing the water phase, the dispersed phase (3% D-limonene) was progressively added into the aqueous phase. After 10 min mixing, the premix was homogenized with a laboratory rotor stator homogenizer (Ultra Turrax T-25, IKA Instruments, Germany) at a speed of 12,000 rpm for 5 min at room temperature to make a coarse emulsion. It was then sonified for 5 min by using a 20 kHz ultrasonic processor (model VCX 750, Sonics & Materials, Inc., USA) operating at a nominal maximum power output of 750 W and equipped with a cylindrical titanium sonotrode (19 mm in diameter) immersed 1 cm below the surface of liquid. The temperature was kept constant at 30 °C throughout sonication by circulating cooling water through the jacket of chamber. A thermometer was also placed inside the sonifying chamber to record the temperature during sonication. Sodium azide (0.02% w/v) was added into the emulsions as an antimicrobial agent. The final pH of emulsions was measured to be 6.8, independent of gum and WPC concentrations.

2.4. Zeta potential

Zeta potential of samples was determined based on their electrophoretic mobility by a combination of laser Doppler velocimetry and phase analysis light scattering technique (Zetasizer Nano ZS, Malvern Instrument, UK). In order to avoid multiple scattering effects, samples were diluted 1000-fold with deionized water. Each measurement was obtained from the average of two readings.

2.5. Emulsion droplet size analysis

Size distribution of the oil droplets was measured by a laser diffraction particle sizer (Fritsch Analysette 22, Germany) relating the scattering of laser beam to the size of droplets as described previously (Koocheki & Kadkhodaee, 2011). The size of droplets was measured immediately after preparation of emulsions and also after 7, 14, 21 and 28 days of storage at 4 °C by the following equations:

$$D_{32} = \frac{\Sigma n_i d_i^3}{\Sigma n_i d_i^2} \ (\mu m) \tag{1}$$

$$D_{43} = \frac{\Sigma n_i d_i^4}{\Sigma n_i d_i^3} \ (\mu m) \tag{2}$$

where n_i is the number of droplets of diameter d_i.

The specific surface area (SSA) was calculated according to Eq. (3):

$$SSA = \frac{6\phi}{D_{32}} \left(m^2 ml^{-1} emulsion\right) \tag{3}$$

where Φ is the oil volume fraction of emulsion.

To determine the distribution width of droplet size, an index known as span was calculated by the following Eq. (4):

$$Span = \frac{[d(v,90) - d(v,10)]}{d(v,50)} \tag{4}$$

where, d (v,10), d (v,50), and d (v,90) are diameters at 10%, 50%, and 90% cumulative volume, respectively. All results were the average of three replications.

2.6. Emulsion stability

The emulsion stability index (ESI) was evaluated by turbidometric technique described by Pearce and Kinsella (1978) with some modifications. 20 ml of emulsion was diluted with 5 ml of sodium dodecyl sulfate (SDS) solution (0.1% w/v) and its absorbance was measured on a spectrophotometer (model 160A, Shimadzu, Japan) at 500 nm. The ESI was calculated after the emulsions were held at 25 °C for 10 min and reanalyzed for turbidity as described previously using the following formula:

$$ESI\ (min) = \frac{A_0}{A_0 - A_{10}} \times 10 \tag{5}$$

where A_0 and A_{10} represent the absorbance at time zero ($t = 0$) and after 10 min, respectively.

The stability of emulsions against creaming and phase separation was also investigated. For this purpose, 10 ml of freshly prepared emulsion was transferred into a test tube, capped, and stored for 28 days at 4 °C. Creaming of emulsions was monitored after 28 days of storage. The total height of the emulsion (HE) and the height of the serum layer (HS) were measured. The measurement was performed in duplicate