



Influence of soybean soluble polysaccharides and beet pectin on the physicochemical properties of lactoferrin-coated orange oil emulsion



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ARTICLE INFO

Article history:

Received 7 March 2014

Accepted 24 October 2014

Available online 4 November 2014

Keywords:

Orange oil

Layer-by-layer emulsion

Lactoferrin

Soybean soluble polysaccharides

Beet pectin

Physicochemical stability

ABSTRACT

Based on electrostatic layer-by-layer deposition, the influence of soybean soluble polysaccharides (SSPS) and beet pectin (BP) on the physicochemical properties of lactoferrin-coated orange oil emulsion was investigated. The physicochemical stability of orange oil emulsions was characterized by droplet size and distribution, zeta-potential, turbidity, rheological behavior, transmission profiles using LUMiSizer and degradation of volatile compounds in the emulsions during the storage. Results showed that the droplet characteristics of secondary emulsions were greatly dependent on the polysaccharides concentration and type. The zeta-potential values were decreased from 39.5 mV to -23.2 mV and -37.6 mV, respectively, for the SSPS and BP concentrations from 0 to 0.75 wt%, which indicated that anionic polysaccharides were adsorbed to the droplet surfaces. SSPS and BP concentration of 0.35 wt% was sufficient to saturate the LF-coated droplets and preferred effectively to increase the physical stability of the emulsion systems. During the storage of 4 weeks at 55 °C, SSPS and BP could significantly ($p < 0.05$) enhance the physical stability of emulsions and protect volatile compounds (decanal, octanal, geranial and limonene) from the oxidation. The least degradation of volatile compounds occurred in LF-BP coated emulsion. These results revealed that the physicochemical stability of LF-coated orange oil emulsions has been improved by the adsorption of anionic polysaccharides.

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1. Introduction

Beverage emulsions are either flavor emulsions to provide the beverage with flavor, cloudiness and color or cloud emulsions to improve only cloudiness (Mirhosseini, Tan, Hamid & Yusof, 2008). A typical beverage emulsion usually contains oil phase and aqueous phase. The oil phase is comprised of flavor oil (such as orange oil, lemon oil et al.) and carrier oil, while the aqueous phase includes water, emulsifier, acid and antimicrobial agent.

In food industry, proteins and polysaccharides are commonly used as emulsifiers to stabilize O/W emulsions. Proteins have the ability to adsorb at the interface between oil and water and stabilize the emulsion through electrostatic and/or steric repulsive forces, however, at the isoelectric point (pI), protein molecules have a net zero charge leading to droplets aggregation (Evans, Ratcliffe, & Williams, 2013). Most polysaccharides could form an extended network in the aqueous phase to stabilize emulsions. Only a few polysaccharide or derivatives have the ability to adsorb at oil-water

interface, such as arabic gum, modified starch and pectin (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012).

Accordingly, there's a growing interest in combining proteins and polysaccharides to form electrostatic complexes to stabilize emulsions. The layer-by-layer (LbL) electrostatic deposition is a promising technique. In this approach, a protein usually adsorbs to the droplet surface to form primary emulsion. At pH below the pI, the protein has positive charges. Then the polysaccharide with opposite charges is added to the system and could adsorb to the droplet to produce secondary emulsion (Aoki, Decker, & McClements, 2005; Guzey & McClements, 2006). This procedure could be repeated to produce multilayer emulsions. Multilayer emulsion is of benefit because it could be prepared with natural food grade ingredients (proteins, lipids, polysaccharides) using simple processing operations (homogenization, mixing) (Li et al., 2010). Multilayer emulsions could protect droplets from aggregation or prevent lipid oxidation. Secondary emulsions containing protein/polysaccharide-coated droplets have better stability to environmental stresses (pH, ionic strength, thermal treatment, and freeze–thaw cycles) than primary ones containing protein-coated droplets (Harnsilawat, Pongsawatmanit, & McClements, 2006; Tokle, Lesmes, & McClements, 2010). On the other hand, it was

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found that SDS-chitosan-stabilized emulsions were more effective to prevent the formation of citral oxidation product, *p*-cymene, than gum arabic-stabilized emulsions. These results could be due to the formation of a cationic and thick emulsion droplet interface that could repel prooxidative metals (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2007). Hou et al. (2010) have found that the physicochemical stability of soybean soluble polysaccharides-coated β -carotene emulsions could be improved by the adsorption of chitosan.

Depletion and bridging flocculation are two important instability mechanisms occurred in multilayer emulsions, which are associated with the polysaccharides concentration. It is necessary to ensure that there are sufficient polysaccharide molecules to cover the droplets and there exist hardly free polysaccharide molecules present in the continuous phase (Guzey & McClements, 2006). The charge density, charge distribution, chain length, rigidity and degree of branching of polysaccharides affect the formation and the properties of multilayer emulsions (Guzey & McClements, 2006). The order of mixing the secondary layer solution and primary emulsion is also important to avoid the aggregation. Adding primary emulsion to the biopolymer solution leads to less aggregation than vice versa (Guzey & McClements, 2006; Zeeb, Zhang, Gibis, Fischer, & Weiss, 2013).

In the present study, lactoferrin (LF) was used to prepare the primary emulsion, then soybean soluble polysaccharides (SSPS) or beet pectin (BP) was added to form the secondary emulsions. Lactoferrin is a globular glycoprotein derived from milk and other mammalian fluids with a relatively high isoelectric point ($pI > 8$), and tends to be cationic at neutral pH, whereas most other globular proteins are anionic (Steijns & van Hooijdonk, 2000; Tokle & McClements, 2011). Soy soluble polysaccharides (SSPS) consist of main rhamnogalacturonan backbone branched with β -1,4-galactan and α -1,3 or α -1,5-arabinan chains, and homogalacturonan covalently bound to a \sim 50 kDa protein moiety that is related to surface activity (Tran & Rousseau, 2013). Due to its high water solubility, low bulk viscosity, thermal stability and its ability to form strong interfacial films, SSPS could be used in flavor emulsions (Furuta & Maeda, 1999; Nakamura, Takahashi, Yoshida, Maeda, & Corredig, 2004). Beet pectin is extracted from sugar beet, which has been reported to have better surface-activity than high-methoxyl or low-methoxyl pectins (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). Some studies suggested that the different properties of beet pectin could be due to the presence of acetyl groups (4–5%) (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003) and proteins (Drusch, 2007).

Beverage emulsions are usually stabilized with polysaccharides, such as gum arabic or modified starch, which are used at relatively high concentrations. To the best of our knowledge, some studies were carried out using the layer-by-layer deposition to prepare beverage emulsions (Harnsilawat et al., 2006; Klein, Aserin, Svitov, & Garti, 2010), but no information is available concerning the effect of LF-polysaccharides complexes on the physicochemical stability of beverage emulsions. The objective of this study was to gain a better understanding of the effect of SSPS or BP adsorption on the physical and rheological properties of orange oil emulsions, meanwhile, to evaluate whether the degradation of volatile compounds in orange oil emulsion could be inhibited during the storage.

2. Materials and methods

2.1. Materials

Orange oil was obtained from Huiyuan Co. Ltd. (Beijing, China), which was from the water-insoluble, lipophilic portion of the

condensed distillate formed when orange juice was thermally concentrated. Canola oil was purchased from Richardson Oilseed Ltd. (Winnipeg, Canada). LF was obtained from Westland Milk Products (Hokitika, New Zealand). The product contained 0.72% moisture, 0.6% ash and 98.68% protein, of which 94% was lactoferrin. SSPS (Lot 131215/001) were obtained from Fuji Oil Co. Ltd. (Osaka, Japan). The product contained more than 70% soluble polysaccharides, 5.4% moisture, 5.2% crude protein and 7.2% crude ash. BP (batch GR93208, Lot 0001005-32) was supplied by CP Kelco (Lille Skensved, Denmark). C8–C20 mixed standard solution was supplied by Sigma–Aldrich Chemicals Co. (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Preparation of biopolymer solutions

LF, SSPS and BP were dispersed in deionized water, respectively and stirred overnight to ensure complete dispersion and dissolution. The pH of the solutions was adjusted to 5.50 (the origin pH for LF solution) using 0.1 mol/L HCl or NaOH. Sodium azide (0.02 wt%) was added as an antimicrobial agent. Oil phase was made by mixing orange oil with canola oil, which was acted as the carrier oil. The ratio was fixed at 1:1 (wt/wt).

2.3. Primary emulsion preparation

Primary emulsions were prepared by mixing LF solution (0.3 wt% \sim 1.5 wt%) with oil phase (10 wt%) at a speed of 10,000 rpm for 6 min using a blender (Ultra Turrax, model T25, IKA Labortechnik, Staufen, Germany). Then the coarse emulsions were further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 60 MPa.

2.4. Secondary emulsion preparation

Secondary emulsions were prepared by diluting primary emulsions with aqueous SSPS (0.05 wt% \sim 0.75 wt%) or BP (0.05 wt% \sim 0.75 wt%) to make the final concentration of 5 wt% oil phase. These emulsion systems were stirred in a blender (Ultra Turrax, model T25, IKA Labortechnik, Staufen, Germany) at a speed of 10,000 rpm for 6 min, followed by three passes at 60 MPa through a two-stage valve homogenizer (Parma, Italy). The pH of primary and secondary emulsions was 5.50. For a comparison, the primary emulsion was diluted with an equal amount of pH-adjusted deionized water to make the oil phase concentration of 5 wt%.

2.5. Droplet size and size distribution measurements

Droplet size of orange oil emulsions was determined by dynamic light scattering using a Zetasizer NanoZS90 (Malvern Instruments, Worcestershire, UK) at a fixed angle of 90°. Emulsions were diluted to a final oil droplet concentration of 0.005 wt% with pH-adjusted deionized water prior to each measurement to minimize multiple scattering effects. Refractive index of 1.45 and 1.33 were used for the oil droplet and the solvent, respectively. Results were described as cumulant mean diameter (size, nm) for droplet size, polydispersity index (PDI) for droplet size distribution. All measurements were performed in triplicate.

2.6. Zeta-potential measurement

Zeta-potential of orange oil emulsions was determined by measuring the direction and velocity of droplet movement in a well-defined electric field using a Zetasizer NanoZS90 (Malvern Instruments, Worcestershire, UK). Emulsions were diluted to a final oil droplet concentration of 0.005 wt% with pH-adjusted deionized

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