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Influence of environmental stresses on the physicochemical stability of orange oil bilayer emulsions coated by lactoferrin–soybean soluble polysaccharides and lactoferrin–beet pectin

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ABSTRACT

Based on layer-by-layer electrostatic deposition, orange oil bilayer emulsions stabilized with lactoferrin (LF)–soybean soluble polysaccharides (SSPS) and lactoferrin (LF)–beet pectin (BP) were prepared. The effect of environmental stresses (ionic strength, pH, freeze–thaw and light) on the physicochemical stability of primary and secondary emulsions was investigated. In the absence of anionic polysaccharides, orange oil emulsion was highly unstable and aggregated at pH 7–9 and NaCl of 0.1–0.5 M. The droplets in LF–SSPS coated emulsion were stable against aggregation at pH range of 3–10 and NaCl concentration less than 0.3 M, while the droplets in LF–BP coated emulsion were stable against aggregation at pH 4–9 and NaCl concentrations of 0–0.5 M. All the primary and secondary emulsions showed the instability after the freeze–thaw treatment and the stability could be improved in the presence of maltodextrin. During the light exposure (0.35 W/m^2, 45 °C) for 8 h, the bilayer emulsions could protect key volatile compounds (decanal, octanal and geranial) from the oxidation compared with the primary emulsions. These results suggested that the layer-by-layer electrostatic deposition could improve the stability of LF-coated emulsion to environmental stresses.

1. Introduction

Orange oil has been used extensively in food and beverage industries as a flavoring agent, however, it has low solubility in water and the volatile compounds are chemically unstable in the presence of air, high temperatures and light. Hence, in order to incorporate orange flavor into beverage, it is beneficial to emulsify orange oil prior to use to prevent the aroma from degradation during processing and storage (Xiao, Yu, & Yang, 2011).

An emulsion is a thermodynamically unstable system consisting of a dispersion of two immiscible liquids in which one of the droplets is homogenously dispersed throughout the other one (Farshchi, Ettelaie, & Holmes, 2013). Proteins are widely used to stabilize O/W emulsions, but the stability of protein-coated emulsions depends on pH and ionic strength, freeze–thaw and dehydration (Zeeb, Gibis, Fischer, & Weiss, 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. Multilayer emulsions usually have an initial ionic protein layer, which is more effective than polysaccharides in producing small emulsion droplet at low concentration and have the ability to form strong adsorbed layer at oil–water interface (Tippetts, Shen, & Martini, 2013). The second layer could be formed by polysaccharides with the opposite charge. Polysaccharides are of benefit in stabilizing emulsions in a wide range of environmental condition and they could modify the rheology of aqueous phase (Tippetts et al., 2013). The adsorption of polysaccharides onto the droplet surface could form an extended network, thereby prevent the droplets from coming in closer contact through steric hindrance (Bouyer, Mekhloufi, Rosilio, et al., 2012).

Relatively thick and highly charged interfaces can be produced through layer-by-layer technique, consequently the steric and electrostatic repulsion between droplets can be increased (Harnsilawat, Pongsawatmanit, & McClements, 2006). Emulsions stabilized by two-layered membranes have better stability against environmental stress than those stabilized by single-layered membranes (Gu, Regnier, & McClements, 2005), such as extreme pH, high ionic strengths, freeze–thaw cycling, thermal processing and lipid oxidation (Aoki, Decker, & McClements, 2005; Chen, Li, Ding, & Rao, 2011; Gu, Regnier, & McClements, 2005; Hou et al., 2010; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005; Li et al., 2010; Perrechil & Cunha, 2013; Thanasukarn, Pongsawatmanit, & McClements, 2006). In addition, it was found that SDS-chitosan-stabilized emulsions were more effective to prevent the formation of citral oxidation product, *p*-cymene, than gum Arabic-stabilized ones. These results could be

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due to the formation of a cationic and thick emulsion droplet interface that could repel prooxidative metals (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2007). Benjamin, Silcock, Leus, and Everett (2012) found that multilayer emulsions stabilized with β-lactoglobulin and pectin were stable and able to retain non-polar volatile organic compounds at pH 5 where the primary emulsion was unstable and volatile release was increased. The improved retention of volatile compounds in multilayered emulsions might be due to the hydrophobic interactions and hydrogen bonds with the dense layer of pectin.

Recently, much attention has been drawn to the application of lactoferrin (LF) in food emulsions. LF is a globular glycoprotein which has an isoelectric point (pI) of 8.5, thus has a positive charge under a very wide pH range in food systems. However, most other milk proteins are usually negatively charged at neutral pH (Ye & Singh, 2006). A few previous papers, based on the use of LF to produce multilayer emulsions, have been published. Tokle, Lesmes, and McClements (2010) investigated the effects of low methoxyl pectin (LMP), high methoxyl pectin (HMP) and alginate on the physicochemical properties and stability of LF-coated droplets. The addition of anionic polysaccharides could greatly improve the thermal stability (30 °C–90 °C) of primary emulsion at neutral pH, while primary emulsion droplets would aggregate above 60 °C. However, in the presence of high calcium levels at certain pH value, the droplet aggregation would occur in secondary emulsions. Among the three kinds of polysaccharides, HMP provided better stability over a wider range of environmental stresses than LMP and alginate. The formation and properties of electrostatic complexes between LF and polysaccharides in aqueous solutions were also reported in several studies (Bengoechea, Jones, Guerrero, & McClements, 2011; Peinado, Lesmes, Andres, & McClements, 2010).

Soybean soluble polysaccharides (SSPS) are acidic polysaccharides extracted from soy cotyledons, containing 18% galacturonic acid (Nakamura, Yoshida, Maeda, & Corredig, 2006). Their main backbones consist of homogalacturonan and rhamnogalacturonan and are branched by β-1,4-galactan and α-1,3 or α-1,5 arabinan chains (Liu, Verespej, Corredig, & Alexander, 2008). Beet pectin (BP) is extracted from sugar beet pulp, and it is different from citrus pectin for the ferulic acid group esterified to some of the neutral sugars in the side-chains of the so-called "hairy" regions (Littoz & McClements, 2008).

To the best of our knowledge, no information is available concerning the application of LF in orange oil emulsion and the effect of polysaccharides on the physicochemical stability of LF-coated orange oil emulsions. In the current study, an electrostatic deposition technique was applied to produce bilayer emulsions coated with LF–SSPS and LF–BP. The purpose of this study was to determine whether secondary emulsions stabilized with LF–BP and LF–SSPS are stable to environmental stresses (pH, ionic strength, freeze–thaw and light) normally found in practice. Besides, the different effects of SSPS and BP on the stability of LF-coated droplets were also compared.

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). 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 orange oil emulsions

2.2.1. Biopolymer solutions

LF, SSPS and BP were dispersed in deionized water, respectively and stirred overnight to ensure complete dispersion and dissolution. Sodium azide (0.02 wt.%) was added as an antimicrobial agent. Oil phase was formed by mixing orange oil with canola oil, which was acted as the carrier oil. The ratio was fixed at 1:1 (wt/wt).

2.2.2. Primary emulsion preparation

Primary emulsion was prepared by mixing LF solution (0.7 wt.%) with oil phase (10 wt.%) at a speed of 10,000 rpm for 6 min using a blender (IKA, Germany). Then the coarse emulsion was further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 60 MPa.

2.2.3. Secondary emulsion preparation

Secondary emulsion was prepared by diluting primary emulsion with aqueous SSPS (0.35 wt.%) or BP (0.35 wt.%) to make the final concentration of 5 wt.% oil phase. These emulsion systems were stirred in a blender (IKA, 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). For a comparison, the primary emulsion was diluted with an equal amount of deionized water to make the oil phase concentration of 5 wt.% as a control.

2.3. Droplet size and size distribution measurements

Droplet size of orange oil emulsions was determined by dynamic light scattering using a Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed angle of 90°. Emulsions were diluted to a final oil droplet concentration of 0.005 wt.% with deionized water prior to each measurement to minimize multiple scattering effects. A refractive index of 1.45 and 1.33 was used for the oil droplet and the solvent, respectively. Results were described as cumulant mean diameter (size, nm) for droplet size, and polydispersity index (PdI) for droplet size distribution. All measurements were performed in triplicate.

2.4. 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 Nano ZS90 (Malvern Instruments, Worcestershire, UK). Emulsions were diluted to a final oil droplet concentration of 0.005 wt.% with deionized water to avoid multiple scattering effects. The data was collected from at least 10 sequential reading per sample after 120 s of equilibration, and the data were calculated by the instrument using the Smoluchowski model. All measurements were performed in triplicate.

2.5. Physical stability measurement of orange oil emulsions

The physical stability of orange oil emulsions was measured with LUMiSizer (L.U.M. GmbH, Berlin, Germany), a novel instrument employing centrifugal sedimentation to accelerate the occurrence of instability phenomena such as sedimentation, flocculation or creaming (Xu, Wang, Jiang, Yuan, & Gao, 2012). The integration graph shows the percentage of light absorbance per hour, the "creaming rate". The rate is correlated to the stability of the emulsion: the higher the creaming rate, the lower the stability. The instrumental parameters used for the measurement were as follows: rotational speed, 4000 rpm; temperature, 25 °C; time interval, 30 s; and time $_{Exp}$, 7650 s.