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A soy protein-polysaccharides Maillard reaction product enhanced the physical stability of oil-in-water emulsions containing citral

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ABSTRACT

The processing parameters for making a Maillard reaction product (SPPMP) from soy protein isolate (SPI) and soy soluble polysaccharide (SSPS) were studied against the yield of the product and its emulsification capacity in an oil-in-water emulsion. The optimized SPPMP was produced by dry-heating the SPI-SSPS mixture (SPP) at a ratio of 3:5, temperature of 60 °C and 75% relative humidity for 3 days. The formation of SPI-SSPS conjugates was confirmed by gel electrophoresis, FTIR spectroscopy and high performance size exclusion chromatography. The citral (10 wt%) oil-in-water emulsions stabilized by SPPMP exhibited superior physical stability than those stabilized by SPI or SPP during prolonged storage, after thermal treatment or under simulated gastrointestinal conditions. At pH 7.0, all the emulsions studied exhibited monomodal particle size distribution initially, however, only those stabilized by SPPMP remained monomodal distribution for up to 70 days during storage at 25 °C. The SPPMP-stabilized emulsion maintained its physical stability to the thermal treatment at 95 °C for 30 min or under simulated gastric conditions for 2 h; while the emulsions stabilized by SPI or SPP exhibited various degrees of instability. The release rate of citral from the emulsion droplets was found inversely related to the stability of emulsion. The emulsion droplets retained approximately 70% of citral after 2 h incubation in simulated gastric fluid, whereas, complete release of citral from the droplets occurred in 4 h in simulated intestinal fluid. These results indicate that SPPMP-stabilized emulsions have a good potential as a carrier system for intestinal delivery of hydrophobic compounds such as citral.

1. Introduction

Protein-stabilized oil-in-water emulsions have been developed and widely used for delivery of hydrophobic bioactive compounds in food and pharmaceutical applications. In such applications, these emulsions may be susceptible to a broad range of instabilities caused by, for examples, changes of temperature, pH, ionic strength, and presences of digestive enzymes and other surface active compounds (Sarkar, Goh, & Singh, 2010). The physical and chemical stabilities of emulsion droplets in these challenging environments have a profound impact on the digestion and adsorption of bioactive compounds they carry (Golding & Wooster, 2010; Xu et al., 2014). Previous studies have demonstrated that emulsions stabilized by proteins, such as whey protein and soy protein isolates, could lose their stability under gastric conditions due to reasons mentioned above (Kato, 1996). It has been demonstrated that addition of polysaccharides to protein-stabilized emulsions, either conjugated or unconjugated with the proteins, improved their stability against thermal treatment and under simulated gastric conditions (Diftis & Kiosseoglou, 2003, 2006; Ray & Rousseau, 2013; Xu et al., 2014). Protein-polysaccharide complexes can be formed via electrostatic or covalent bonds, however, only covalently linked complexes are expected to be more stable in adverse conditions. The protein and polysaccharide complexes prepared by Maillard reaction showed high tolerance to the changes of pH, temperature and ironic strength, and high resistance against proteolytic reaction and oxidation (Akhtar & Dickinson, 2003; Corzo-Martinez, Soria, Belloque, Villamiel, & Moreno, 2010; Dickinson & Semenova, 1992; Drusch et al., 2009; Kasran, Cui, & Goff, 2013; Lesmes & McClements, 2012; Oliver, Augustin, & Sanguansri, 2009). Presumably, the effectiveness of these conjugates to

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stabilize emulsions depends on the nature of the composed proteins and polysaccharides, as well as their processing conditions. By selecting the Maillard-type protein and polysaccharide complexes it is possible to control the release of emulsified bioactives in the gastrointestinal tract (Head, Sanguansri, & Augustin, 2005; Lesmes & McClements, 2012; Oliver, Augustin, & Sanguansri, 2009).

Soy protein isolate (SPI) is commercially available and widely used as an emulsifier in food industry. Soy soluble polysaccharide (SSPS) is a highly branched complex polysaccharide extracted from the by-products of SPI or soy milk processing. Incorporating SSPS into SPI stabilized-emulsions improved the emulsion stability against low pH, thermal treatment and peptic and tryptic digestions (Fafaungwithayakul, Hongsprabhas, & Hongsprabhas, 2011; Ray & Rousseau, 2013; Roudsari, Nakamura, Smith, & Corredig, 2006). However, the emulsification capacity of conjugated SPI-SSPS, especially their performance in the gastrointestinal environments, have not been reported.

Citral is the major compound of citrus-derived essential oils which has shown selective inhibition capacity towards common pathogens found in the animal intestines over beneficial bacteria above a certain concentration (Si et al., 2009). However, citral has not been widely applied for pathogen control in the animal intestines largely owing to a lack of suitable carrier to prevent its loss during processing, storage and gastric transition. Although formulations of citral-in-water emulsions have been the subject of many studies (Choi, Decker, Henson, Popplewell, & McClements, 2009; Djordjevic, Cercaci, Alamed, McClements, & Decker, 2007, 2008; Yang, Tian, Ho, & Huang, 2011), plant-derived protein and polysaccharide complexes were rarely used as emulsifiers and stabilizers in these studies. In addition, the citral concentration in the previous studies was low (<2% (w/w)) and the stability and release characteristics of citral containing emulsions under simulated gastrointestinal conditions were not reported. In order to enhance the antimicrobial ability of citral and other similar essential oils, there is a need to prepare emulsions with high citral loading capacity and high stability during processing, storage and gastric passage, so that sufficient amount of citral can be delivered to the intestine. Therefore, the objectives of the current study were to: (1) optimize the production parameters of a soy protein-polysaccharide Maillard reaction product (SPPMP) with regard to their emulsification capacities, and (2) investigate the storage and thermal stabilities of SPPMP-stabilized citral oil-in-water emulsions, as well as their performance under simulated gastrointestinal conditions.

2. Materials and methods

2.1. Materials

Soy protein isolate and soy soluble polysaccharide were provided by Shandong Gushen Industrial and Commercial Co., Ltd (Shandong, China). The sample of SPI contained 92.0% protein (Dumas combustion method), 0.33 mmol/g free $-NH_2$ (measured as described by Liu, Xiong, & Butterfield, 2000); the sample of SSPS contained 85% total sugar (phenol-sulfuric acid method), 0.48 mmol/g reducing sugar (3,5-di-nitrosalicylic acid method, glucose as standard), 4.0% protein (Dumas combustion method), and the average molecular weight (Mw) was 542 kDa (high performance liquid chromatography with standard PS-82 from Showa Denko, Japan). Citral (mixture of cis and trans isomers, 95% pure), pepsin from porcine gastric mucosa (lot# SLBC 4920V, 280 ± 6 units/mg solid as tested during this study period, ≥250 units/mg solid as reported by the manufacture), pancreatin from porcine pancreas (lot# 071M1639V, 4×United States Pharmacopeial specifications as reported by the manufacture) and porcine bile extract (Lot# 031M010V) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Hexane (HPLC grade) was purchased from Caledon Laboratories Ltd (Georgetown, ON, Canada). All other reagents and chemicals were of analytical grade unless otherwise specified. 2 M HCl and 2 M NaOH were used to adjust the pH. Ultrapure water produced by Milli-Q® Reference Water Purification System (Lit No: PB0015ENUS) was used to prepare all solutions.

2.2. Preparation of soy protein-polysaccharides mixture (SPP) and soy protein-polysaccharide Maillard reaction product (SPPMP)

Soy protein isolate (10%, w/w) and soy soluble polysaccharide (10%, w/w) were dispersed in water with stirring for 4 h at 25 °C, respectively, followed by pH adjustment to 7.0 and storage at 4 °C overnight. The dispersions of SPI and SSPS were centrifuged at 15,000 *g* for 30 min at 25 °C, separately, and the supernatants were collected as the soluble SPI and SSPS solutions, the solid contents of which were determined. The SPI and SSPS solutions were then mixed at SSPS:SPI ratios of 3:8, 1:2, 3:5 and 4:5, respectively (dry weight basis). These mixed solutions were freeze dried and grind to powders (particle diameters < 0.125 mm), to yield soy protein-polysaccharides mixture (SPP). A portion of SPP was placed in an capped container in the presence of saturated sodium chloride (relative humidity of 75%), heated at 55, 60 or 65 °C, respectively for 36–96 h, to induce Maillard reaction. The mixture was then dispersed into water with stirring for 4 h, followed by centrifugation at 15,000 *g* for 30 min. The supernatant was collected and freeze dried, yielding the soy protein-polysaccharide Maillard reaction product (SPPMP). SPP and SPPMP were sealed and stored at 4 °C until further use.

2.3. Preparation of oil-in-water emulsions

Samples of SPI, SPP or SPPMP (3.7%, w/w) were dissolved in water with stirring at 400 rpm for 4 h at 25 °C, separately, followed by adjusting pH to 7.0 with 2 M NaOH or 2 M HCl. Citral (10%, w/w) was slowly poured into the SPI, SPP or SPPMP solution with stirring at 400 rpm, respectively. Sodium azide (0.02% w/w) was added to prevent microbial growth. These citral dispersions were pre-homogenized with a Polytron® (PT 2500E, EQUL-kinematica) for 3 min at the speed of 20,000 rpm, followed by homogenization with a laboratory jet homogenizer (Nano DeBEE 8710, B.E.E. International Inc.) at the pressure of 7500 psi for 3 passes. Portions of the emulsions were adjusted to pH 4.5 or 2.0 with 2 M HCl with stirring at 250 rpm for 30 min. Triplicates were prepared for each emulsion.

2.4. Storage and thermal treatment of emulsions

Freshly made emulsions were stored in 20 mL capped amber glass vials, each with approximately 10 mL headspace, in the dark at 25 °C for up to 140 days. Some of the freshly made emulsions (in 20 mL amber glass vial) were heated to 90 °C at pH 7.0 in a water bath for 30 min (Xu & Yao, 2009) and then rapidly cooled to 25 °C in an ice bath before storage.

2.5. Droplet characterization

The ζ-potential of oil droplets was determined at 25 °C using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK) which measures the direction and velocity of droplet movement in an applied electric field. The droplet size distribution was determined using the same instrument by dynamic light scattering and Z-average diameter was recorded as a measure of droplet size. 100 μL aliquot from the middle part of each emulsion was taken by pipetting, followed by