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Formation of thymol nanoemulsions with combinations of casein hydrolysates and sucrose stearate

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ABSTRACT

Casein hydrolysates (CHs) have the improved emulsifying activity than caseins, and the emulsion stability can be principally improved by co-adsorbing sucrose stearate (SS). The objective of this work was to fabricate thymol nanoemulsions using the combination of CH and SS. CH produced by 10 min pancreatic hydrolysis was used at 2% and mixed with SS to prepare emulsions by shear homogenization and heating at 90 °C for 20 min. Addition of SS from 0 to 1.0% increased the thymol content in nanoemulsions ($d_{3,2} \approx 50$ nm) from 1 to 3%. Excess SS however increased the emulsion turbidity and instability due to its poor solubility at ambient conditions. Heating improved the solubility of SS, strengthened its hydrophobic interactions with CH, and thus greatly improved the emulsion storage stability and the stability at pH 3.0 and 5.0. Therefore, the combination of CH and SS provides a novel approach to prepare nanoemulsions.

1. Introduction

Oil-in-water emulsions are frequently studied to deliver lipophilic bioactive components such as beta-carotene (Qian et al., 2012), curcumin (Pan et al., 2013), and essential oils (Chen and Zhong, 2015; Luo et al., 2014b) in aqueous products. Nanoemulsions have advantages when compared to conventional emulsions. Droplets in nanoemulsions are relatively small (<200 nm in diameter) and are not as effective in scattering visible light, which provide the unique opportunity of nanoemulsions for use in transparent products (McClements, 2011). The small dimension of droplets also improves the stability against droplet aggregation and gravitational separation (McClements, 2011). Microemulsions are another group of oil–water-surfactant mixtures with structures smaller 100 nm (McClements, 2011). Different from nanoemulsions, microemulsions are thermodynamically stable and isotropic, and the structures of the dispersed phase are not always spherical (McClements, 2011). The ultra-low interfacial tension in microemulsions can enable their formation spontaneously (McClements, 2011). However, the surfactant: oil ratio in microemulsions is typically higher than in nanoemulsions, and the type of surfactants forming food grade microemulsions is limited.

Nanoemulsions have been studied extensively using synthetic surfactants and surface-active biopolymers (Courthaudon et al., 1991b). For food applications, generally-recognized-as-safe (GRAS) emulsifiers such as polysaccharides, proteins, and certain sugar-fatty acid esters are preferred to meet regulatory requirements (McClements, 2011). Whey protein isolate (Chanamai and McClements, 2002), soy protein isolate (Sofos and Allen, 1977), and sodium caseinate (NaCas) (Hu et al., 2003) are commonly studied as emulsifiers (McClements, 2011). NaCas has excellent emulsification properties due to its copolymer-like flexible structure (Robson and Dalgleish, 1987). However, with the isoelectric point (pI) being pH 4.6, NaCas has few net charges around pH 5.0 and precipitates due to the weakened electrostatic and steric repulsions, and thus the prepared emulsions can be destabilized (Tuinier et al., 2002). Additionally, biopolymeric surfactants are not as effective as small molecular surfactants in adsorbing onto new interfaces and reducing interfacial tension during emulsification, and therefore are not as effective in reducing droplet dimension to form nanoemulsions (Jafari et al., 2007).

To improve functionalities of proteins, hydrolysis of proteins to smaller molecules is a common approach (Mahmoud et al., 1992). The reduction of molecular weight improves the flexibility and solubility of proteins (Luo et al., 2014a; O'Regan and Mulvihill, 2010), as well as antioxidant properties (Taghvaei et al., 2014). Hydrolysis of caseins using proteases is the most commonly studied

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method to improve functional properties such as inhibition of angiotensin I-converting enzyme and antihypertensive properties (Miguel et al., 2009), hypersensitiveness (Sampson et al., 1991), antioxidant activity (Gómez-Ruiz et al., 2008), and antimicrobial activity (Hayes et al., 2006). Therefore, it is of great interest to prepare nanoemulsions using casein hydrolysates (CHs) as not only an emulsifier but also a functional ingredient.

However, there are a few limitations of using CHs to prepare nanoemulsions. The stabilization of emulsions via the stearic repulsion mechanism of NaCas is weakened, and the stability of the prepared emulsions around pH 4.6 can still be poor (Luo et al., 2014a). The elasticity of interfacial films and therefore the stability of emulsions can also be reduced when the molar mass of proteins/peptides decreases (Dwyer et al., 2013). To overcome these limitations, nonionic emulsifiers may be used to co-emulsify oils with CHs, because the nonionic head groups provide steric hindrance to stabilize emulsions and are insensitive to pH and ionic strength (Anarjan and Tan, 2013b).

The objective of the present work was to fabricate thymol nanoemulsions using CHs and sucrose stearate (SS). SS is produced by esterification of sucrose with edible stearic acid (Fanun, 2008) and is GRAS (Hasenhuettl, 2008). Because there are eight hydroxyl groups available on one sucrose molecule, different amounts of stearate molecules can be grafted on sucrose to form mono-, di-, tri-, or polyesters to obtain a wide range of hydrophile-lipophile-balance (HLB) values (Glatter et al., 2001). Furthermore, because the conformation of stearate is a function of temperature (Fanun, 2008), temperature can be used as an additional factor to fabricate emulsions. Thymol, the major component in the essential oil extracted from *Carum copticum* or *Thymus vulgaris* (Deb et al., 2011), was chosen as a model lipophilic compound because it is classified as GRAS by the U.S. Food and Drug Administration (Weiss et al., 2009). The excellent antioxidation (Aeschbach et al., 1994) and antimicrobial activities (Guarda et al., 2011) of thymol have been widely reported. The solubility of thymol in water is 0.75 mg/mL at 21 °C, and nanoemulsification of thymol by NaCas can improve its antimicrobial activity in complex food matrices such as milk (Pan et al., 2014).

2. Materials and methods

2.1. Materials

NaCas, thymol (>99% purity), and pancreatin (catalogue number P1750) were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Propylene glycol (PG) was purchased from Fisher Scientific (Pittsburgh, PA, USA). The commercial SS product (S1170) donated by Mitsubishi-Kagaku Foods Corporation. (Tokyo, Japan) contained 55% monoesters and 45% di-/tri-/poly-esters, according to the manufacturer.

2.2. Preparation of casein hydrolysates

NaCas was dissolved at 5% w/v in 200.000 mL of 0.010 M sodium phosphate-buffered saline (PBS), and pancreatin was dissolved at an enzyme: substrate mass ratio of 1:1000 (Mahmoud et al., 1992). The mixture pH was adjusted to 8.0 before incubation in a shaking water bath (C 76 classic series, New Brunswick Scientific Co., Inc., Edison, NJ, USA) operating at 120 rpm and 37 °C. Fifty milliliters of samples were withdrawn after hydrolysis for 10 min, 1 h, 4 h, and 20 h without pH adjustment. The withdrawn samples were heated at 95 °C for 10 min to inactivate the enzyme. After cooling in an ice/water bath, the samples were centrifuged (SORVALL RC5B Plus centrifuge, DuPont, Wilmington, DE, USA) at 5000 *g* for 20 min to remove insoluble contents. The supernatants were freeze-dried (VirTis AdVantage Plus EL-85 benchtop freeze dryer, SP Scientific Inc., Gardiner, NY, USA), and the powder was collected and stored at −20 °C before further use.

2.3. Preparation of emulsions

Thymol was dissolved in PG at different concentrations. SS was mixed with CH solutions, followed by stirring overnight to ensure compete hydration. The thymol/PG solution was added into the emulsifier dispersion. The acidity was adjusted to pH 6.8. The mixtures were homogenized at 10,000 rpm for 2 min using an Ultra-Turrax high speed homogenizer (model IKA T25 digital, IKA® Werke GmbH & Co., Staufen, Germany) to prepare coarse emulsions. The coarse emulsions were heated at 90 °C for 20 min in a water bath, followed by quenching in an ice/water bath under static conditions. The first variable was studied for conditions of preparing CHs, by preparing emulsions using fixed concentrations of SS and thymol. The next group of variables were then studied for emulsions prepared with a fixed CH concentration but varying SS and thymol concentrations.

2.4. Turbidity and particle size of emulsions

Sample turbidity was measured for absorbance at 600 nm (Abs_{600}) as an indicator using a UV–Vis spectrophotometer (model Biomate 5, Thermo Electron Corporation, Woburn, MA, USA). Deionized water was used as a blank.

The particle size of samples was measured using a Delas Nano particle analyzer (Beckman Coulter, Fullerton, CA, USA). All samples were diluted to an appropriate concentration in deionized water prior to analysis to meet the required sensitivity of the instrument. The area-volume ($d_{3,2}$) mean diameters were calculated using Eq. (1).

$$d_{3,2} = \frac{\sum_{i=1} n_i d_i^3}{\sum_{i=1} n_i d_i^2} \quad (1)$$

where d_i is the diameter of the *i*th group of droplets and n_i is the corresponding number of droplets.

2.5. Atomic force microscopy (AFM)

The morphology and dimension of particles were characterized using a Multimode VIII microscope (Bruker Corporation, Billerica, MA, USA). Nanoemulsions were diluted to 50 ppm CH using deionized water. Five microlitters of the diluted sample was dropped on a freshly cleaved mica disk and dried under ambient temperature (21 °C) in a chemical fume hood overnight. Images were collected at the tapping mode and analyzed using the instrument software.

2.6. Fluorescence spectroscopy

The interactions between SS and CH were studied by fluorescence spectroscopy using a spectrofluorometer (model RF-1501, Shimadzu Corp., Kyoto, Japan). The solubility of SS in water was limited at room temperature (21 °C), but aqueous dispersions with 0–4 mg/mL SS and 0.5 mg/mL CH adjusted to pH 6.8 were overall transparent after 2-h incubation at both 21 and 90 °C. Furthermore, no difference in fluorescence spectra was observed for samples heated at 90 °C for 2 h or 20 min after cooling down to room temperature in an ice/water bath. Fluorescence spectra were collected at an excitation wavelength of 280 or 295 nm. The slit