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Influence of pH and cinnamaldehyde on the physical stability and lipolysis of whey protein isolate-stabilized emulsions

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ABSTRACT

Cinnamaldehyde (CA), a common hydrophobic flavor, was encapsulated in oil-in-water emulsions that were stabilized by whey protein isolate (WPI). The impact of CA content and pH on the physical stability and lipolysis of the emulsions was then investigated. The presence of CA gave the emulsions a creamy yellow color, which became darker during storage. Emulsions formed using only CA as the oil phase contained large droplets that were physically unstable to particle growth and phase separation. The addition of medium chain triglyceride oil (MCT) improved the stability of emulsions containing CA, which was attributed to inhibition of Ostwald ripening effects. Fluorescent microscopy indicated that the adsorption of the protein to the droplet surfaces led to a thicker adsorbed layer in the presence of CA. The stability of the emulsions to droplet flocculation and coalescence depended on the CA level in the oil phase and the pH of the aqueous phase. An *in vitro* model was used to assess the impact of oil phase composition and pH on lipid hydrolysis and emulsion microstructure under simulated gastrointestinal tract conditions. The rate of lipid hydrolysis was highly dependent on CA level and pH. These results may facilitate the fabrication of emulsions with controlled GIT fate that are suitable for use in functional foods and beverages.

1. Introduction

Essential oils, usually extracted from plants, are increasingly being utilized as functional ingredients in foods because of their potent antimicrobial, antioxidant, and antiradical activities (Asbahani et al., 2015). These oils are complex mixtures of both non-volatile and volatile compounds that vary in their lipophilicity and water-insolubility. Those compounds include alkaloids, flavonoids, isoflavones, monoterpenes, phenolic acids, carotenoids, and aldehydes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Seow, Yeo, Chung, & Yuk, 2014). The relatively low water-solubility of essential oils means that they need to be incorporated into colloidal delivery systems before they can be introduced into aqueous-based foods. However, these delivery systems must be carefully designed to maintain the biological activity of the active components, and to ensure that they do not adversely impact the desirable sensory characteristics of foods and beverages, such as appearance, texture and flavor (Buranasuksombat, Kwon, Turner, & Bhandari, 2011).

Emulsion-based delivery systems, which can easily be formulated from food-grade ingredients, are a popular vehicle used to encapsulate, deliver and protect lipophilic functional components (McClements & Li, 2010b). The selection of an appropriate oil phase and emulsifier is critical to creating an emulsion-based delivery system with the desired functional attributes. One of the major challenges that needs to be overcome when fabricating essential oil-loaded emulsions is the fact that the oil phase contains many components that are sufficiently soluble in water, promoting droplet growth through Ostwald ripening, which then accelerates creaming and phase separation (Chang, McLandsborough, & McClements, 2012; Tian, Lei, Zhang, & Li, 2016). Ostwald ripening occurs due to the diffusion of oil molecules from small droplets to large droplets through the intervening aqueous phase. The incorporation of water-insoluble oils (such as medium or long chain triglycerides) into the oil phase can suppress Ostwald ripening through an entropy of mixing effect known as compositional

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ripening. Consequently, the oil phase composition has to be carefully controlled to produce essential oil-loaded emulsions that are physically stable.

The nature of the emulsifier is also important because it determines the initial size of the droplets produced during homogenization, their subsequent stability to aggregation, and their gastrointestinal fate (Speranza et al., 2013). There is a growing trend in the food industry towards developing "clean-label" products containing only natural ingredients. For this reason, many food manufacturers aim to develop emulsion-based delivery systems using natural emulsifiers (McClements & Gumus, 2016). In this study, we used a milk protein (whey protein isolate, WPI) as an emulsifier to form the essential oil-loaded emulsions because it is already widely used in the food industry to stabilize emulsion-based food products (Giang et al., 2015; Qiu, Zhao, Decker, & McClements, 2015; Sarkar et al., 2016). Unlike many other food oils, essential oils contain compounds that have chemically reactive functional groups that can covalently interact with protein molecules, such as aldehydes (Sarika, Anil Kumar, Raj, & James, 2015). Consequently, it is important to understand how these chemical interactions between oil phase components and emulsifiers influence the formation and stability of the emulsion-based delivery systems formed.

Cinnamaldehyde (3-phenyl-2-propenal, CA), which is the major active constituent in cinnamon oil, has been shown to have broad-spectrum antimicrobial activity against bacteria, yeasts, and molds (Otoni et al., 2014; Shen et al., 2015). CA is a hydrophobic aromatic aldehyde that has been approved by the FAO/WHO Expert Committee on Food Additives (JECFA) for use as a food-flavoring agent. Encapsulation of CA using emulsion-based delivery systems may be used to enhance its water-dispersibility, control its volatility, and control its flavor profile (Shao, Shao, Jiang, & Sun, 2016). CA has a free carbonyl group that can react with free amino groups on protein molecules through a Schiff-base reaction. Our previous study showed that CA encapsulated in oil droplets could react with chitosan molecules in the surrounding aqueous phase, which promoted the formation of a gel network when pH was higher than 4.3 (Lei et al., 2015). In this study, we hypothesized that the encapsulated CA may interact with protein molecules adsorbed to the oil droplet surfaces, thereby altering their functional properties. For this reason, we prepared CA emulsions using WPI as an emulsifier and medium chain triglycerides (MCT) as a ripening inhibitor. The physical properties and lipolysis of protein-stabilized emulsions were investigated in the present study. The rate of the Schiff-base reaction depends on solution pH (Li, Shao, Shi, & Hu, 2013), and so the physical stability and gastrointestinal fate of CA emulsions were evaluated at different pH values. The knowledge gained from this study may be useful for the formulation of natural essential oil-loaded emulsions that can be used as functional food components, such as antimicrobials or antioxidants.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, Hilmar™ 9410) was obtained from Hilmar Ingredients (Turlock, CA, USA). It consisted of 93% protein, 1.0% fat, 4.5% moisture and 2.0% ash. Medium chain triglyceride (MCT) oil was purchased from Boxing Chemical Reagent Co. Ltd. (Wuhan, China). MCT are the triglycerides formed by glycerol and Caprylic (C8:0) and Capric acid (C10:0), which has a density of 950 kg m^{-3}. The molecular weight was selected as 521 g/mol in the present study. Cinnamaldehyde (3-phenyl-2-propenal, 95%, CA) was purchased from Aladdin Reagent Co. (Shanghai, China). The molecular weight is 132 g/mol. Pepsin and mucin from porcine stomach were obtained from Yuanye Biotechnology Co. Ltd. (Shanghai, China). Lipase from porcine pancreas was obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Bile salt (porcine) was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Rhodamine B (analytical grade), fluorescein isothiocyanate (FITC), nile red were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultra-pure water used in the preparation of all solutions and emulsions was obtained from a Milli-Q-water purification system (Millipore, MA, USA). All other reagents used were of analytical grade.

2.2. Emulsion preparation

An aqueous phase containing 2 wt% WPI as an emulsifier was prepared by mixing 8 g WPI and 392 g ultra-pure water, stirring at room temperature for 2 h, and then holding at 4 °C overnight to ensure complete hydration. To prepare the emulsions, 90 g of aqueous phase and 10 g of oil phase were homogenized together in an ice bath using a high-speed disperser (T18 digital ULTRA TURRAX, IKA Instruments Ltd, Germany) operated at 12,000 rpm for 3 min. Oil phases with three different compositions were tested: pure MCT, 1:1 mass ratio of MCT and CA, and pure CA. The corresponding emulsions are referred to as "MCT emulsion", "MCT/CA emulsion" and "CA emulsion", respectively. The obtained emulsions were placed in sealed glass tubes to investigate their physical stability when held at room temperature (around 25 °C).

2.3. Physical stability

After preparation, the emulsions were adjusted to pH 3.0, 7.0 and 11.0, and then the emulsions were stirred for 15 min. Before analysis, these emulsions were placed in sealed glass tubes for different periods.

2.4. In vitro gastrointestinal model

A simulated gastrointestinal tract (GIT) model was used to evaluate the impact of CA on the potential gastrointestinal fate of the emulsions. This model consisted of simulated mouth, stomach, and small intestine phases (Chen et al., 2015; McClements & Li, 2010a).

2.4.1. Mouth phase

Human saliva is a complex system, which mainly consists of water (~99.5%), various proteins (~0.3%), small organic compounds and inorganic salts (Humphrey & Williamson, 2001). According to the previous studies (Sarkar, Goh, & Singh, 2009; Guo, Ye, Lad, Dalgleish, & Singh, 2014; Minekus et al., 2014), the parameters to simulate oral conditions were selected as follow. Simulated saliva fluid (SSF) containing 0.5 wt% mucin and various salts was prepared as described previously (Sarkar et al., 2009). To start the digestion experiment, 9.0 mL emulsion containing 4 wt% oil was mixed with 9.0 mL SSF; the final mixture contained 2 wt% oil and 0.25% mucin. The pH of the mixture was then adjusted to 6.8 and the mixture was shaken continuously at 100 rpm in a magnetic stirring water bath (Model DF-101S, Changcheng Science and Engineering Instruments, Zhengzhou, China) (37 °C) for 10 min to mimic oral conditions. It was noted that 10 min was selected for the convenience of the experiment, which did not indicate the real oral administration. After the mouth phase, 15 mL of the mixture were used to continue the gastrointestinal fate, while the rest was collected to observe its microstructure.