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Improving the stability of phosphatidylcholine-enhanced nanoemulsions using octenyl succinic anhydride-modified starch

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ABSTRACT

Phosphatidylcholine-coated emulsions are unstable and prone to phase separation under certain environmental stresses, such as low pH and moderate ionic strength (>100 mmol/L NaCl). This study investigated the formation and stability of octenyl succinic anhydride-modified starch (OSAS)-stabilized nanoemulsions enhanced with phosphatidylcholine for the encapsulation of soybean oil. The effect of variables (OSAS/phosphatidylcholine weight ratio, oil composition, emulsifier concentration, etc.) and environmental stresses (pH, temperature, ionic strength, etc.) on the stability of phosphatidylcholine-enhanced nanoemulsions stabilized by OSAS was examined. Adequate addition of OSAS (OSAS/phosphatidylcholine weight ratio = 3:7) led to the formation of a more stable nanoemulsion. An emulsion at pH 2 was unstable against creaming, and no phase separation occurred after 130 h; presumably, OSAS has a certain degree of steric repulsion that protects the nanoemulsion from oiling-off. The phosphatidylcholine-enhanced nanoemulsions stabilized by OSAS were stable at high NaCl concentrations (≤800 mmol/L) and temperatures from 30 to 90 °C, which was attributed to steric and electrostatic repulsions. Transmission electron microscopy images showed that OSAS and phosphatidylcholine were both adsorbed around the surface of the emulsion. These results showed that adding OSAS could improve the formation and stability of phosphatidylcholine-stabilized emulsions.

1. Introduction

Oil-in-water emulsion (O/W emulsion) systems are found in many food products, such as a variety of dairy products, functional beverages and salad dressings. In recent decades, the production and stability of O/W emulsions containing functional lipophilic components (such as curcumin, vitamin E and γ-oryzanol) have been evaluated in many studies [1–3]. The type and concentration of emulsifiers are key factors that influence the formation and stabilization of emulsions [4]. Emulsifiers, such as protein, phosphatidylcholine and Tween series compounds, are commonly used to stabilize emulsions [5,6]. The main limiting factor to the widespread use of emulsions stabilized by protein, phosphatidylcholine and Tween series compounds is their potential instability caused by various environmental factors. For example, emulsions formed with whey protein isolate are unstable at its isoelectric point and are easily denatured at approximately 75–80 °C [7]. Emulsions produced using Tween 80 and Span 20 were found to be unstable at higher temperatures (≥70 °C) [3]. Although phosphatidylcholine is one of the most widely used emulsifiers in the food, cosmetic and pharmaceutical industries due to its biocompatibility, phosphatidylcholine-coated emulsions are susceptible to oxidative deterioration [8]. In addition, phosphatidylcholine-coated nanoemulsions were observed to be highly unstable against phase separation at low pH values and ionic strengths above 100 mmol/L NaCl [9]. Instead of using a single emulsifier in an emulsion formulation, using more than one type of emulsifiers is an alternative that can form a thick and dense droplet layer and improve emulsion stability under certain types of environmental variables [10]. A combination of protein, carbohydrate and phosphatidylcholine was reported to stabilize emulsions [11]. However, little information is available about using octenyl succinic anhydride-modified starch (OSAS) to improve the stabilization of emulsions enhanced with phosphatidylcholine for the encapsulation of oils.

The OSAS as an emulsifier has received considerable attention due to its low price, good emulsifying ability and better stability under environmental conditions (i.e., temperature, a wide range of pH and ionic strength) [10]. The possibility of using OSAS to form and stabilize emulsions was also reported in other studies [12–14]. Steric repulsion plays a more significant role in the stabilization of emulsions by the OSAS than electrostatic repulsion [12,15]. However, lipid droplets coated with regular emulsifiers (i.e., protein and phosphatidylcholine) which are primarily stabilized by electrostatic repulsion are relatively unstable under acidic conditions because of a reduction in surface charge and electrostatic repulsion [9].

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The objective of the present study was to evaluate the formation and stability of OSAS-stabilized nanoemulsions enhanced with phosphatidylcholine for the encapsulation of soybean oil. First, the effect of major factors (OSAS/phosphatidylcholine weight ratio, oil composition, emulsifier concentration, etc.) on the formation and stabilization of emulsions was examined. Second, the influence of environmental factors involved in food processing and the food system on the stability of phosphatidylcholine-enhanced emulsions stabilized by OSAS was investigated.

2. Materials and methods

2.1. Materials

Medium-chain triglycerides (MCTs) and γ-oryzanol (purity >98%) were purchased from the Shanghai Yiji Industrial Co., Ltd. (Shanghai, China) and Dalian Meilun Biotech Co., Ltd. (Dalian, China), respectively. Phosphatidylcholine (CAS No. 8002-43-5) was supplied by the Hefei Bomei Biotechnology Co., Ltd. (Hefei, China). OSAS (trade name: Purity Gum 2000) was purchased from the National Starch and Chemicals (Shanghai, China). All the other reagents used were of analytic grade.

2.2. Nanoemulsion preparation

2.2.1. General

Initially, MCTs, soybean oil, γ-oryzanol (1% by mass, in MCTs and soybean oil) and phosphatidylcholine were added to a beaker and mixed by magnetic stirring for 1 h at 50 °C, the resultant solution was the organic phase (oils and lipophilic components). The aqueous phase was prepared by dissolving OSAS in ultrapure water and stirring at 433 rpm at a temperature of 30 °C for 1 h. Then, the organic phase and the aqueous phase were mixed together at 100 rpm and 30 °C for 30 min. Finally, the mixture was then emulsified to produce an emulsion using an Ultra Turrax T18 homogenizer (IKA, Staufen, Germany). Sodium azide solution was added to emulsions in order to inhibit microbial growth during the investigation.

2.2.2. Effect of main variables on the formation and stabilization of nanoemulsions

The effect of OSAS/phosphatidylcholine weight ratio on the z-average diameter, polydispersity index (PDI) and stability of emulsions was investigated by varying OSAS/phosphatidylcholine weight ratio (0:10, 1:9, 3:7, 5:5 and 7:3), while the other conditions were 7% emulsifiers and 4% oil (MCT/soybean oil weight ratio = 5:5). Emulsions were then formed by homogenization at 20,000 rpm for 2 min.

The oils included MCTs and soybean oil. The effect of MCTs/soybean oil weight ratio on the physical stability of emulsions was studied by fixing oil concentration at 4% and emulsifiers (OSAS/phosphatidylcholine weight ratio = 3:7) concentration at 7% while MCTs/soybean oil weight ratio was set at 0:10, 3:7, 5:5, 8:2 and 10:0. Emulsions were then formed by homogenization at 20,000 rpm for 2 min.

The influence of oil concentration on the physical stability of emulsions was investigated by varying oil (MCT/soybean oil weight ratio = 3:7) concentration from 1% to 5% while emulsifiers concentration (OSAS/phosphatidylcholine weight ratio = 3:7) was 7%. Emulsions were then formed by homogenization at 20,000 rpm for 2 min.

The effect of the homogenization time on the physical stability of emulsions was studied by varying the homogenization time from 2 to 10 min. This was conducted while the other conditions of emulsion preparation were set at 7% emulsifiers (OSAS/phosphatidylcholine weight ratio = 3:7) and 3% oil (MCT/soybean oil weight ratio = 3:7).

2.3. Physicochemical stability testing of nanoemulsions

The physical stability of nanoemulsions obtained with the optimized conditions was investigated by determining changes in z-average diameter, PDI and ζ-potential under various environmental stresses. The influence of pH was evaluated by adding 1 mol/L HCl or NaOH to the nanoemulsions for a final pH of the nanoemulsions of 2 to 10. The influence of heat treatment was studied by keeping the nanoemulsions in a water bath (30–90 °C for 20 min) followed by immediate cooling in an ice-water bath to room temperature. The influence of ionic strength was studied by adding various amounts of NaCl or $CaCl_2$ solution to the nanoemulsions.

A nanoemulsion obtained with the optimized conditions was stored at 4, 25 and 37 °C for 45 days. Nanoemulsion samples were withdrawn at various time intervals and degassed with nitrogen. The samples degassed were stored at −80 °C until further analysis. The chemical stability of the emulsions was studied by determining changes in primary and secondary lipid oxidation products. Peroxide and anisidine values measures were obtained to represent the primary and secondary lipid oxidation products, respectively. The peroxide value was determined according to the method outlined by Shantha and Decker [16] and the procedure was described in detail in a previous study [3].

The anisidine value of nanoemulsions during storage was determined according to the standard method ISO 6885 [17].

2.4. Particle size, PDI and ζ-potential measurements

The diameter of particles, PDI and ζ-potential of emulsion samples, obtained as described in sections "2.2. Nanoemulsion preparation" and "2.3. Physicochemical stability testing of nanoemulsions", were measured by dynamic light scattering on a Malvern Zetasizer Nano ZS90 instrument (Malvern Instruments, Worcestershire, UK). All the emulsion samples were diluted 1000 times with ultrapure water to prevent multiple scattering effects. Each individual measurement was an average of 13 runs. All the samples were measured at least in duplicate at 25 °C. The mean particle size is expressed as the z-average diameter.

2.5. Morphology measurement

Emulsions prepared using various emulsifiers (i.e., OSAS, phosphatidylcholine, and a mixture of OSAS and phosphatidylcholine in a weight ratio of 3:7) were prepared under the following conditions: 7% each type of emulsifier, 3% oil (MCT/soybean oil weight ratio = 3:7) and 20,000 rpm homogenization speed for 8 min. The morphological characteristics of the emulsions prepared with the different emulsifiers were observed by transmission electron microscopy (JEOL JEM-1400, JEOL Ltd., Japan). The emulsion samples were diluted 10 times with water, and the diluted sample solutions were adsorbed onto a carbon-coated copper grid for 1 min. Then, excess solution was removed by filter paper. Finally, a drop of 2% phosphotungstic acid solution was applied to the grids for 1 min. Excess phosphotungstic acid was removed by filter paper. Finally, the grids were observed by the instrument operating at an acceleration voltage of 100 kV.

2.6. Statistical analysis

The data are presented as the mean ± standard deviation. Statistical analysis of the data was performed using SPSS software (version 14.0 demo; SPSS Inc., Chicago, IL). The significance of differences between the measured mean values was evaluated at the 0.05 probability level.

3. Results and discussion

3.1. Effect of the formulation parameters on the formation and stabilization of nanoemulsions

3.1.1. Effect of OSA-modified starch/phosphatidylcholine weight ratio

Two emulsifiers, OSAS and phosphatidylcholine, were used to form emulsions by high-speed homogenization. The z-average diameter of