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Development of a palm olein oil-in-water (o/w) emulsion stabilized by a whey protein isolate nanofibrils-alginate complex

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ABSTRACT

An oil-in-water (o/w) emulsion is a system where the oil droplets are dispersed within a watery phase. The most important function of emulsions is their ability to incorporate lipophilic components into food matrices. Thus, it is crucial to develop an emulsion that is highly stable. This work was aimed at developing a palm olein o/w emulsion stabilized by a whey protein isolate nanofibrils-alginate complex, as well as evaluating the influence of oil load and the homogenization process (both pressure and cycle) on the characteristics of the o/w emulsions. Emulsions were analyzed for droplet size, zeta potential, viscosity, creaming stability, and morphology. The results showed that an increase in oil load led to a larger droplet size, less negative zeta potential, and emulsions that were more viscous and less stable. On the other hand, increasing homogenization pressure and the number of homogenization cycles resulted in a smaller droplet size, more negative zeta potential, and emulsions that were less viscous and more stable. Emulsions with a smaller droplet size and better stability resulted from lower oil load, high homogenization pressure and more homogenization cycles.

1. Introduction

Proteins are widely used as emulsifiers in food products. However, protein-stabilized emulsions are highly sensitive to environmental stresses such as pH, ionic strength, and temperature, which limit their application in many foods (Das & Kinsella, 1989; McClements, 2004; Monahan, McClements, & German, 1996). Mixing of protein with hydrocolloids was found to improve the resistance of these protein-stabilized emulsions to environmental stresses (Diftis & Kiosseoglou, 2003; Hattori, Ogino, Nakai, & Takahashi, 1997). In fact, the addition of polysaccharide caused the formation of an interfacial complex with the adsorbed protein layer after homogenization, which could improve the emulsion stability (Dickinson, 1995; McClements, 2004).

Under the appropriate conditions, whey proteins have the ability to self-assemble into long, semi-flexible structures, which are known as amyloid fibrils (Rogers et al., 2006). β-Lactoglobulin (β-lg) is the most abundant protein (approximately 50%) present in whey protein, which self-assembles into amyloid-like fibrils by heat-induced hydrolysis under high-temperature thermal treatment at a low pH (Aymard, Nicolai, Durand, & Clark, 1999). Alginate as its sodium salt, sodium alginate, is able to form complexes with proteins such as WPI (Harnsilawat, Pongsawatmanit, & McClements, 2006).

Emulsions are typically classified according to the spatial distribution of the oil and water phases relative to each other. An oil-in-water (o/w) emulsion is a system where the oil droplets are dispersed within a watery phase. The most important function of emulsions is their ability to incorporate lipophilic components into food matrices. Thus, it is crucial to develop an emulsion that is highly stable. In the food industry, a homogenization process, which usually subjects the liquids to mechanical agitation (e.g., high speed blenders, high-pressure valve homogenizers, and colloid mills), is carried out to improve the stability of emulsions

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(Perrier-Cornet, Marie, & Gervais, 2005).

The objectives of this work were to develop a palm olein o/w emulsion stabilized by a whey protein isolate (WPI) nanofibrils-alginate complex and also investigate the effects of the formulation and processing parameters to the emulsion characteristics, as to produce an emulsion that is highly stable. In the present work, the effect of oil load, homogenization pressure and homogenization cycles on the characteristics of the o/w emulsions were investigated. Three different sets of o/w emulsions were studied by altering the oil load (10%, 20%, and 30%). To determine the effects of homogenization process on the emulsions, a series of experiments were carried out at three different homogenization pressures (50, 75, and 100 MPa) and homogenization cycles (3, 5, and 7 passes). The concentration of the emulsifier was fixed at 2% of WPI nanofibrils-alginate complexes, which dispersed in the aqueous phase. The characteristics of the emulsions include mean droplet size, zeta potential, viscosity, creaming index and microstructure.

2. Material and methods

2.1. Materials

BiPRO whey protein isolate (WPI) rich in β-lg (approximately 70%) was obtained from Davisco Foods International, Inc. (Eden Prairie, MN). Sodium alginate (GRINSTED® Alginate FD 155) was obtained from Danisco (Australia). Additive-free RBD palm olein oil was purchased from Moi Foods Sdn. Bhd. (Selangor, Malaysia). All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparing WPI solutions and producing WPI fibrils

WPI fibril solutions were prepared according to the established method with slight modification (Serfert et al., 2014). In brief, 2.5% (w/w) of the WPI was dissolved in deionized water, and the pH of the WPI solutions was adjusted to 4.6 with 6.0 M HCl to precipitate the denatured protein. The WPI solutions were centrifuged at 6000 × g for 30 min at 25 °C (Heraeus Multifuge, Thermo Fisher Scientific, Dreieich, Germany) and subsequently subjected to vacuum filtration using 0.2 μm regenerated cellulose filter paper (Sartorius, Kuala Lumpur, Malaysia). Native WPI solutions were formed by adjusting the WPI solutions to a pH of 2.0 with 6.0 M HCl. For fibrillation to occur, native WPI solutions were heated at 80 °C in a temperature-controlled water-bath (Memmert WNB 14, Gemini BV, Apeldoorn, Netherland) for 20 h under 400 rpm (2Mag Magnetic Motion, Gemini BV, Apeldoorn, Netherland). The fibrillar WPI solutions were rapidly cooled in an ice water bath for 30 min after 20 h of heating.

2.2.2. High pressure homogenization of the whey protein isolate fibrils solution

Prior to high pressure homogenization, the WPI fibril solutions were diluted with pH 2.0 deionized water in a 1:1 w/w ratio. The solutions were then homogenized in a high-pressure homogenizer (HomoGENIUS, GEA Niro Saovi, Italy) at 70 MPa for 5 cycles. The resulted solutions contained 1% (w/w) of WPI nanofibrils. The particle size of the WPI nanofibril solutions was determined by using a Zetasizer Nano system (Malvern Instruments Inc., Worcester, UK). The refractive index of the fibrils sample was set at 1.450 with the water as dispersant. The measurements were performed at 25°C, at 10 s intervals, measuring the backscatter at 173°. The particle size of the WPI nanofibrils was 38.7 ± 0.6 nm.

2.2.3. Fibril complexation with sodium alginate

Sodium alginate solution was prepared by dispersing 1.0 wt% powdered sodium alginate in deionized and distilled water and stirring for at least 2 h. Sodium azide (0.04 wt%) was also added to this solution to prevent microbial growth. The WPI nanofibrils-alginate complexes from the mixing of WPI and alginate dispersions at different polysaccharide/protein weight ratios were obtained by the post-blending acidification method (Hosseini et al., 2013). The pH of the WPI nanofibril solution was adjusted to pH 3.0 using 1.0 M NaOH solution. While stirring, the 1.0% (w/w) of sodium alginate solution was added to 1.0% (w/w) of WPI nanofibrils solution. These solutions were stirred for 1 h and then allowed to equilibrate at ambient temperature for 18–24 h prior to emulsion preparation.

2.2.4. Emulsion preparation

Palm olein oil (organic phase) was added into the complex solution (aqueous phase), with different ratios (w/w) of organic and aqueous phases (1:9, 2:8, and 3:7). Sodium azide (0.01%) was added to each emulsion as a preservative. These mixtures were then sheared using a shear homogenizer at 5000 rpm for 5 min to yield a coarse emulsion premix. These premix samples were then processed into fine emulsions by passing them through high pressure homogenizer under various pressures (50, 75, and 100 MPa) and various cycles (3, 5, and 7 passes). The homogenizer was chilled throughout the homogenization procedure to avoid excessive heating of the emulsions.

2.3. Emulsion characterization

2.3.1. Droplet size measurement

The emulsions droplet size $D_{4,3}$ (volume weighed mean or volume mean diameter) was measured using a laser light scattering instrument (Mastersizer, Malvern Instruments, Worcestershire, UK). The emulsion sample was measured 5 min after high pressure homogenizing to cancel any creaming effect, and diluted under stirring to about 1/1000 with distilled water in the wet sample dispersion unit. The droplet size is determined by means of light scattering.

2.3.2. Zeta potential

All of the samples were diluted to 0.1% v/v by using deionized water before zeta potential measurements with a Zetasizer Nano system (Malvern Instruments Inc., Worcester, UK). The refractive index of the sample was set at 1.450 with the water as dispersant. The viscosity of the sample was set at 0.8872 cP, which was assumed to be the same as that of water. The measurements were made at 25 °C and an equilibration time of 120 s. The Smoluchowski model was applied to derive the zeta potential. The measurement settings and the voltage were set to auto to allow the software to determine the optimum voltage for the measurement and also for the number of runs performed per measurement.

2.3.3. Emulsion viscosity

The apparent viscosities of the WPI nanofibrils solutions were measured immediately after the sample preparation at 25 °C using a rheometer equipped with a concentric cylinder measuring system (Rheolab QC, Anton Paar, USA) at a fixed shear rate of 1550.0 s^{-1}. The viscosity measurement from the rheometer appears as mPa·s.

2.3.4. Emulsion stability

The emulsion stability of each emulsion was accessed according to Winuprasith and Suphantharika (2015). Immediately after preparation, 15 g of each emulsion was transferred into a transparent glass test tube (20 mm diameter and 70 mm height) and sealed with a plastic cap. The sample tubes were kept at room temperature, and the movement of any creaming boundary was