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Influence of surfactin on physical and oxidative stability of microemulsions with docosahexaenoic acid

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ABSTRACT

Docosahexaenoic acid (DHA), one of the most important omega-3 polyunsaturated fatty acids (PUFAs), shows significant health benefits for human beings. In order to stabilize nutrients like DHA, microemulsion is normally used through the addition of surfactant, and surfactin as a natural peptide biosurfactant shows strong surface activity. In this study, we investigated the effects of surfactin on the stability of docosahexaenoic acid single cell oil (DHASCO) microemulsions. The microemusion region was significantly increased with elevated surfactin concentration from 0 to 0.2 mmol/L, and reached a maximum. The o/w region of DHASCO microemulsion could significantly increase and the diameters of microemulsion particles were reduced from 140 to 15 nm after addition of 0.2 mmol/L surfactin into emulsion system. Generally, the physical and anti-oxidation stability of the o/w DHASCO microemulsion with surfactin was highly enhanced. DHA oxidation in microemulsion with surfaction was significantly reduced even stored at 37 °C for 60 days as compared to non-surfactin. The excellent properties of microemulsion with surfactin could be useful in functional food and medicine.

1. Introduction

Micro algae oils are natural mixtures of PUFAs which were extracted from algae [1]. Docosahexaenoic acid single cell oil (DHASCO) is one of the characteristic functional ingredients in micro algae (*Crypthecodinium Cohnii*) oils. DHASCO can reduce the risks of cardiovascular diseases, inflammation, and cancer, and improve the brain development [2,3]. Currently, DHA added into food and dietary supplements are mainly obtained from fish oils and micro algae oils. In comparison of fish oils, micro algae oils have higher level of DHA and lower level of eicosapentaenoic acid [4,5]. The DHASCO is the only source of children's DHA supplement recognized by the US food and drug administration (FDA) with no fishy smell and less heavy metal pollution. However, DHASCO also shows several drawbacks that greatly limits its application in food processing, such as 1) DHASCO is sensitive to external factors such as light, heat and oxygen; 2) Oxidized DHASCO is easy to lose nutrition and physiological active function [6,7].

In order to stabilize nutrients like DHASCO, microemulsion is normally used through the addition of amphiphilic molecules which act by decreasing the oil-water interfacial tension between the phases, increasing the steric hindrances and/or the electrostatic repulsion between the micelles [8,9]. In this method, lipids are encapsuled in the micelles to isolate from the influence of oxygen in oil-in-water (o/w) microemulsions [10]. The oxidation of lipids is usually raised from the interaction of lipid hydroperoxides located at the surface of the oil droplets [11,12]. In the process of oxidation, lipid hydroperoxides are decomposed to hydrogen peroxide, and produce free radicals to affect other unoxidized lipid molecules [13,14]. External factors, such as temperature, light exposure, oxidizers, reductants and ionic strength will also influence oxidation rate of lipids [15,16]. To prevent against these external factors, selection of appropriate amphiphilic molecules or addition of certain antioxidants can form more stable micelles in order to protect lipid from oxidation.

Amphiphilic molecules commonly used in microemulsion are surfactants, such as sodium dodecyl benzene sulfonate (SDS, an anionic surfactants), quaternary ammonium salt (a cationic surfactants), lecithin (a zwitterionic surfactants), Tweens (a non-ionic surfactants). Unfortunately, uses of these artificial surfactants have already caused serious damage to the environment [17]. In order to protect the environment, researchers have been focusing on studying the natural source of biodegradable surfactant. Among these surfactants, surfactin is one of the sustainable and environmental-friendly surface active agents low toxicity, and it is used as a model to study the adsorption of biosurfactants at hydrophobic and

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hydrophilic solid-liquid interfaces as well as the air-liquid interface [18]. The Hwang's research on mutagenic rate and animal cell micronucleus rate showed that surfactin (500 mg/kg body weight (BW) per day) did not cause genetic and developmental toxicity, and its LD_{50} was over 5,000 mg/kg BW, which belongs to the non-toxic level [19].

Surfactin is formed from a lactone ring containing seven amino acid residues (L-Glu → L-Leu → D-Leu → L-Val → L-Asp → D-Leu → L-Leu), linked to a β-fatty acid (lipidic) via a lactone bond [20,21]. The two amino acids (Glu and Asp) provide the hydrophilic domain, while other amino acids and fatty acid chain provide the hydrophobic moiety [22]. This nature promotes surfactin folding into a β-sheet structure, resembling a horse saddle, which explains its wide biological and interfacial activity [23]. For instance, surfactin can reduce the interfacial tension of the water-air and water-hexadecane systems from 72.8 to 27.9 mN m^{-1} [23,24]. Its properties promote the use of surfactin in several industrial applications such as detergent formulations [25], emulsions, health care products, cosmetics [26,27], pharmaceuticals and oil recovery [28]. It is also well-known that surfactin shows significant antimicrobial activities against bacteria, fungi and viruses, and also exhibits antitumor and fibrinolytic activity [29,30].

Since surfactin has stronger surface activity than most of other natural surfactants which added into food emulsions and no report on application of food microemulsion with the addition of surfactin is studied. Therefore, the main objective of this study is to investigate the effects of surfactin added in o/w DHASCO microemulsion on the physical stability and oxidative stability.

2. Materials and methods

2.1. Materials

Docosahexaenoic acid single cell oil (DHASCO) was obtained from Zhejiang Several Billion Technology Co., Ltd (Zhejiang, China), and testing results showed that DHA content was over 46.8%. Isopropyl myristate (IPM) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Tween 80 and glycerol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sucrose ester of fatty acid-11 and lecithin were all food grade, which were purchased from Zhejiang Deyer Chemicals Co., Ltd (Zhejiang, China). All chemicals except food-grade surfactants used in this study are analytical grade. Surfactin was fermented and purified in our laboratory. The purity of surfactin was about 88.6% after purification [31].

2.2. DHASCO microemulsion formation

The appropriate proportion of oil phase (IPM:DHASCO = 3:1, m/m) and surfactants (Tween 80: Glycerol = 2:1, m/m) were blended at 60 °C, and kept stirring at the speed of 20 rpm with magnetic stirrer while adding aqueous phase (10 mmol/L NaCl solution, pH = 8.0 ± 0.2) dropwise (not faster than 1 mL/min). The influence of preparation process and the areas of microemulsion in pseudo ternary phase diagram were measured after adding 0.2 mmol/L surfactin in aqueous phase.

2.3. Microemulsion type determinations and conductivities measurements

Staining method was used to determine the microemulsion type [32]. The water-soluble dye methyl orange and lipid-soluble dyes Sudan Red were added in equal amounts to the microemulsion samples, respectively. The mixtures were shaken slowly and diffusion and staining effects of dyes were observed. Water-soluble dye diffused faster and stained more complete in o/w microemulsion, and vice versa. Conductivities of deionized water, oil phase (IPM:DHASCO = 3:1) and microemulsion preserved at 60 °C for 30 min were detected. Each state was detected after 5 min after the preparation of microemulsion, then the microemulsion region was roughly divided based on the measured electric conductivities [33].

2.4. Centrifugal and static stability

Microemulsion samples were diluted 5 times with deionized water. Samples were centrifuged at 2,000, 3,000, 4,000, 5,000, 7,000, 9,000, 12,000, 15,000, 18,000 rpm, respectively, for 30 min at room temperature. After the centrifugation, observed whether the samples were layered or not and whether they could keep clear and transparent. Then, all the samples were stored in 4 °C for 30 days, and 37 °C for 60 days, respectively. OD_{600} of the samples was also determined [34] by UV–vis spectrophotometer (Shimadzu Co., China) to measure the turbidity.

2.5. Particle size of DHASCO microemulsion

Microemulsion samples were diluted 5 times with deionized water for follow-up experiments. Particle sizes were tested by NanoBrook 90Plus Zeta Particle Size Analyzer (Brookhaven Instruments Co., US). Microstructures were observed by H-7650 Transmission Electron Microscope (Hitachi High-Technologies Co., Japan).

2.6. Rheological characteristics

The viscosities of DHASCO microemulsion were determined under the conditions of constant shear rate ($\dot{\gamma}$) = 1 s^{-1}, temperature (T) range from 4 to 96 °C.

Furthermore, the viscosity of tested samples under the conditions of constant temperature (T) = 25 °C, shear rate ($\dot{\gamma}$) range from 0 to 1000 s^{-1} was also tested.

Data can be analyzed by Ostwald–de Waele power law:

$$\eta_a = \frac{\tau}{\dot{\gamma}} = K\frac{\dot{\gamma}^n}{\dot{\gamma}} = K \cdot \dot{\gamma}^{n-1} \quad (1)$$

where η_a is apparent viscosity, τ is sheer stress, $\dot{\gamma}$ is sheer rate, K is flow consistency index, n is flow behavior index. According to the equation fit out, n = 1 is a Newtonian fluid, n > 1 is a dilatant fluid and n < 1 is a pseudoplastic fluid [35].

2.7. DHA stability—TBARS and peroxide value (POV) test

The microemulsion samples was separated into several portions and treated in different conditions, respectively. Treating conditions include heat treatment at room temperature (10 °C), 37 °C, 90 °C for 30 min in the dark, and in direct sunlight (light intensity is about 10^6 Lux) at room temperature (10 °C) for 30 min. In addition, the malondialdehyde (MDA) contents of each experiment group by TBARS assay were determined by comparing with oxidation degree of DHA for each sample.

Titrimetric method was used to detect the peroxide value. Microemulsion samples were dissolved in 30 mL acetic acid/trichloromethane (3:2, V/V), then 1 mL saturated solution potassium iodide was added in each sample. After 30 s, 100 mL of deionized water and 1 mL of 0.1 g/L starch solution was added. Then, samples were mixed evenly and 0.002 mol/L sodium thiosulfate was used to titrate until it turned to colorless. Volume