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Roles of Fucoidan, an Anionic Sulfated Polysaccharide on BSA-Stabilized Oil-in-Water Emulsion

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Abstract: Fucoidan, a sulfated polysaccharide derived from brown seaweed, is an important material valued for its various biological functions, including anti-coagulation, anti-aging, and immune system support. In this study, we examined the potential of fucoidan as a novel emulsifying agent in BSA (bovine serum albumin)-stabilized emulsion at a neutral pH. We measured the dispersed oil-droplet size, surface zeta-potential and creaming formation of 0.5 wt% BSA emulsion (20 wt% oil fraction) in the absence and presence of fucoidan. The average particle size and zeta-potential value were 625.4 nm and -30.91 mV in only BSA-stabilized emulsion and 745.2 nm and -44.2 mV in 1.0 wt% fucoidan-added BSA emulsion, respectively. This result suggested that some positive charges of the BSA molecules interacted with the negative charges of fucoidan to inhibit the flocculation among the oil droplets. The creaming rate calculated from the backscattering data measured by Turbiscan dramatically decreased in 1.0 wt% fucoidan-added BSA emulsion during storage. Accordingly, the repulsion forces induced among the oil particles coated with 1.0 wt% fucoidan in emulsion solution resulted in significantly increased emulsion stability. The turbidity of the BSA-stabilized emulsion at 500 nm decreased during five days of storage. However, the fucoidan-added BSA emulsion exhibited a higher value of turbidity than the BSA-stabilized emulsion did. In conclusion, an anionic sulfated fucoidan lowered the surface zeta-potential of BSA-coated oil droplets via the electrostatic interaction, and subsequently inhibited the flocculation among the oil droplets, thereby clearly minimizing the creaming and phase separation of the emulsion.

Keywords: fucoidan, surface zeta-potential, electrostatic interaction, emulsion stability.

Introduction

Emulsions have important roles in various industry fields such as beverages, cosmetics, pharmaceutical products, cleaning agents and so on. In any case, emulsions consist of oil phase-dispersed into aqueous phase, vice versa, and these are thermodynamically unstable to be subject to phase separation with time due to the change in density between two phases. Commonly, physical phenomena occurred in emulsions are flocculation, coalescence and creaming, leading to destabilization of emulsion.[1] To retard these destabilization and enhance emulsion stability during storage, the surface active materials such as proteins, carbohydrates, small molecules and so on can be used. Protein as a bioactive surfactant has been well known to lower the surface or interface tension and to enhance the stability of dispersion into the continuous phase. Especially, bovine serum albumin (BSA) is widely used as emulsifying agent in food colloidal system because of its amphiphilic structure and flexible conformation. Another important type of surface-active component is the polysaccharide utilized as thickening, gelling and water-holding agents in food industry. Among food polysaccharides, gum arabic is distinguished by its emulsifying properties under acidic conditions, and for this reason it is commonly used in soft drink industry.[2] Recently, many researchers demonstrated that the emulsion made with 0.5 wt% fenugreek gum has large surface area and the oil droplets over 70% were under 1 μm in diameter.[3] Besides, so many polysaccharides on the emulsifying properties have been investigated, but fucoidan has not been used ever. Fucoidan is a sulfated heteropolysaccharide obtained from brown seaweed, and commonly produced in as health-promoting supplements in many countries because of its diverse biological activities such as anticoagulant,[4,5] antivirus,[6] anticancer activity[7,8] and antioxidant activity.[9,10] Generally, it is known that fucoidan consisted mainly of α-1,3-linked L-fucose residues. The brown seaweeds such as *Ascophyllum nodosum*, *Undaria pinnatifida*, *Fucus evanescens*, and *Laminaria japonica* are well known as an abundant source of fucoidan. According to the previous studies, monosaccharide composition of fucoidan isolated from *L. japonica* contains the major components of fucose, galac-

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tose and minor of mannose, glucose, xylose and rhamnose.[11] Although the dynamic viscoelasticity of fucoidan is increased linearly with an increase of fucoidan concentration up to 2% (w/w), the fucoidan solution can be stable over the wide range of pH (5.8 to 9.5). This indicates that the fucoidan molecules are stable under both acidic and alkaline conditions, and then it is suitable for stabilizing the food colloidal system.[12] However, most polysaccharides have a little surface activity, and therefore there is limitation of using alone as an emulsifying agent. Thus, many researchers tried to make protein-polysaccharide complexes, and also layer-by-layer (LBL) technique was used to improve the emulsion stability against environmental stresses such as pH, ionic strength and thermal processing. The LBL electrostatic deposition method means to attachment of a polymeric layer to the surface of the emulsion droplet, come to that charged polysaccharides anchor to the surfaces of oppositely charged protein-coated oil droplets.[13-17] In this study, we intended to study the effect of fucoidan on the emulsifying activity and stability of BSA-stabilized O/W emulsions and to investigate its potent possibility as novel emulsifying stabilizer for functional dairy beverage, cosmetics and other industrial use.

Experimental

Materials. Lyophilized bovine serum albumin (BSA, Fraction V, minimum 96% by agarose gel electrophoresis, product #A4503) was purchased from Sigma Chemical Co., stored in refrigerator. Sodium phosphate dibasic anhydrous was purchased from Samchun Pure Chemical Co., Ltd (Kyeongki-do, Korea) and sodium dihydrogen phosphate anhydrous was purchased from Kanto Chemical Co., Inc (Tokyo, Japan). Corn oil was purchased from a local supermarket and used as the oil phase. Powdered fucoidan was obtained from Haewon Biotech, Inc (Seoul, Korea). Also, the compositional information analyzed by Japan Food Research Laboratories was provided by the manufacturer. Briefly, sulphate content and crude protein of fucoidan was assayed by ion chromatography and Kjeldahl method, respectively. The contents of total sugars and monosaccharide contents were determined by the HPLC method. The extracted fucoidan composed of carbohydrates (59.4%) and sulphates (24.3%) with a little amount of proteins (5.4%). Monosaccharide composition showed that fucose (14.9%) and galactose (17.9%) were major sugar and other monosaccharides such as glucose, xylose, rhamnose and mannose were also contained (Table I). Distilled water from a water purification system was used for the preparation of all solutions.

Preparation of Buffer and BSA Solution. A buffer solution was prepared by mixing 9.5 mL 0.2 M NaH_2PO_4 and 40.5 mL 0.2 M Na_2HPO_4 and diluted using distilled water (100 mM, 10 mM). BSA stock solution was prepared by dispersing BSA powder into buffer solution (10 mM phosphate buffer, pH 7.4).

Emulsion Preparation. Oil-in-water emulsions were prepared by pre-mixing 20 wt% corn oil and 80 wt% aqueous phase (BSA stock solution; 5 mg/mL, 100 mM phosphate buffer; pH 7.4, 10% fucoidan stock solution and distilled water) using an Ultra-turrax T25 homogenizer (IKA Labortechnik, Germany) for 2 min at 13,500 rpm. Then the probe sonicator (Model GE 50, Ultrasonic Processor 50 W model, Thomas Scientific, Swedesboro, New Jersey, USA) of power generation 20 kHz with 3 mm diameter tip was used for further emulsification.[18,19] To avoid temperature effects, the tube with emulsion prepared by homogenization was placed into a beaker with iced water.

Determination of Particle Size and Zeta-Potential. The particle size and charge distribution of emulsion were determined using Delsa™ Nano C (Beckman Coulter, Sandiego, U.S.A.). This instrument simultaneously measured both size and zeta potential distribution of dispersed particles with a duo laser light. For size analysis, Delsa Nano C used photon correlation spectroscopy (PCS) which determines particle size by measuring the rate of fluctuations in laser light intensity scattered by particles as they diffuse through a fluid. And, for zeta potential determination, it used electrophoretic light scattering (ELS), which determines electrophoretic movement of charged particles under an applied electric field from the Doppler shift of scattered light. All samples were analyzed immediately after preparation in triplicate. Prior to analysis, the emulsions were diluted to a droplet concentration of approximately 40 times using buffer solution (pH 7.4, 10 mM phosphate) to avoid multiple scattering effects only in zeta potential.

Spectroturbidity Measurements. Emulsifying properties were measured through turbidimetry according to the

Table I. Chemical Composition of Fucoidan and its Fractions Isolated from *L. japonica*

Moisture	8.5 g/100 g
Protein*1	5.4 g/100 g
Fat	2.5 g/100 g
Ash	24.2 g/100 g
Carbohydrates*2	59.4 g/100 g
Glucose*3	0.7 g/100 g
Xylose*3	0.4 g/100 g
Rhamnose*3	0.3 g/100 g
Fucose*3	14.9 g/100 g
Mannose*3	1.4 g/100 g
Galactose*3	17.9 g/100 g
Sulfate*4	24.3 %

*1Nitrogen-to-protein conversion factor: 6.25.
*2Formula: 100-(Moisture+Protein+Fat+Ash).
*3Acid hydrolysis was performed, and the determination was made. Hydrolysis condition: stirring for 1hour at room temperature in 72% sulfuric acid, and then at autoclave (121 °C) for 1 h in 4% sulfuric acid.
*4After boiling with hydrochloric acid, the test was conducted.

method of Pearce and Kinsella with a slight modification.[20] Before measurements, twenty microliters of the emulsion was taken from the bottom of the container and diluted with 15 mL of 0.1 M NaCl solution containing 0.1% SDS and then transferred into 1 cm path length plastic spectrophotometer cuvettes. The emulsifying activity was measured as absorbance at 500 nm immediately after the emulsion was made. The emulsifying activity index (EAI) was calculated using the following equation.[21]

$$EAI = 2T/(1-\Phi)C$$

Where Φ is oil fraction and C refers to the protein content in milligrams per milliliter. The turbidity, T, is calculated as 2.303 A/L, where A is the observed absorbance and L is the path length of the cuvette.

The emulsion stability index (ESI) was determined as follows.[22]

$$ESI\ (min) = T_0 t/T'$$

Where T_0 refers to turbidity at 0 min after emulsification, T' denotes the change in turbidity between 0 and 30 min, and t is the time interval.

The change in turbidity of emulsions during 5 days was determined using UV-visible spectrophotometer (Ultrospec® 2100 pro, Amersham Biosciences, Uppsala, Sweden) equipped with xenon lamp and wavelength range from 190 to 900 nm. The absorbance was read at 500 nm and distilled water being used as a reference. All samples were run in triplicate and averaged.

Creaming Stability Measurements. The creaming rate was monitored by measuring in a TurbiScan Lab (Formulaction, Toulouse, France). The detection head of turbiscan composed of a pulsed near infrared light source (880 nm) and two synchronous detector scans the 55 mm cylindrical glass cell height of the sample. The advantage of turbiscan is to characterize creaming phenomena of emulsion without dilution. Emulsions were sampled immediately after preparation and the sample was placed into cylindrical glass cell (55 mm) and analyzed by a light beam which scanned the glass cell. The samples were scanned every 6 min for one day at 25 °C and we analyzed delta backscattering and mean value kinetics.

Results and Discussion

Measurements of Particle Size and Zeta-Potential. We, first, examined the effect of an anionic sulfated polysaccharide, fucoidan on the dispersion properties of BSA-stabilized emulsion at fresh state and during storage. In our experiment, the effect of sonification on the dispersion properties of BSA-stabilized emulsion in the absence and presence of fucoidan was carried out. As a result, the emulsions prepared by using only homogenizer (Ultra-turrax T25) at 13,500 rpm had bimodal to polymodal dispersion profiles, whereas using combination of homogenizer and sonifier generated the mostly monomodal dispersion patterns at any concentration of fucoidan (data not shown). Thereby, the homogenization and subsequent sonification was used to make the emulsions. Figure 1 showed the cumulant mean diameter (μm) of dispersed oil droplet in the emulsions prepared with different concentrations of fucoidan (0-1.0 wt%). We obtained the similar patterns in particle size distribution of the emulsions formulated with and without fucoidan. Eventhough the emulsions were stored for 24 h, any significant changes in particle size were not observed at any fucoidan concentrations. Therefore, we measured the zeta potential value to determine whether an anionic sulfated fucoidan would alter the surface charges through interacting with BSA coated at the interface. Figure 1 shows that the protein-coated oil droplets at pH 7.4 were negatively charged (ζ= -30.91 mV), due to BSA is known to have approximately -18 mV of surface charge at the neutral pH. As the fucoidan concentration in the aqueous phase of the emulsions increased, the electrical charge on the droplets became more negative, until it finally reached -43.82 mV at the concentration of 1.0 wt% fucoidan in fresh emulsion. The steepness of the initial change in ζ-potential with increasing the fucoidan concentration up to 0.3 wt% was shown, but no remarkable change in ζ-potential occurred at the concentration from 0.3 to 1.0 wt% fucoidan, suggesting the BSA-coated interface saturated with fucoidan molecules. This result seems to be contradictory to the report that if protein and polysaccharides have similar electrical charges at pH 7, polysaccharides were not likely to be adsorbed the surfaces of the protein-coated droplets.[23] However, in our experiment, even though both BSA and fucoidan are known to be negatively charged at a neutral pH, the zeta potential value of the interface coated with mixture of BSA and 1.0 wt% fucoidan was much lowered than with BSA only. Therefore, we confer that the some positive charges on the BSA were initially interacted with the anionic sulfated

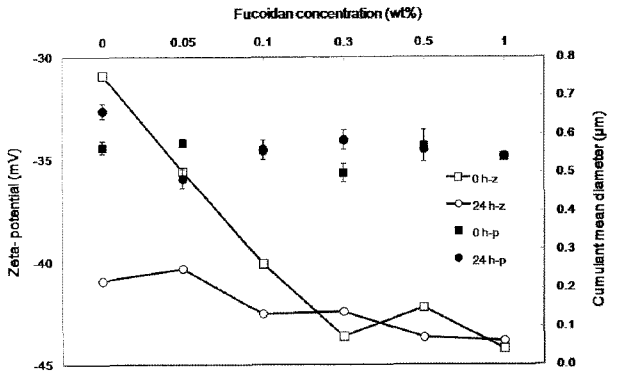

Figure 1. Influence of fucoidan concentration (0-1.0 wt%) on the measured cumulant mean diameter (closed symbols) and zeta potential (open symbols) of emulsions at 25 °C (0 h: squares, 24 h: circles).

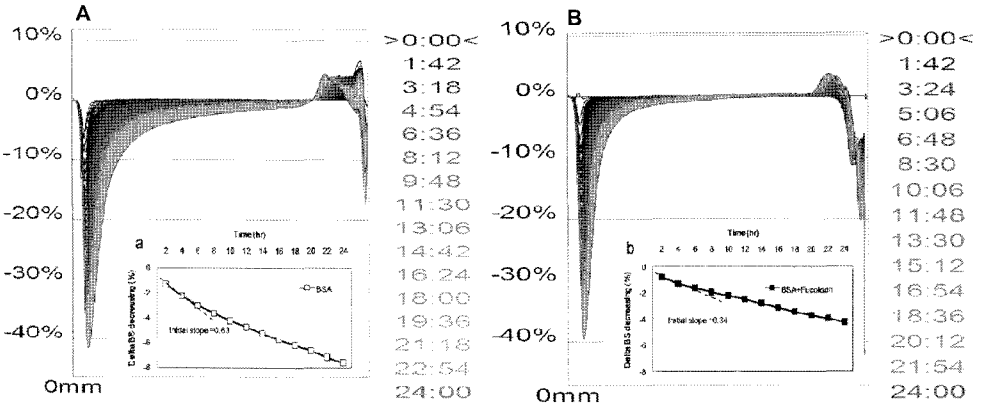

Figure 2. The creaming profiles of BSA-stabilized emulsion in the absence (A) and presence (B) of fucoidan (1.0 wt%) during 24 h. The mean value kinetics of emulsion as a function of time (a, b).

groups of fucoidan, and saturated with excessive negative charges enough to prevent droplets flocculation by forming a strong electrostatic repulsion. Also, the surface potentials of BSA only and BSA-fucoidan complex emulsion were measured with time course. Interestingly, ζ-potential value of only BSA stabilized interface was dramatically lowered after 24 h. Thus, we presumed that the molecular structure of BSA at the interface was rearranged and simultaneously, charge integrity at the interface was changed. Therefore, very little change in ζ-potential was observed even in the presence of fucoidan after 24 h. Nevertheless, addition of 1.0 wt% fucoidan lowered ζ-potential of interface in 24 h-aged emulsion, suggesting 1.0 wt% fucoidan would be enough to stabilize the protein-coated interface under any circumstances.

Therefore, it is believed that the repulsion force formed among the emulsion droplets saturated with fucoidan molecules prevented the oil droplets from accelerating flocculation and subsequent coalescence in oil-in-water emulsions. However, the compactness and integrity of BSA-fucoidan coated layer at the interface was not investigated in this study. And, further investigation is needed to determine the interfacial properties of BSA-fucoidan stabilized emulsion as a function of pH.

Creaming Stability Analysis. The creaming stability of the emulsions made from both BSA only and BSA-fucoidan complex solution was investigated using a Turbiscan (Formulaction, France). In this work, we scanned at preset intervals (6 min) during the period of 24 h (1 day) at 25 °C and analyzed delta backscattering profiles. At the beginning of the scanning (purple line), the emulsion was homogeneous but over a period time, delta backscattering increased at the top part of cylindrical glass cell and decreased at the bottom part (Figures 2(A), 2(B)). These results indicated that particle concentration at top of emulsion was increased with particle migrations, and subsequently creaming layer was formed. Generally, creaming profile analysis in delta backscattering data was evaluated through upper part of sample that was occurred creaming phenomena but in case of need, analyzing of creaming profiles was determined from the results of the front of delta backscattering plot contrary to general cases. Because of the effect of dependent diffusion, concentration of emulsion was above the saturation concentration of creaming layer, due to the distance of neighboring particles was close. In the absence of fucoidan, the BSA-stabilized emulsion was found to have a high creaming rate during 24 h, whereas the emulsion containing 1.0 wt% fucoidan was stabilized, although it displayed a similar tendency of profiles as the BSA-stabilized emulsion. And the extent of creaming in BSA-stabilized emulsion was more widely observed than fucoidan-BSA emulsions. In repeated experiments, the measurements both BSA-stabilized and fucoidan-BSA emulsions generated the same results. The evidence of creaming was also displayed with the change of creaming rate with time (Figures 2(a), 2(b)). Considerable change in the slope of mean value kinetics at the initial point (from 0 to 6 h) was shown in both samples, and the creaming rate of BSA-stabilized emulsion had 2 times higher than that of fucoidan-BSA emulsion. However, creaming rate was gradually reduced in process of time. Therefore, we confer that the fucoidan can stabilize the oil droplets against flocculation by forming a high negative charge layer around the BSA-coated interface.

Spectroturbidity Measurement. Figure 3 showed the time course of turbidity of emulsion. During the storage time from 0 to 96 h, the turbidity of emulsions showed a reduction, and the turbidity reduction of BSA-stabilized emulsion was maximal. On the other hand, fucoidan-BSA emulsion decreased much slower in storage time than BSA-stabilized emulsion. Interestingly, there were time-dependent differences from the turbidity change in emulsions. Remarkably, at storage times longer than 48 h, the turbidity of emulsion at low concentration of fucoidan(<0.5%) was lower than BSA emulsion. Conversely, at high concentrations (>0.5%),

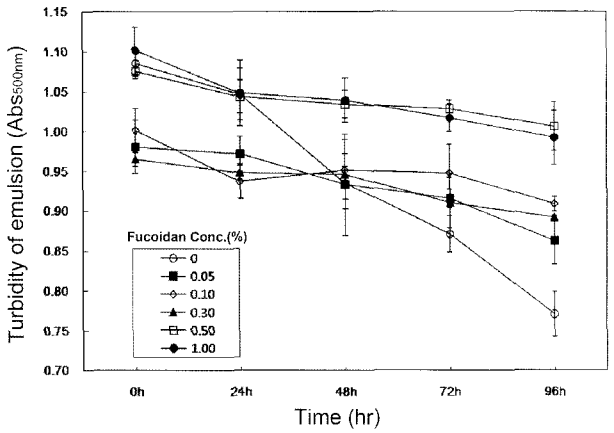

Figure 3. Spectroturbidity of 20 wt% corn oil-in-water emulsions containing different concentration of fucoidan (wt%): 0 (○); 0.05 (■); 0.1 (◇); 0.3 (▲); 0.5 (□); 1.0 (●).

the turbidity of emulsion was higher than BSA emulsion. These results were contradictory to the general theory that the turbidity value was closely correlated with particle size. Consequently, these results suggested that only BSA-stabilized emulsion remained stable for 24 h and started destabilized after 24 h rapidly. On the other hands, fucoidan-added emulsions showed a similar tendency in turbidity reduction, but there was no dramatic change in emulsion stability, indicating the positive role of fucoidan for emulsion stability at a neutral pH.

Conclusions

An anionic sulfated polysaccharide, fucoidan, significantly lowered the surface zeta-potential of 0.5 wt% BSA-stabilized oil-in-water emulsion, and subsequently inhibited the phase separation of the emulsion at pH 7.4. BSA-coated oil droplets negatively charged (ζ= -30.91 mV) became more negative until reached -43.82 mV at the concentration of 1.0 wt% fucoidan added in fresh emulsion. According to the measurement of creaming stability, the migration rate of oil phase in fucoidan-added emulsion was twice slower than that of only BSA-stabilized emulsion. Therefore, we believe that the fucoidan can stabilize the BSA-coated interface with hydrostatic interaction, and highly negative charged and thick layer around the interface leads to resistant to coalescence and stability against creaming. Accordingly, we confer that even though the particle size of fucoidan-added emulsion was similar to BSA only-stabilized emulsion, a highly negative charged and thick layers around the oil droplets coated with BSA and fucoidan can be more resistant to form the flocculations and further coalescence, resulting in delaying of phase destabilization in fucoidan-added emulsion during storage.

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