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Emulsifying and Foaming Properties of Ultraviolet-Irradiated Egg White Protein and Sodium Caseinate

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ABSTRACT: The physicochemical and functional properties of ultraviolet (UV)-treated egg white protein (EW) and sodium caseinate (SC) were investigated. UV irradiation of the proteins was carried out for 30, 60, 90, and 120 min. However, the SC samples were subjected to extended UV irradiation for 4 and 6 h as no difference was found on the initial UV exposure time. Formol titration, SDS-PAGE, and FTIR analyses indicated that UV irradiation could induce cross-linking on proteins and led to improved emulsifying and foaming properties ($P < 0.05$). These results indicated that the UV-irradiated EW and SC could be used as novel emulsifier and foaming agents in broad food systems for stabilizing and foaming purposes.

KEYWORDS: egg white protein, sodium caseinate, ultraviolet irradiation, SDS-PAGE, emulsification, foaming

INTRODUCTION

Many natural and processed foods consist of dispersions in the form of emulsions and foams. Emulsions refer to the dispersions of two or more immiscible liquids.[1] In other words, emulsions are heterogeneous systems consisting of one or more phases dispersed in a continuous phase. Stabilization of an emulsion system is achieved by amphiphilic surface active agents (emulsifiers) possessing an affinity for both phases.[2] From a fundamental point of view, emulsifiers forming the interfacial films in an emulsion can be modeled as a monolayer. Thus, the interactions between droplets of dispersed phase determine the stability of the emulsion, and the stability depends on the characteristics of this monolayer.[3] Additionally, the emulsifiers used must be placed at the interface, forming a film around the particles. These emulsifiers decrease the surface tension, and thus the free energy of the system, and impart kinetic stability to the emulsion.[4] This is known as the Marangoni mechanism.[5]

Proteins are able to stabilize emulsions because of their amphiphilic nature. These protein molecules concentrate at the oil−water interface, with the lipophilic portion in the nonpolar phase (oil) and the hydrophilic portion in the polar (water) phase.[5] The stabilization by protein is effective when the proteins form a solid viscoelastic layer. The proteins adsorb, partially unfold, and form strong interactions, which in turn result in a viscoelastic adsorbed layer that has been well correlated with emulsion stability.[6,7] The unfolding of proteins at interfaces is influenced by the structure in solution, such that flexible proteins will unfold quickly and rapidly lower the interfacial tension,[2,7] whereas globular proteins unfold more slowly as they have more intramolecular bonds stabilizing their structure.[5] Unfolded proteins tend to form stronger intermolecular interactions and stabilize against coalescence very effectively.[7] Therefore, changing the structure of proteins by various means has been used as a tool for improving protein functionality, probably by inducing a change in adsorbed conformation.

A typical foam is composed of millions of bubbles each encapsulated by a protein film and separated by thin water-filled canals (lamella).[2] According to Belitz et al.,[1] the protein is adsorbed at the interface via hydrophobic areas during whipping, followed by partial unfolding (surface denaturation). The protein adsorption then gives rise to a decrease of the surface tension and facilitates the formation of new interfaces and more gas bubbles (foams). Foams can be conveniently categorized into two subclasses: (1) bubbly foams (e.g., ice cream), in which the gas-to-liquid ratio is low enough for the gas cells to retain their roughly spherical shape; and (2) polyhedral foams (e.g., beer foams), in which the bubbles are more closely pressed together in a honeycomb-type structure.[8,9] Bubbles in foam are stabilized by a bilayer of protein (emulsifier) molecules separated by the continuous phase. Thus, the characteristics of this thin film (lamella) determine the stability of the foam.[5,6] Therefore, maintenance of the lamella is essential for foam stability because contact between adjacent bubbles results in disproportional coalescence and collapse of the foam.[2]

The foaming properties of proteins depend on a variety of factors, such as protein concentration, pH, ionic strength, aqueous phase composition, temperature, and method of foam production.[3] Modification of proteins such as chemical and physical modification may increase the foaming properties. In addition, partial enzymatic hydrolysis produces smaller molecules of higher diffusion rate, better solubility, and higher surface hydrophobicity in whey proteins.[10] Improved foaming ability and foaming stability were also observed on egg white proteins upon heat or high-pressure treatment.[11] These authors explained that the heat-induced conformational changes, by enhancing the protein−protein interactions at the interface, have given rise to improved foaming properties.

Ultraviolet (UV) irradiation appears to be a cost-effective, nonthermal, and environmentally friendly technology for the physical modification of protein structures. UV irradiation has been shown to modify proteins in general[12] and collagen in

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particular.[13] Our previous studies showed significant improvement on gel strength of fish gelatin[14] and improved functional properties of gum arabic[15] when treated with UV irradiation. Other than this, UV irradiation has shown significant tensile strength improvement on films made with egg white protein (EW) and sodium caseinate (SC) due to UV-induced cross-linking.[16] However, to our knowledge, no studies have been undertaken to investigate the effects of UV irradiation on EW and SC in their powder form and the functional properties, particularly emulsifying and foaming properties. From the literature, UV irradiation has shown the possibility to induce cross-linking in proteins. Therefore, we hypothesized that UV irradiation would induce structural changes and cross-linking on the protein component in EW and SC and subsequently improve their emulsifying and foaming properties. The main objectives of the present study were to investigate the effects of UV treatment on EW and SC and to explore the changes in the physicochemical and functional properties of EW and SC to be applied in the food industry.

MATERIALS AND METHODS

Materials. The egg white protein with 95.0% protein content was procured from Sim Co. Sdn. Bhd. (Penang, Malaysia); meanwhile, the sodium caseinate used in this study was a gift from NZMP—New Zealand Milk Products (Fonterra Co-operative Group Limited) with 94.5% protein content. Other chemicals were all of reagent grade and were used without further purification.

Modification of Food Proteins. The EW and SC samples were subjected to UV irradiation by following the method described by Bhat and Karim.[14] Briefly, ~15 g samples were spread in a thin layer on sterile oven dishes (15 × 15 cm) and subsequently exposed to a UV light source (253.7 nm; 30 W, Sankyo Denki, Kanagawa, Japan) positioned 30 cm away in a laminar flow cabinet (Pro-Lab, Neston, U.K.) for 0, 30, 60, 90, and 120 min. However, the SC samples were subjected to extended UV irradiation for 4 and 6 h as no difference was found in the results obtained from formol titration, SDS-PAGE, and FTIR analyses at initial UV exposure time. The samples were then kept in sterile polyethylene bags and stored for the succeeding analysis.

Total Free Amino Group Measurement (Formol Titration). All of the food protein samples were subjected to formol titration analysis for free amino group determination following the method of Denis et al.[17] Briefly, 0.5 g of sample (P) was placed in a 100 mL beaker and 20 mL of deionized water was added. The suspension was stirred for 5 min until complete dissolution occurred, and the pH was then adjusted to 7.4 ± 0.1 with 0.05 N NaOH. The pH was tested using a pH-meter (Delta 320, Mettler Toledo, Greifensee, Switzerland). The formol reagent was prepared by diluting 500 mL of the commercial solution with 200 mL of deionized water and thoroughly stirring the solution. The pH was adjusted to 7.4 ± 0.1 with 0.05 N NaOH just before use. Subsequently, 35 mL of the formol reagent was added to the suspension to be tested. The mixture was stirred for 5 min, and it was titrated to pH 9.2 ± 0.1 with 0.05 N NaOH using a 25 mL buret. The volume V (mL) of NaOH required was recorded. The quantity of total free amino groups [N_t (mmol/g)] present was then determined as

$$N_t = 0.05 \times \frac{V}{P}| \quad (1)$$

Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was performed by employing a method described by Laemmli.[18] A 4% acrylamide stacking gel and a 12% separating gel were used. EW and SC samples were standardized to 0.5% protein with deionized water. The standardized protein solutions were diluted with sample buffer containing 5% β-mercaptoethanol at ratio of 1:2. The mixtures were then heated at 90 °C for 5 min before loading into the sample wells. Approximately 15 mL of sample was loaded into each well in the gel. Subsequently, the samples were run at 150 V and 50 mA in the Mini-PROTEAN II electrophoresis cell (Bio-Rad Laboratories, Singapore) for 1.5 h until the blue band reached the bottom of the gel. The gel was stained with 0.1% Coomasie Brilliant Blue R-250 in 40% methanol and 10% acetic acid mixed solution and destained in a solution containing 40% methanol and 10% acetic acid.

The stained gel was then captured by using a chemiluminescence imaging system (LAS-3000, Fujifilm, Tokyo, Japan) controlled by using image reader software (LAS-3000, Fujifilm). The densitometry and molecular weight quantification were then carried out by using Multi Gauge version 2.2 analysis software (Fujifilm), and the protein fractions were identified using Bio-Rad's Precision Plus Protein Standards with molecular masses of 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa.

FTIR Spectroscopy Analysis. The total reflectance method was applied to obtain the Fourier transform infrared (FTIR) transmission spectra by employing a FTIR spectrometer (System 2000, Perkin-Elmer, Wellesley, MD). Briefly, 3 mg of sample was mixed with 200 mg of KBr and ground for 30 s using a stainless steel ball mill. A pellet was prepared using a press and was immediately put into the sample holder. The FTIR spectra were then recorded in the region of 4000—400 cm^{-1} for 20 scans. Corrected band heights were used for FTIR analysis and were obtained using the software Spectrum for Windows version 5.0.1 (Perkin-Elmer).

Emulsification Properties. The emulsification properties of the irradiated and unirradiated samples were determined according to the method previously described by Pearce and Kinsella,[19] which entailed the formation of an emulsion and then determination of the absorbance of a dilution series at 500 nm. The emulsion was formed by transferring 1.0 mL of palm oil (Felda Iffco Sdn. Bhd., Selangor, Malaysia) into 3.0 mL of 0.1% w/v sample solution in 100 mM sodium phosphate buffer at pH 7.4. The mixture was then homogenized in an Ultra-Turax T25 basic (Ika-Works, Malaysia) at 12000 rpm for 1 min at 25 °C. A 100 μL aliquot of the emulsion sample was taken from the bottom of the test tube at 0, 1, 2, 3, 5, 10, and 20 min and immediately diluted with 5 mL of 0.1% SDS solution (prepared with 100 mM sodium phosphate buffer at pH 7.4). The absorbance (A) of the diluted emulsion was then determined at 500 nm by using a UV—visible spectrophotometer (UV-160A, Shimadzu, Tokyo, Japan).

For a spectrophotometer in which none of the light scattered by the turbid sample reaches the photodetector and for a sample that does not adsorb light, the turbidity of the sample is given by

$$T = \frac{2.303A}{l} \quad (2)$$

where A is the absorbance and l is the path length of the cuvette.

The interfacial area of a dilute dispersion is given by

$$\text{interfacial area} = 2T \quad (3)$$

The emulsifying activity index (EAI) is a product of the area of interface stabilized per unit weight of protein used.

Oil Droplet Size Distribution. EW and SC emulsions were prepared in the same manner described above, and the oil droplet distributions were measured with a Malvern MSS laser diffraction system (Malvern Instruments Ltd., Worcestershire, U.K.); data were analyzed using Mastersizer-S (V 2.19, Malvern Instruments Ltd.) software. The emulsion was transferred into the instrument's dispersion circulator tank, which contained deionized water, after 20 min of standing at room temperature. The emulsion then was fed into the diffraction cells. Sufficient sample was added to yield an obscuration factor within 10—15% before measurement. Particle size was then

Table 1. Total Free Amino Group for UV-Irradiated Egg White Protein and Sodium Caseinate

exposure time (min)	total free amino group[a] (mmol/g)
EW	
0 (control)	1.08 ± 0.03 a
30	0.98 ± 0.04 ab
60	0.97 ± 0.03 ab
90	0.82 ± 0.02 c
120	0.74 ± 0.04 d
SC	
0 min (control)	1.12 ± 0.02 a
30 min	1.11 ± 0.04 a
60 min	0.98 ± 0.04 ab
90 min	0.98 ± 0.02 ab
120 min	0.98 ± 0.04 ab
4 h	0.91 ± 0.02 c
6 h	0.82 ± 0.02 d

[a] Results are expressed as the mean ± standard deviation; n = 3. Different letters in the same column are statistically different ($p < 0.05$).

expressed as the volume mean diameter $D[4,3]$

$$D_{[4,3]} = \sum n_i d_i^4 / \sum n_i d_i^3 \quad (4)$$

where n_i is the number of particles with diameter d_i. All particle size distributions were measured in triplicate.

Foaming Properties. Foaming properties were determined by using the method described by Song et al.[20] with some modifications. Briefly, a volume of 30 mL of 1% w/v sample solution was added into a 100 mL cylinder. Aeration was performed by using an Ultra-Turax T25 basic (Ika-Works, Malaysia) at 12000 rpm for 1 min at 25 °C. The foaming activity (% volume) was measured in terms of volume of the liquid. Foam stability (% volume) was expressed as percent liquid drainage in relation to initial liquid volume as a function of standing time for 30 min. Both foaming ability and foaming stability were calculated from the following equation:

$$\% \text{ volume} = \frac{(\text{volume of prepared foam} - \text{volume of liquid drainage})}{\text{original volume of liquid}} \times 100\% \quad (5)$$

Statistical Analysis. All experiments were conducted in triplicate. All data analyses were performed using SPSS for Windows version 12.0 (SPSS, Chicago, IL). Differences between means were assessed using a one-way analysis of variance (ANOVA) with a post hoc determination by Tukey's test. The α level was set at 0.05.

RESULTS AND DISCUSSION

Total Free Amino Group Measurement (Formol Titration). The total free amino group measurement was carried out to examine the possibility of UV irradiation to induce cross-linking on EW and SC samples. Table 1 illustrates the total free amino groups for UV-irradiated and unirradiated EW and SC samples. From Table 1, it can be observed that the values of total free amino group for EW and SC decreased significantly ($p < 0.05$) upon the increase of UV irradiation exposure time. This decreasing trend indicates the evidence of UV-induced cross-linking on EW and SC samples. As stated by Delincée[21] and Stewart,[22] the

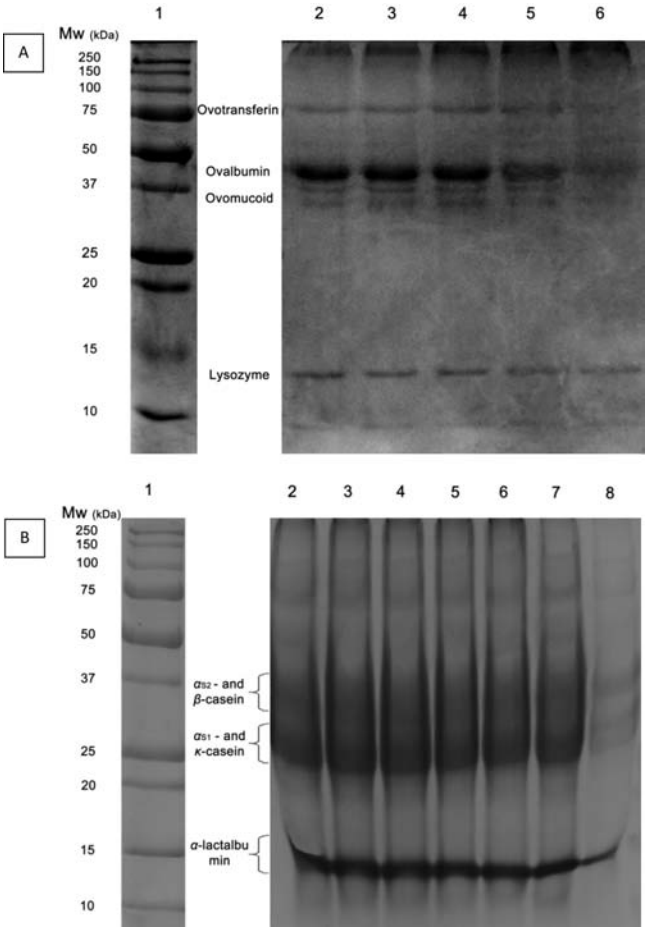

Figure 1. SDS-PAGE patterns for control and UV-irradiated (A) egg white protein (lane 1, molecular weight standard; lane 2, control; lanes 3−6, UV exposure times of 30, 60 90, and 120 min, respectively) and (B) sodium caseinate (lane 1, molecular weight standard; lane 2, control; lanes 3−8, UV exposure times of 30, 60 90, and 120 min and 4 and 6 h, respectively).

radiation-induced reactions in proteins are either depolymerization or cross-linking, or both. Both splitting and aggregation of proteins that occur on irradiation are related to the disturbance of the secondary and tertiary structures that expose reactive groups to the action of radiolytic products of water, such as hydrated electron, hydrogen atom, and hydroxyl radical.[22,23] In this context, it is hypothesized that the cross-linking reaction in EW and SC samples was more preferable upon UV irradiation.

SDS-PAGE. SDS-PAGE was employed to monitor structural changes of EW and SC subunits after UV irradiation. Figure 1 shows SDS-PAGE patterns of UV-irradiated and unirradiated EW and SC samples. The SDS-PAGE patterns of the control EW sample (Figure 1A, lane 2) exhibited the characteristic bands for the constituents of EW, which are ovotransferin, ovalbumin, ovomucoid, and lysozyme. These bands are in good agreement with the results reported by Gennadios et al.[24] Ovalbumin is the principal EW that contributes 54% in mass.[25] Other than this, ovotransferin and lysozyme contribute lesser percentages of protein in EW, which are 12.5% and 3.5% in mass, respectively.[25] Generally, EW begins to coagulate at 62 °C. Ovotransferin, lysozyme, and ovalbumin denatured at 61, 75, and 84 °C, respectively, at pH 7.[26] On the other hand, the separated patterns

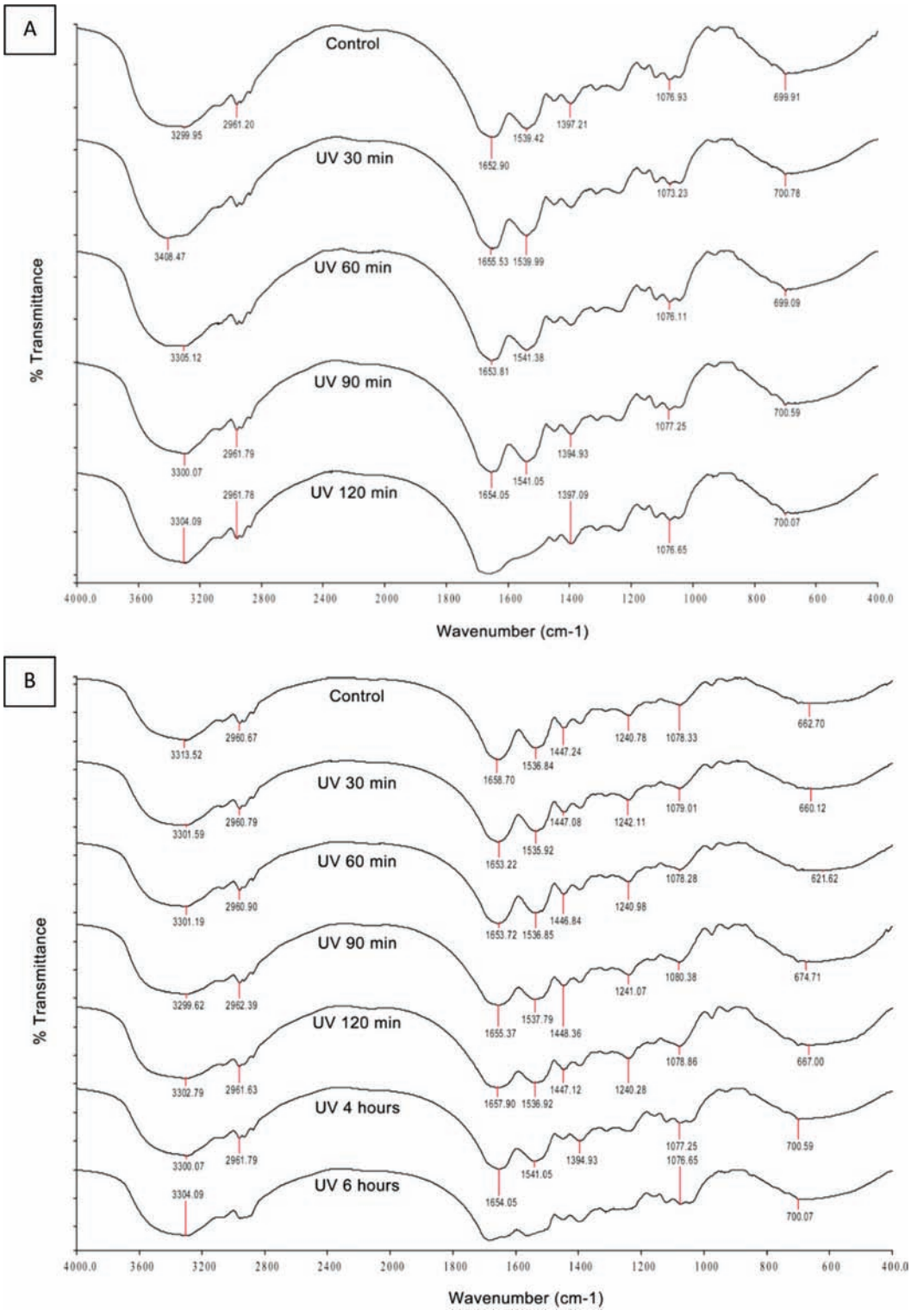

Figure 2. FTIR spectra of the control and UV-irradiated (A) egg white protein and (B) sodium caseinate samples.

of control SC samples showed the major fractions of casein (Figure 1B, lane 2), which are in agreement with the main fractions α_{S1}-, α_{S2}-, β-, and κ-caseins as well as α-lactalbumin present in the milk casein.[27] The α_{S1}- and β-caseins are two major caseins that comprise around 75% of total milk casein. On the basis of their amino acid sequences, α_{S1}- and β-caseins might be expected to share many properties in common and contribute to the functional properties of SC.[27] From Figure 1, it can be observed that the electrophoretic patterns of the UV-irradiated EW and SC samples were related to the UV exposure time.

For EW samples, the SDS-PAGE profiles (Figure 1A, lanes 5 and 6) revealed that UV irradiation caused a slight breakdown of

the polypeptide chain upon increased UV exposure time for 90 and 120 min, as evidenced by the disappearance on the intensity of the bands around 10−75M_w. At the same time, the formation of polymers was observed at the top of the separating gel. This aggregate was attributed to the difficulty of these polymers to penetrate the running gel, presumably as a result of UV-induced cross-linking. For SC samples (Figure 1B), no significant changes in the major bands were observed at the initial UV irradiation exposure time for 30−120 min. However, upon extended UV irradiation exposure time for 4 h (lane 7), the intensity of the bands around 75−150M_w was slightly reduced compared to the control sample. Consequently, the disappearance of all major bands was distinctively observed on extended UV irradiation time for 6 h as an indication of UV-induced cross-linking.

Upon irradiation on proteins, fragmentation and aggregation are two types of radiation damage that occurred.[21] These protein molecules can be converted to higher molecular weight aggregates due to the generation of interprotein cross-linking reactions, hydrophobic and electrostatic interactions, and the formation of disulfide bonds. Consequently, this cross-linking process results in the formation of chemical bonds between two adjacent protein molecules.[28] Therefore, the cross-linking increased the molecular weight of the protein and bound into an insoluble three-dimensional network, as indicated in Figure 1, where the cross-linked proteins were unable to penetrate the running gel, causing the disappearance of the major bands. Furthermore, cross-linking usually occurred on globular proteins, and degradation often occurred on fibrous proteins.[21] Ovalbumin and casein are major components and examples of globular protein present in EW and SC, respectively.[29,30] Therefore, it is possible that EW and SC might have undergone the UV-induced cross-linking as shown by our results. This finding concurs with the results obtained from total free amino groups, where a reduction was observed with increasing UV exposure time. The cross-linking-induced polymerization on EW film upon UV irradiation has been reported by Rhim et al.,[16] and on caseinates upon γ-irradiation in either powder state or film by Lacroix et al.,[31] Vachon et al.,[32] and Cieśla et al.[33]

FTIR Spectroscopy Analysis. The FTIR analysis spectra obtained from UV-irradiated and unirradiated EW and SC samples were taken in the spectral region of 4000−400 cm^{-1} for 20 scans (Figure 2). For EW samples undergoing UV irradiation for 120 min (Figure 2A), marked changes were observed in the intensities of the band at the region of 1400−1700 cm^{-1} (amide I and amide II), whereas no significant changes were seen for EW samples UV-irradiated for 30−90 min. On closer examination, the FTIR spectrum showed the disappearance of the peak at 1652.90 cm^{-1} for the EW sample UV-irradiated for 120 min compared with the control sample; this change was attributed to the changes in amide I, particularly CO stretching. Another marked change of peak disappearance for the EW sample UV-irradiated for 120 min at 1539.42 characterized the change in amide II, particularly NH deformation. These changes correspond to the β-sheet structure, suggesting the reorganization of the structure after UV irradiation. In addition, rearrangement of the β-sheet after UV irradiation also can be confirmed, where changes of the band intensities occurred in the region of 1600−1700 cm^{-1}. The changes in conformation can be related to the tendency of the protein to adopt a better ordered structure after UV-induced cross-linking.[33] Therefore, the results obtained from FTIR were in good agreement with results obtained from formol titration and SDS-PAGE.

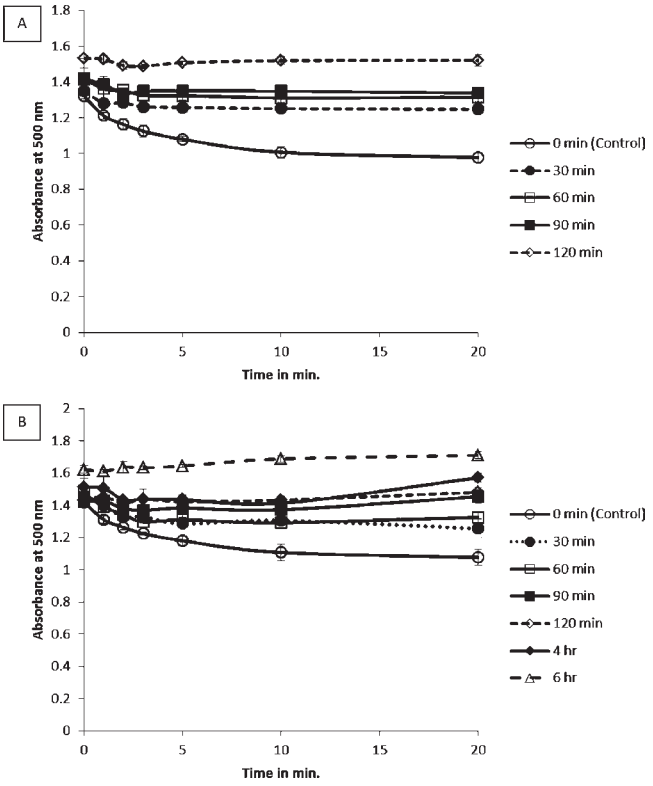

Figure 3. Effect of UV irradiation on emulsification properties of (A) egg white protein and (B) sodium caseinate. 0, control; 30, 60, 90, and 120, exposure time in minutes; 4 and 6, exposure time in hours. Each plotted point is the mean ± standard deviation; n = 3.

For all of the FTIR spectra for SC samples (Figure 2B), the presence of primary or secondary amines and amides can be detected by absorption due to stretching of NH_2 or NH groups between 3350 and 3200 cm^{-1}. It can be observed that no major differences can be obtained for any of the SC samples except the sample treated with extended UV irradiation for 6 h. SC samples undergo substantial chemical changes starting from extended UV exposure time for 4 h. The changes were attributed to the peak disappearance of the band at 1240 cm^{-1}, indicating CN stretching in aromatic amines. As previously pointed out by Delincée[21] and Stewart,[22] aromatic amines are the most sensitive to irradiation and react avidly with the $^{\bullet}H$ radical, a hydrolytic product of irradiation. The $^{\bullet}H$ radical will subsequently attach to the aromatic amine by a hydroxylation process. Dimerization of two hydroxylated aromatic amines would then take place and induce cross-linking. Upon extended UV irradiation for 6 h, major differences in peak change and band disappearance were observed (Figure 2B). On closer examination, the FTIR spectra of SC UV-irradiated for 6 h showed a marked peak disappearance at 2960 cm^{-1}, indicating CH antisymmetric and symmetric stretching. Furthermore, another disappearance of the peak at 1658 cm^{-1} indicated the CO stretch of the amide I band, particularly in primary and secondary amides. Another peak disappearance has been observed at 1536 cm^{-1} for SC samples UV-irradiated for 6 h compared with control sample, indicating the changes in the amide II band, particularly NH deformation. Peaks at 1447 and 1240 cm^{-1} were not found for SC samples treated with UV irradiation for 4 and 6 h, indicating changes in COO symmetric and CN stretch in aromatic amines,

Table 2. Emulsifying Activity Index (EAI) for Control and UV-Irradiated Egg White Protein and Sodium Caseinate Emulsions Standing at Room Temperature for 0, 1, 2, 3, 5, 10, and 20 min

	EAI ($m^2\ g^{-1}$) for emulsions standing at room temperature[a]						
sample	0 min	1 min	2 min	3 min	5 min	10 min	20 min
EW							
UV 0 min	150.77 ± 1.72 d	139.41 ± 2.01 d	133.88 ± 3.70 c	129.54 ± 3.99 d	124.28 ± 1.65 d	115.88 ± 3.78 e	112.42 ± 3.37 d
UV 30 min	155.52 ± 1.76 c	147.53 ± 1.15 c	147.86 ± 2.66 b	145.12 ± 3.19 c	144.71 ± 2.40 c	144.03 ± 2.90 d	143.59 ± 0.89 c
UV 60 min	163.43 ± 3.22 b	156.68 ± 7.83 b	155.76 ± 0.15 b	152.57 ± 1.15 b	152.26 ± 3.05 b	150.94 ± 2.90 c	151.28 ± 3.05 b
UV 90 min	162.97 ± 2.90 b	159.68 ± 1.33 b	153.67 ± 3.70 b	155.17 ± 1.33 b	155.30 ± 1.15 b	155.20 ± 2.34 b	154.02 ± 2.55 b
UV 120 min	176.29 ± 0.63 a	175.72 ± 2.30 a	171.77 ± 2.30 a	171.31 ± 1.33 a	173.45 ± 1.15 a	174.91 ± 2.46 a	175.11 ± 3.70 a
SC							
UV 0 min	163.43 ± 2.42 c	150.93 ± 2.42 c	145.40 ± 1.73 e	141.06 ± 1.15 d	135.80 ± 3.68 d	127.39 ± 5.87 e	123.94 ± 5.53 f
UV 30 min	168.12 ± 1.15 b	161.06 ± 2.30 bc	153.00 ± 3.91 cd	153.07 ± 4.03 c	148.20 ± 3.68 c	150.39 ± 4.61 d	144.63 ± 6.73 e
UV 60 min	169.96 ± 1.15 b	159.98 ± 1.73 bc	154.73 ± 3.22 cd	149.23 ± 0.12 c	150.73 ± 4.61 c	148.74 ± 2.88 c	152.57 ± 4.03 d
UV 90 min	163.82 ± 3.68 bc	164.12 ± 6.91 bc	158.94 ± 4.38 cd	157.53 ± 1.15 c	159.17 ± 6.91 bc	157.99 ± 1.15 c	167.35 ± 4.61 c
UV 120 min	165.06 ± 2.30 bc	167.13 ± 2.42 bc	162.74 ± 0.69 b	165.55 ± 6.91 b	163.86 ± 2.39 bc	164.98 ± 2.42 b	170.50 ± 2.99 c
UV 4 h	173.99 ± 6.68 a	173.42 ± 5.83 b	164.86 ± 0.37 b	165.55 ± 1.15 b	165.39 ± 3.05 bc	162.67 ± 2.90 b	180.87 ± 3.05 b
UV 6 h	186.81 ± 2.90 a	185.43 ± 1.33 a	188.41 ± 3.70 a	187.97 ± 1.33 a	189.23 ± 1.15 a	194.32 ± 2.34 a	196.99 ± 2.55 a

[a] Results are expressed as the mean ± standard deviation; $n = 3$. Different letters within the same column are statistically different ($p < 0.05$).

respectively. Similar results were observed with casein and whey protein film treated with γ-irradiation.[33,34] According to Lacroix et al.,[34] these observed changes most likely resulted from α-helix and β-sheet conformation. Consequently, these results suggest that UV irradiation could induce cross-linking on protein and lead to the conformational alteration in protein, which concurs with the results obtained from formol titration and SDS-PAGE. Again, these changes could be related to a tendency of the proteins to adopt a more stable structure after cross-linking.

Emulsification Properties and Oil Droplet Size Distribution. Emulsifying ability and emulsion stability are important parameters generally used to investigate the emulsifying properties of proteins in food emulsion systems. Figure 3 and Table 2 show improved emulsifying ability on UV-irradiated EW and SC compared with control sample. The emulsifying ability of a protein emulsifier depends on its ability to form adsorption films around the oil globules and to lower the interfacial tension at the oil−water interface. Emulsion stability is the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing, and flocculation.[35] Therefore, the improved stability could be explained by the decreased droplet size of UV-irradiated EW and SC after standing for 20 min at room temperature (Figure 4). Figure 4 shows that the oil droplet size of UV-irradiated samples was smaller than that of control sample and decreased ($p < 0.05$) with increasing UV exposure time. This implies that there was significant flocculation and coalescence of the control sample, leading to increased oil droplet size and emulsion destabilization. On the other hand, a better emulsion stability was observed for UV-irradiated EW and SC samples. The improved emulsifying properties of EW and SC could be attributed to changes in the conformational and surface properties of protein structures upon treatment with UV irradiation. According to Jambrak et al.,[36] protein denaturation and changes on the conformational structure would affect surface hydrophobicity and subsequently lead to better adsorption of the oil-in-water emulsion system. UV-induced protein cross-linking and conformational changes are indicated by the results obtained from formol titration, SDS-PAGE, and FTIR. These results suggest that significant conformational changes on the surface properties of EW and SC upon UV irradiation would have given rise to an improvement of the emulsifying properties.

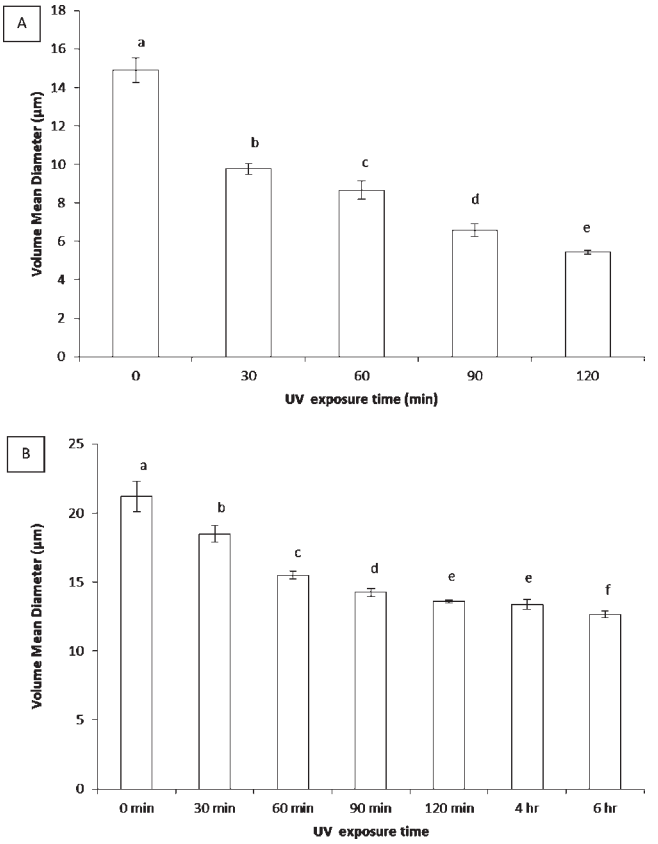

Figure 4. Effect of UV irradiation on droplet size of the oil-in-water (A) egg white protein and (B) sodium caseinate emulsion after standing for 20 min at room temperature. Results are expressed as the mean ± standard deviation; $n = 3$. Different letters denote statistical difference ($p < 0.05$).

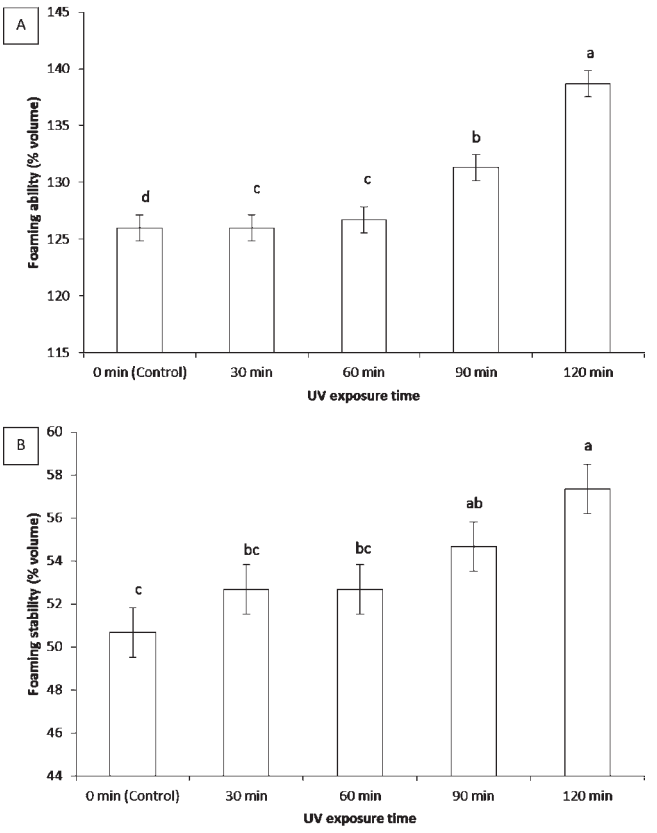

Figure 5. Effect of UV irradiation on foaming properties of egg white protein: (A) foaming ability and (B) foaming stability after standing at room temperature for 20 min. Each bar shows the mean ± standard deviation; $n = 3$. Different letters denote statistical difference ($p < 0.05$).

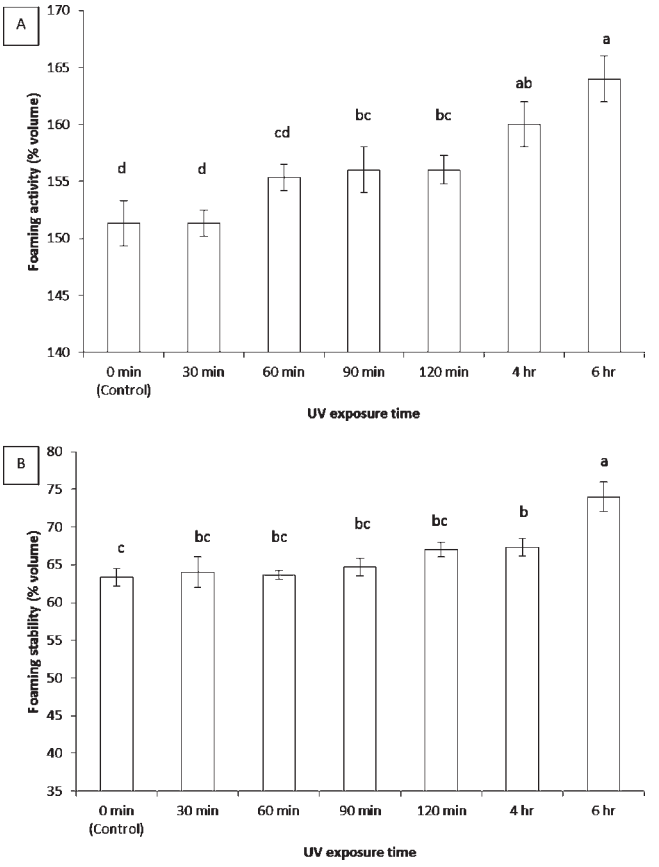

Figure 6. Effect of UV irradiation on foaming properties of sodium caseinate: (A) foaming ability and (B) foaming stability after standing at room temperature for 20 min. Each bar shows the mean ± standard deviation; $n = 3$. Different letters denote statistical difference ($p < 0.05$).

Foaming Properties. The foaming ability was determined for fresh foams after whipping, whereas the foaming stability was determined 30 min after whipping. The foaming ability and foaming stability of all the control and UV-irradiated samples are presented in Figures 5 (EW) and6 (SC). The foaming ability and foaming stability of EW and SC samples increased with increasing of UV irradiation exposure time. Ma et al.[37] reported that the foaming ability of egg white was improved upon γ-irradiation due to the conformational changes of proteins in EW, which increased surface hydrophobicity. Additionally, Clark et al.[38] reported improved functional properties in spray-dried EW irradiated with γ-irradiation and small changes in secondary structure. It has been previously reported by Partanen et al.[39] that the improvement of foaming properties of SC is attributed to the transglutaminase-induced cross-linking. They explained that the cross-linking would have altered the interfacial elasticity and enhanced the foam formation by preventing coalescence and disproportionation. As previously indicated by our FTIR analysis, the UV irradiation caused changes in the secondary structure of EW and SC due to the tendency of the proteins to adopt better ordered structure after UV-induced cross-linking. Moreover, it has been indicated by Ali et al.[40] that the polymerization and cross-linking of protein could improve the foaming properties of protein due to the increased ability to form a better interfacial protein network during foam forming, because its high molecular size and cross-linked structure are more resistant to excessive denaturation than the native protein at the high speed of the homogenization used to make emulsions and foams. Moreover, reduced electrostatic repulsion as the result of decrease in the number of amino group could have enhanced protein−protein interaction and therefore protein adsorption on the interface. Our results also showed a reduction in the total amino groups upon increasing UV exposure time. On the basis of these findings, we postulate that the protein cross-linking has substantially changed the protein conformation in EW and SC, which led to an effective unfolding of the protein during foam forming. On the other hand, the increased apparent viscosity (data not shown) was also strongly related with the improved foam stability.[39,41] The increased apparent viscosity caused by protein cross-linking could enhance the stability of foams and emulsions due to the formation of more elastic foam networks at the air−water interfaces.

In conclusion, the results obtained in this study indicated the possibility of using UV irradiation to induce cross-linking of proteins (i.e., EW and SC). The conformation changes resulted in the enhancement of emulsifying and foaming properties. Therefore, these changes suggest that the UV-irradiated EW and SC could serve as novel emulsifier and foaming agents to be used in broad food systems.

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