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Special Issue: Bioactive Surface Functionalization

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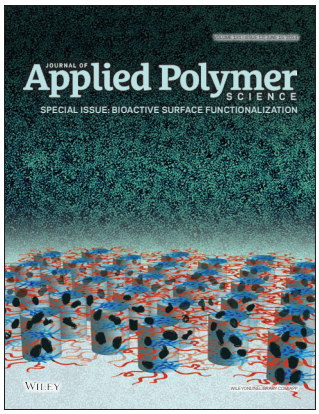

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Surface Activity of Pepsin-Solubilized Collagen Acylated by Lauroyl Chloride Along with Succinic Anhydride

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ABSTRACT: The surface activity of pepsin-solubilized type I collagen acylated by lauroyl chloride and succinic anhydride was investigated in this article. Compared with native collagen, acylated collagen exhibited better surface activity such as oil absorption capacity, emulsion activity and stability, foam expansion, and foam stability but presented lower water absorption capacity. Acylated collagen also had higher emulsion activity and stability than Tween 80, whereas Tween 80 displayed higher foam expansion and stability than acylated collagen. After acylation, the surface tension of collagen decreased with the storage time increased, the hydrophobicity and the wetting power increased with the increase of the concentration. Meanwhile, the emulsion activity and stability of acylated collagen decreased with the increase of NaCl concentration ranging from 20 to 100 mmol/L. Under neutral pH value, acylated collagen reached higher emulsion activity and stability at acylated collagen concentration of 0.4 mg/mL and lower temperature (<35°C).
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KEYWORDS: proteins; surfactants; foams; acylated collagen; surface activity

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INTRODUCTION

Since proteins are biopolymers composed of both hydrophobic and hydrophilic amino acid residues, most of the protein molecules have amphipathicity that are somewhat similar to those of polymer surface materials. However, native proteins often exhibit a limited surface activity since the surface activity of proteins depends on the characteristics of molecular structure and the amount of the hydrophobic groups together with the polar moiety. Therefore, chemical modification of proteins might serve as a tool for improving their surface activity.[1,2]

Aewsiri et al.[3] reported that the introduction of fatty acid residues (C_{10}-C_{14}) to the cuttlefish skin gelatin caused an increase of surface activity of the gelatin derivates, as determined by surface tension and emulsion activity. Meanwhile, Lin et al.[4] prepared gelatin derivates by the reaction of gelatin hydrolysate with alkenylsuccinic acid anhydride and found that the surface tension of gelatin derivates increased with the increase of pH value, indicating that gelatin derivates were more surface-active in acidic range than under alkaline condition. Additionally, casein-derived surfactants were also found to decrease the surface tension of water up to around 15 mN/m after the introduction of tetradecanoic acid residues to the free amino groups of the casein molecules.[5]

Type I collagen, with a molecular weight of about 300 kDa,[6] is characterized by the presence of a triple helix structure that is composed of three polypeptide chains.[7] Gómez-Guillén et al.[8] reviewed that native collagen might have surface activity due to the collagen sequence containing hydrophobic and hydrophilic amino acid residues as well as the presence of charged groups in the side chains. As early as 1991, Montero et al.[9] demonstrated that the emulsion activity of collagen extracted from the skin of hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) was the highest at pH values ranging from 1.0 to 3.0. Subsequently, the emulsion activity of the acid-soluble collagen extracted from the skin of Pacific whiting was found to be slightly lower than that of Tween-80, as described by Kim and Park.[10] Compared with other surface active materials including sodium dodecyl sulfate (SDS) [11] and sorbitan surfactants,[12] however, native collagen might have a limited surface activity due to its hydrophilicity.[6]

To broaden the application of collagen-based materials to cosmetics, drug delivery, and foods, etc., it would be beneficial to improve the surface activity of native collagen. In our previous

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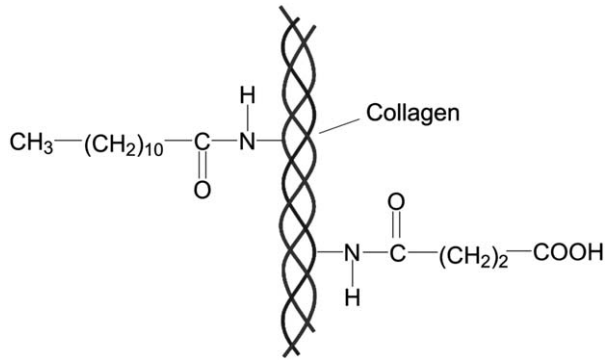

Figure 1. Structural scheme of acylated pepsin-solubilized type I collagen. Lauroyl and succinic residues were introduced on the ε-NH_2 of lysine groups of native collagen molecules for providing the hydrophobic and hydrophilic groups, respectively.

report,[13] the authors prepared acylated collagen based on the method of the reaction of ε-NH_2 of lysine groups of native collagen molecules with lauroyl chloride and succinic anhydride, and mainly focused on the structural properties, thermostability, and surface morphology for acylated collagen. In this work, the comprehensive surface activity for this acylated collagen was investigated, which included hydrophobicity, surface tension, water absorption capacity, oil absorption capacity, emulsion properties, foaming properties, and wetting power.

EXPERIMENTAL

Materials

Type I collagen was extracted from calf skin by the method of Zhang et al.[14] The obtained collagen solution was lyophilized by a freeze dryer (Labconco Freeze Dryer FreeZone 6 Liter, USA) at −50°C for 2 days and stored at 4°C until used. The analysis of extracted collagen by sodium dodecyl sulphate-polyacryamide gel electrophoresis (SDS-PAGE) was investigated in our laboratory to verify its purity and structural integrity, and the results showed that the electrophoresis patterns of the extracted collagen displayed two α bands (α1 and α2) and one β band, and the molecular weight was about 300 kDa. Additionally, lauroyl chloride, succinic anhydride and 8-anilino-1-naphthalenesulfonate (ANS) were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of Acylated Collagen

The structural scheme of acylated collagen is presented in Figure 1 and the preparation could be found in previous report described by the authors.[13] Briefly, 1.0 g lauroyl chloride was added dropwise into 100 mL dimethyl sulfoxide (DMSO) solution containing 1.0 g lyophilized collagen and two equivalent of triethy amine. After 24 h, the intermediate product was dialyzed against 0.1 mol/L acetic acid for 3 days. Subsequently, 0.2 g succinic anhydride dissolved in 10 mL DMSO was added into the intermediate product solution with a constant pH value of 9.0 to react for 4 h. Finally, acylated collagen was purified by dialyzed against deionized water for 3 days and the purified acylated collagen solution was lyophilized by a freeze dryer at −50°C for 2 days and stored at 4°C until used. The preparation of acylated collagen was done by three times and native collagen was used as a reference.

Determination of Hydrophobicity

The hydrophobicity of acylated collagen solution was determined using 8-anilino-1-naphthalenesulfonate (ANS) as a probe, as described by Kamyshny et al. [15] A series of acylated collagen solutions with the concentrations of 0.01, 0.05, 0.1, 0.2, 0.4, and 0.6 mg/mL were obtained by dissolving different weight of lyophilized acylated collagen in 0.01 mol/L phosphate saline buffer (PBS, pH 7.4), whereas ANS was dissolved in 0.1 mol/L sodium phosphate buffer (pH 7.0) to obtain ANS concentration of 8 mmol/L. Then, 2 mL acylated collagen solution was mixed with 20 μL ANS solution and the mixture was stirred vigourously for 10 min. After that, the fluorescence spectrum was recorded at 20°C using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, USA). The excitation wavelength was 380 nm and the emission spectra were measured in the wavelength region from 400 to 650 nm.

Surface Tension Measurements

Prior to running tests with acylated collagen solutions, the surface tensionmeter (dataphysics OCA-H200, Germany) was calibrated with ultra-pure water. After acylated collagen solutions with various concentrations (3×10^{-3}, 4×10^{-3}, and 5×10^{-3} mg/mL) were prepared by dissolving lyophilized acylated collagen in 0.01 mol/L PBS (pH 7.4), the surface tension was immediately recorded with triplicate for 0 $\sim$ 5000 s at 20°C, until a constant reading was obtained.

Water and Oil Absorption Capacities

Water and oil absorption capacities were determined by the method of Cho et al.[16] with slight modification. For measuring water absorption capacity, one centrifuge tube was weighed and was placed with 0.05 g lyophilized acylated collagen and 10 g distilled water. Subsequently, the samples were mixed with vortex for 5 s every 15 min at 20°C within 1 h and then were centrifuged at 5000 $\times$ g for 20 min. The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper at a 45° angle. The total weight of the centrifuge tube and the samples were weighed again to obtain the weight of the absorbed water, and the water absorption capacity was expressed as the wt % of lyophilized acylated collagen. The operation steps for oil absorption capacity were similar to those for water absorption capacity except that 10 g distilled water was substituted for 10 g vegetable oil. The water and oil absorption capacities were detected with triplicate and lyophilized native collagen was used as a control.

Emulsifying Properties of Acylated Collagen

Emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen, a rough estimate of the particle size of the emulsions based on the interfacial area per unit of protein, were calculated by turbidity, as described by Aewsiri et al. [17] with some modification. Lyophilized acylated collagen was dissolved in 0.01 mol/L PBS (pH 7.4) to obtain the concentration of 0.4 mg/mL, and then 20 mL acylated collagen solution was homogenized with 2 ml vegetable oil at a speed of 2,000 rpm for 10 min at 20°C. Emulsions were pipetted out at 0 and 10 min and were diluted with 0.1% SDS at a suitable fold, and then the mixture was mixed thoroughly using a vortex mixer for 10 s. Finally, the resulting dispersion was measured using a

UV-VIS spectrophotometer (Lambda 25, USA) at 500 nm. EAI was calculated by eq. (1):

$$\mathrm{EAI}\,(\mathrm{m^2/g}) = \frac{2\times 2.303\times A\times \mathrm{DF}}{l\varphi C} \quad (1)$$

where A is the absorbance measured at 500 nm, l is path length of cuvette (m), DF is the dilution factor, φ and C are oil volume fraction and protein concentration in aqueous phase ($\mathrm{g/m^3}$), respectively; meanwhile, ESI was calculated by eq. (2):

$$\mathrm{ESI}\,(\mathrm{min}) = \frac{A_0}{\Delta A}\times \Delta t \quad (2)$$

where A_0 is the absorbance measured at 0 min, $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min.

Native collagen and Tween 80 were used as the controls and all the measurements were done by three times.

Emulsifying Properties Under Different Conditions

Emulsions were prepared as described above, whereas emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen were investigated under different conditions.

Acylated collagen solutions with the concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were prepared by dissolving lyophilized acylated collagen in 0.01 mol/L PBS (pH 7.4). Subsequently, the emulsions were prepared as described above and then EAI and ESI were investigated under various acylated collagen concentrations. The influence of ionic strength on EAI and ESI was measured at acylated collagen concentration of 0.4 mg/mL, whereas, NaCl was added to the emulsions to obtain the final NaCl concentrations of 20, 40, 60, 80, and 100 mmol/L. Also, the pH value of acylated collagen solution (0.4 mg/mL) was adjusted to 3.0, 4.0, 5.0, 6.0, and 7.0 using 1.0 mol/L HCl or NaOH. After that, the emulsions were prepared as described above and then EAI and ESI were investigated under different pH values. To study the effect of temperature on EAI and ESI, 0.4 mg/mL of acylated collagen solution was used to prepare the emulsions, but the emulsions were prepared in water bath at 25, 30, 35, 40, and 45°C for 30 min. After incubation, the emulsions were cooled under running tap water and EAI and ESI under various temperatures were calculated as described above.

Foaming Properties of Acylated Collagen

Foam expansion (FE) and foam stability (FS) of Tween 80, native and acylated collagen solutions were tested according to the method of Aewsiri et al.[17], with some modification. Tween 80 and acylated collagen were dissolved in 0.01 mol/L PBS (pH 7.4) to obtain the concentration of 0.4 mg/mL, whereas native collagen solution with the concentration of 0.4 mg/mL was prepared by dissolving lyophilized native collagen in 0.1 mol/L acetic acid. Then, sample solutions (20 mL) were left at 20°C for 30 min followed by homogenization at a speed of 2000 rpm for 10 min at 20°C to incorporate air efficiently. The whipped samples were immediately transferred into 50 mL cylinders and were allowed to stand for 0 and 5 min. FE and FS were then calculated with triplicate using the eqs. (3) and (4), respectively:

$$\mathrm{FE}\,(\%) = \frac{V_T}{V_0}\times 100 \quad (3)$$

$$\mathrm{FS}\,(\%) = \frac{V_t}{V_0}\times 100 \quad (4)$$

where V_T is total volume after whipping; V_0 and V_t are the original volume before whipping and total volume after leaving for 5 min, respectively.

Wetting Power of Acylated Collagen

Wetting power of acylated collagen was estimated by the value of contact angles, similar to the method reported by Lin et al.[2] Lyophilized acylated collagen was dissolved in 0.01 mol/L PBS (pH 7.4) to obtain the concentration of 0.2, 0.4, and 0.6 mg/mL. After equilibrating for about 36 h, the contact angles between acylated collagen solutions and acrylic plastic sheet or unscoured cotton fabric were obtained with triplicate using a goniometer (dataphysics OCA-H200, Germany) at 20°C. Pure water was used as a control and the contact angle between water and acrylic plastic sheet or unscoured cotton fabric was also detected.

Differential Scanning Calorimetry (DSC)

Acylated collagen solution (10 mg/mL) was prepared by dissolving lyphophilized acylated collagen in 0.01 mol/L PBS (pH 7.4). Then, ~10 mg of acylated collagen solution was sealed in an aluminum pan for DSC, and an equal weight of PBS (0.01 mol/L, pH 7.4) in another pan was used as a reference. The maximum transition temperature (T_m) of acylated collagen was determined by a differential scanning calorimetry apparatus (Netzsch DSC 200PC, Germany). The endothermal curve of acylated collagen was recorded from 20 to 60°C at a scanning rate of 5°C/min in a nitrogen atmosphere.

RESULTS AND DISCUSSION

Determination of Hydrophobicity

8-Anilino-1-naphthalenesulfonate (ANS), a fluorescent hydrophobic probe, is so sensitive to the polarity of environment in solutions that its fluorescence intensity rises with a little reduction of polarity surrounding it.[18] Figure 2 presents the fluorescence intensity of ANS in the presence of acylated collagen with various concentrations. It was apparent that the fluorescence intensity of ANS in the presence of acylated collagen was higher than that of ANS without acylated collagen. With the increase of the concentration of acylated collagen, the fluorescence intensity increased and a blue shift ranging from 521 to 507 nm in the maxima peak of the emission spectrum occurred, indicating that the hydrophobicity (or nonpolarity) of acylated collagen increased. Wu et al.[19] also found that the fluorescence intensity of ANS increased due to the formation of hydrophobic microdomains brought by the aggregation of collagen molecules. Kamyshny et al.[15] reported that the binding of ANS to proteins with the increase of hydrophobicity was equivalent to the decrease of polarity, which should lead to a blue shift in the maxima peak in the emission spectrum and an increase of the fluorescence intensity. Lauric acid, which is hydrophobic in nature, contributed to the improvement of hydrophobicity (or nonpolarity) for acylated collagen. Theoretically, the emission maximum in the fluorescence spectrum of ANS on the

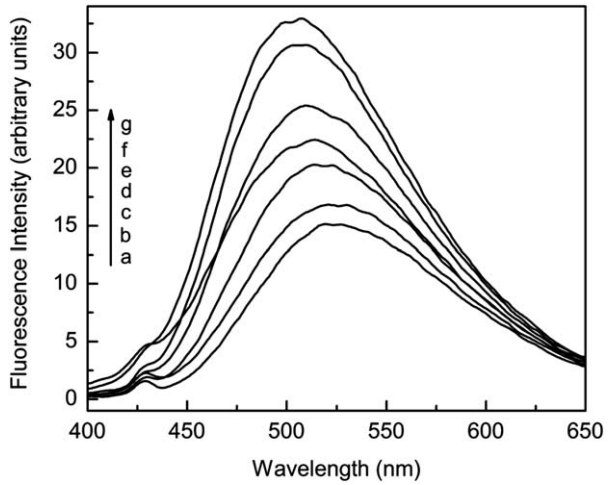

Figure 2. Fluorescence spectra of ANS in 0.1 mol/L PBS, pH 7.4 (a), and ANS in the presence of acylated collagen with various concentrations (b: 0.01 mg/mL, c: 0.05 mg/mL, d: 0.1 mg/mL, e: 0.2 mg/mL, f: 0.4 mg/mL, g: 0.6 mg/mL).

environmental polarity or nonpolarity depends on the reorientation of the solvent shell around the chromophore.[20] When ANS was added into a nonpolar solvent, ANS molecules with aromatic rings bound with the hydrophobic regions tightly and then a higher energy photon (relative to a polar environment) was emitted, which might contribute to the increase of the fluorescence intensity.

Surface Tension Measurements

In aqueous solutions, amphiphilic compound molecules were localized or accumulated at the surface and reduced the surface tension.[21] Additionally, the surface tension of amphiphilic compound solutions decreased with the storage time increased, as investigated by Aewsiri et al.[3] The surface tension for acylated collagen at the air-water interface is shown in Figure 3. It was apparent from the kinetic curves that the surface tension decreased with the storage time increased and finally kept equilibrium. At the concentration of 5×10^{-3} mg/mL, the equilibrium surface tension was about 58 mN/m after 3500 s, whereas at low concentration (3×10^{-3} mg/mL), the equilibrium surface tension (60.5 mN/m) was reached at about 4500 s, demonstrating that the higher concentration of acylated collagen, the more rapid approach toward the equilibrium surface tension. Although the equilibrium surface tension was higher than that of other polymer surface active materials including dextrin[22] and polyester derivates,[23] it was similar to that of polysaccharide derivates[24] and was lower than that of pure water,[13] suggesting that acylated collagen had surface activity to some degree, which might promote the dispersion and the sustained release for hydrophobic drugs.[25,26]

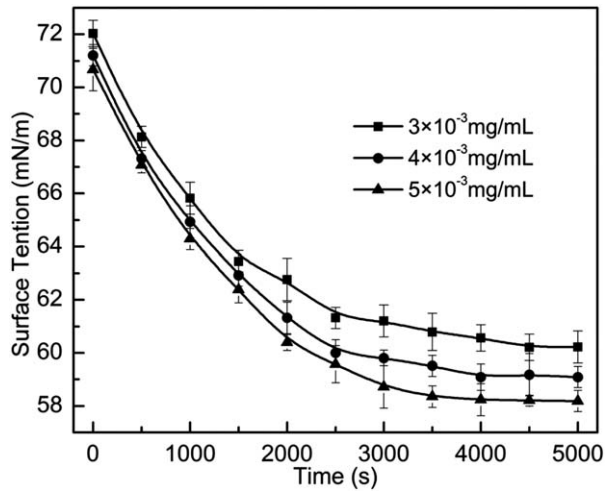

Figure 3. Surface tension of acylated collagen with various storage time and concentrations.

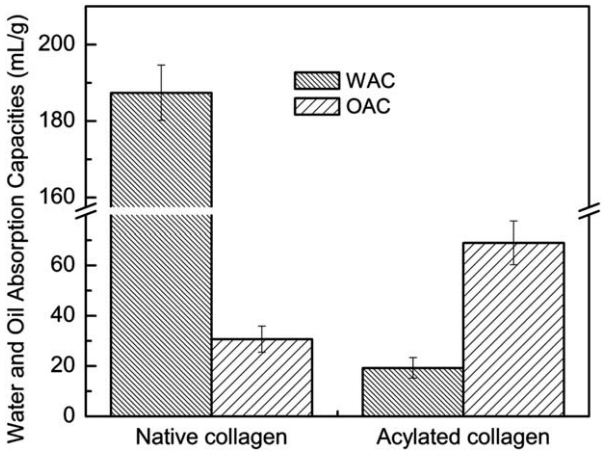

Figure 4. Water absorption capacity (WAC) and oil absorption capacity (OAC) of native and acylated collagens.

Water and Oil Absorption Capacities

Water and oil absorption capacities of a protein are functional properties that refer to the ability of the protein to imbibe water or oil and retain it against gravitational force. Figure 4 shows water and oil absorption capacities of native and acylated collagens. It could be found that water and oil absorption capacities of native collagen were 187.41 and 30.66 g/g, followed by 19.26 and 68.91 g/g for acylated collagen, respectively. It was obvious that water absorption capacity for native collagen was approximately ten times than that of acylated collagen, whereas oil absorption capacity was much lower than that of acylated collagen. Hermansson[27] reported that highly soluble protein had low water absorption capacity. Meanwhile, Cho et al.[16] investigated that the hydrophobicity contributed to improve oil absorption capacity of gelatin extracted from shark (*Isurus oxyrinchus*) cartilage. Being a fibrous protein, native collagen obtained by the treatment of acetic acid (pH 2.5–3.0) could not dissolve in water, which might promote the improvement of water absorption capacity. However, the isoelectric point (pI) for acylated collagen was 4.93,[13] which endowed acylated collagen with better solubility in water and then resulted in lower water absorption capacity. In the case of oil absorption capacity, acylated collagen exhibited stronger hydrophobicity than native collagen, which helped to improve oil absorption capacity for acylated collagen.

Emulsifying Properties of Acylated Collagen

Emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen, native collagen, and Tween 80 expressed as the turbidity of emulsions at the wavelength of

Table I. Emulsion Activity Index (EAI), Emulsion Stability Index (ESI), Foam Expansion (FE), and Foam Stability (FS) of Tween 80, Native, and Acylated Collagens

Sample	EAI_0 (m^2/g)	EAI_{10} (m^2/g)	ESI (min)	FE (%)	FS (%)
Native collagen	28.29 ± 3.19	11.82 ± 1.93	17.15 ± 0.63	109.17 ± 2.89	103.33 ± 1.44
Tween 80	25.33 ± 1.27	14.35 ± 0.73	23.25 ± 2.05	163.33 ± 1.44	158.33 ± 1.44
Acylated collagen	264.73 ± 10.05	224.62 ± 10.31	66.03 ± 3.11	115 ± 2.5	111.5 ± 1.73

EAI_0 and EAI_{10} referred to the emulsion activity index at 0 and 10 min, respectively.

500 nm are shown in Table I. In comparison with Tween 80, native collagen displayed slightly higher EAI_0, but exhibited slightly lower EAI_{10}; consequently, ESI for native collagen was slightly lower than that of Tween 80. Also, Kim and Park[10] found that type I collagen derived from skin of Pacific whiting presented slightly lower emulsion activity than Tween 80, whereas type I collagen extracted from muscle of Pacific whiting exhibited slightly higher emulsion activity than Tween 80. After the introduction of lauroyl and succinic residues, however, EAI and ESI of acylated collagen increased markedly. Kato and Nakai[28] reported that the emulsion activity of proteins including bovine serum albumin, ovalbumin, and lysozyme increased with the increase of the hydrophobicity. After acylation, the hydrophobicity of collagen increased and then resulted in higher EAI and ESI. Therefore, acylated collagen might be prepared as microparticles for delivery of drugs including retinol,[29] glucocorticosteroids,[30] and progesterone,[31] etc.

Emulsifying Properties Under Different Conditions

Emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen at different concentrations are presented in Figures 5(a) and 6(a). With the increase of acylated collagen concentration, EAI, and ESI increased firstly (<0.4 mg/mL),

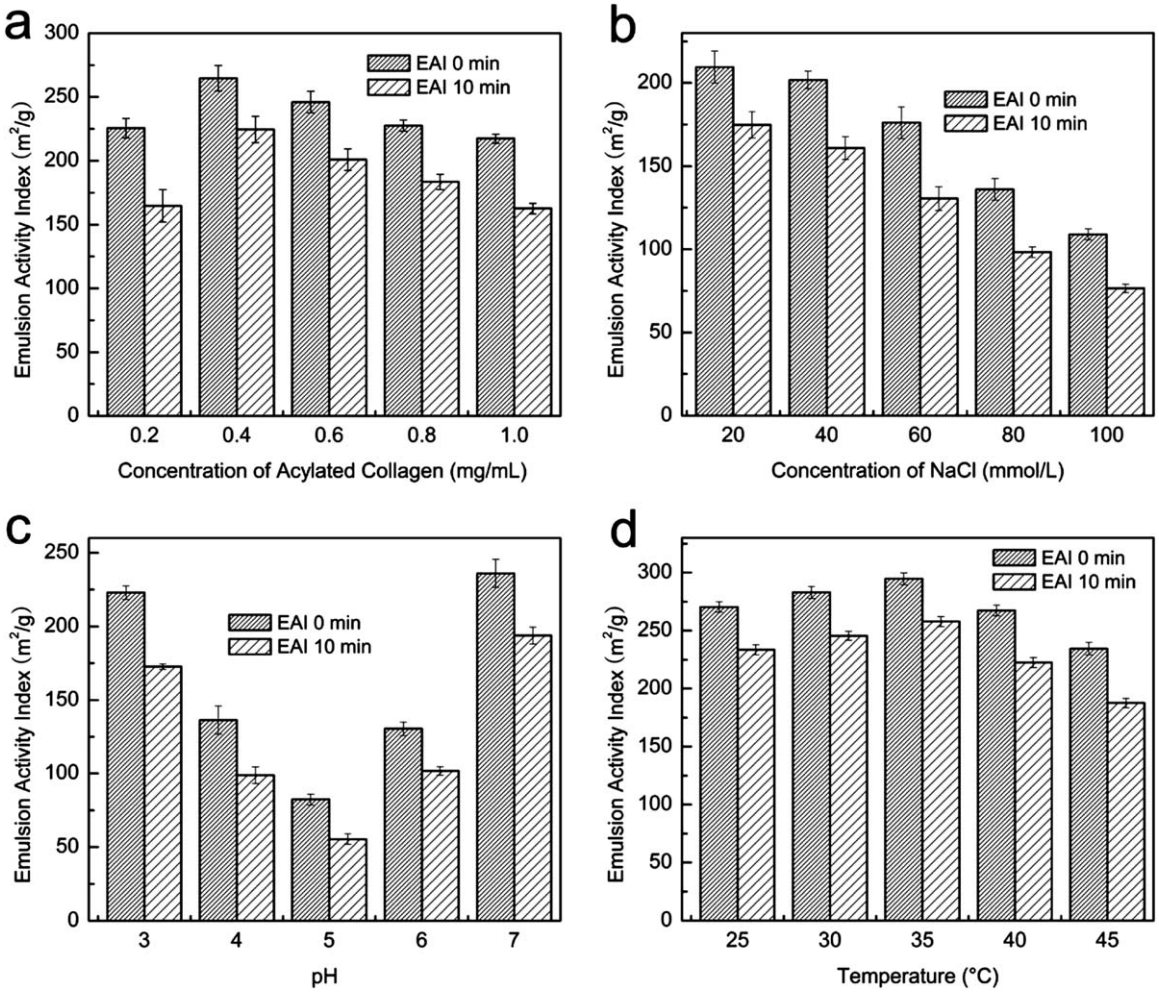

Figure 5. Emulsion activity index (EAI) of acylated collagen at different acylated collagen concentrations (a), NaCl concentrations (b), pH (c), and temperatures (d).

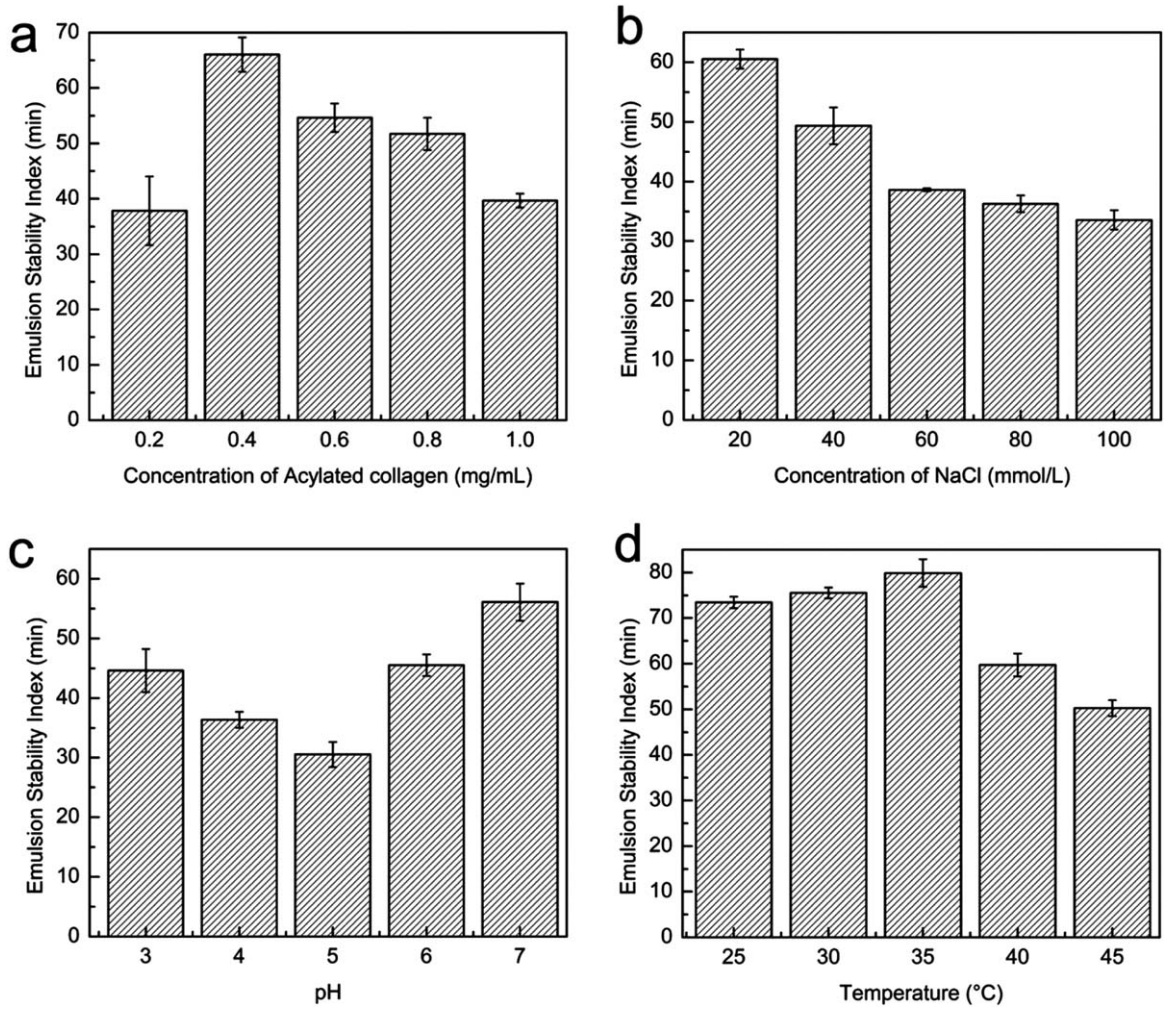

Figure 6. Emulsion stability index (ESI) of acylated collagen at different acylated collagen concentrations (a), NaCl concentrations (b), pH (c), and temperatures (d).

but gradually decreased at higher concentration (>0.4 mg/mL). At the concentration of 0.4 mg/mL, EAI_0, $EAI_{10,}$ and ESI reached the highest values, which were 264.73 m^2/g, 224.62 m^2/g, and 66.03 min, respectively. Although EAI and ESI for tuna fin gelatin increased with the increase of gelatin concentration,[32] the tendency of emulsion activity for acylated collagen was similar to that of mucuna bean protein concentrate derivates[33] and gelatin extracted from Bigeye snapper (*Priacanthus hamrur*) skin.[34] At lower concentration (<0.4 mg/mL), acylated collagen aggregates during the shearing involved in the emulsifying process might be broken easily, which might promote the uniformity and the stability of the emulsion droplets. Meanwhile, the hydrophobicity increased with the increase of acylated collagen concentration and then led to the increase of EAI and ESI. At higher concentration (>0.4 mg/mL), however, acylated collagen molecules assembled easily, which might increase the size of the emulsion droplets. Furthermore, the balance of hydrophobic interactions between acylated collagen molecules and oil droplets might be lost due to the increase of acylated collagen concentration. Therefore, EAI and ESI for acylated collagen decreased at higher concentration (>0.4 mg/mL).

Figures 5(b) and 6(b) show emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen at various NaCl concentrations. It was observed that EAI and ESI of acylated collagen decreased with the increase of NaCl concentration ranging from 20 to 100 mmol/L. In previous studies, the emulsion stability for cuttlefish skin gelatin [3] and chickpea protein derivates [35] was also found to decrease with the increase of NaCl concentration. The reason might be due to the salting out effect,[9] which might increase the size of the emulsion droplets. Meanwhile, Aewsiri et al.[3] investigated that in the presence of NaCl, the electrostatic repulsion between the adjacent droplets of emulsions decreased since the net charge of emulsions was reduced by electrostatic screening, in which the repulsion between the adjacent droplets was no longer sufficiently strong to overcome the attractive force (e.g., vander Waals and hydrophobic interaction) between the adjacent droplets. Therefore, it would be expected that EAI and ESI of acylated collagen decreased with the increase of NaCl concentration.

Figures 5(c) and 6(c) present emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen at various pH

values. As the pH value increased, EAI and ESI decreased and remained the lowest at pH 5, finally increased with the increase of pH value. At the pH value of 5, EAI_0, EAI_{10}, and ESI were the lowest, which were 82.33 m^2/g, 55.31 m^2/g, and 30.55 min, respectively. When the pH value of the solution was far from the pI values of proteins, the solubility and the electrostatic repulsion increased, which might contribute to the increase of emulsion activity.[3,9] The authors had reported that the pI value for acylated collagen was 4.93,[13] which was approached to the pH value of 5. Therefore, the solubility and the electrostatic repulsion of acylated collagen were the lowest at pH 5, which was responsible for the lowest EAI and ESI for acylated collagen. According to the results, we might conclude that acylated collagen emulsions might be applied in fields including foods, medicines, and cosmetics under neutral pH value.

Emulsion activity index (EAI) and emulsion stability index (ESI) of acylated collagen at various temperatures are shown in Figures 5(d) and 6(d). It was found that EAI and ESI of acylated collagen increased slightly when the temperature increased from 25 to 35°C, and then decreased with the temperature increased. Lower temperature might promote the exposure of hydrophobic associations which were normally buried in the interior of protein molecules.[35] Therefore, these hydrophobic associations might lead to the increase of EAI and ESI for acylated collagen at lower temperatures (25–35°C). However, when the temperature increased higher than 35°C, it might be suggested that some partial desorption of acylated collagen molecules from the droplet surfaces might result in flocculation or coalescence of emulsion droplets.[36] According to the result of DSC, T_m for acylated collagen was about 39°C, then it would be expected that protein denaturation at higher temperature (≥40°C) might also lead to the decrease of the hydrophobicity,[35] which resulted in lower EAI and ESI for acylated collagen.

Foaming Properties of Acylated Collagen

Foams could be defined as two-phase systems composed of air bubbles surrounded by a continuous liquid lamellar phase. Generally, foam formation is controlled by transportation, penetration, and reorganization of protein molecules at the air-water interface. Table I shows foam expansion (FE) and foam stability (FS) of acylated collagen, along with native collagen, and Tween 80 as the controls. FE and FS of acylated collagen were 115% and 111.5%, respectively, which were slightly higher than those of native collagen but were much lower than those found in Tween 80. As early as 1983, Townsend and Nakkai[37] confirmed that foam formation capacity and foam stability of food proteins increased with the increase of hydrophobicity. FE and FS of proteins might also be improved by exposing more hydrophobic residues and by increasing its capacity to decrease surface tension.[38] Compared with native collagen, acylated collagen had stronger hydrophobicity, which might promote the increase of FE and FS. Compared with traditional surfactant, Tween 80, lower FE, and FS for native and acylated collagens might be due to the higher molecular weight causing a considerable increase of area per molecule and producing less cohesive force on the surface.[21] With time increased, the value for FE (115%) of acylated collagen decreased to 111.5% for FS, the reason might be due to the gravitational drainage of liquid from the lamella and disproportionation of gas bubbles via interbubble gas diffusion, which led to the film thinning and rupture.[17]

Table II. Wetting Power of Acylated Collagen and Water at the Surface of Acrylic Plastic or Cotton Fabric

Sample	Concentration (mg/mL)	Contact angle (°)	
		Acrylic plastic	Cotton fabric
Water	-	78 ± 2.77	116 ± 5.12
Acylated collagen	0.2	73 ± 0.85	99 ± 2.12
	0.4	71 ± 0.89	97 ± 0.78
	0.6	68 ± 1.19	90 ± 0.85

Wetting Power of Acylated Collagen

Wetting behavior of fibrous materials determined their liquid transport and absorbent characteristics and was crucial to their manufacturing processes, functional properties, and end-use performances. The contact angle could be used as a measurement for the degree of wetting. Table II shows the contact angles formed between the aqueous solutions of acylated collagen at various concentrations (or pure water) and the surface of an acrylic plastic sheet or cotton fabric. It was appeared that the contact angles between acylated collagen solution and acrylic plastic or cotton fabric were smaller than those found in water and decreased with the concentration increased, suggesting that all the solutions possessed the power to wet acrylic plastic and the cotton fabric. Lin et al.[2,39] and Wang et al.[21] also reported that the wetting power of soy protein, dextrin, and gelatin derivatives increased due to the decrease of contact angles after hydrophobicity modification. Consequently, the hydrophobic interactions might also promote the wetting power of acylated collagen solution on the surface of an acrylic plastic sheet or cotton fabric.

CONCLUSIONS

The surface activity of acylated collagen, containing lauroyl and succinic residues on the ε-NH_2 of lysine groups of native collagen molecules, was investigated and the results showed that acylated collagen exhibited good surface activity such as hydrophobicity, surface tension, oil absorption capacity and wetting power, especially emulsion activity and stability, which were much higher than those of native collagen and Tween 80. Due to its better surface activity, this kind of acylated collagen might be widely used in fields including medicines, cosmetics, foods, and chemical industries.

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