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Research Article

Boosting foam performance of egg white protein: synergy between supercritical carbon dioxide and sucrose for superior freeze-thaw stability

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Abstract: This study investigated the synergistic effects of supercritical carbon dioxide (SCCD) combined with sucrose treatment on the foaming properties and freeze-thaw stability of egg white protein (EWP). The results demonstrated that SCCD-sucrose treatment significantly enhanced the foaming capacity, achieving a maximum of 139.5% (4.6-fold increase over the control) at 9 MPa for 60 min with 10 g/100 mL sucrose, while maintaining the foaming stability (FS) which was compromised by SCCD treatment alone. Furthermore, the treated EWP exhibited markedly improved stability against repeated freeze-thaw cycles. Mechanism analysis revealed that sucrose promoted the formation of larger protein aggregates, as evidenced by increased particle size, and significantly reduced surface tension, enhancing adsorption at the air-water interface. Fourier transform infrared spectroscopy indicated a rise in α-helix relative content, contributing to structural ordering, while rheological measurements showed improved elastic modulus (G'), supporting FS. Scanning electron microscopy further revealed a more cohesive and dense protein network of SCCD-sucrose treated group. These synergistic modifications counteracted the structural loosening induced by SCCD and facilitated the formation of a robust interfacial film. The findings provide an effective and promising physical modification strategy for enhancing the functional performance of frozen-stored egg white liquid in the egg processing industry.

Keywords: foaming properties; egg white protein; sucrose; supercritical carbon dioxide; rheological properties

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1 Introduction

The excellent foaming property of egg white protein (EWP) is widely used in baking food, such as cakes, ice cream and chocolate mousses[1]. For these food products, foams of EWP provide unique and desirable textures and largely affect the final quality[2]. However, egg white or yolk in the form of liquid, frozen or dried are available for ease transport and storage due to the increase of food industry's demand for eggs and egg products. The unstable foams of egg white liquid are sensitive to many factors, such as temperature, storage time and freezing[3]. A series of studies have demonstrated that prolonged storage or repeated freeze-thaw cycles under conditions of refrigeration at 4 °C or freezing at –20 °C can induce irreversible alterations in protein structure, thereby impairing their foaming properties[4–5].

EWP is composed of two macroscopically different fractions: the firm albumen and the thin albumen. In the process of frozen storage, the firm albumen gradually thin, while the content of the thin albumen would increase[6]. Foaming capacity (FC) and foaming stability (FS) are two important indicators for evaluating protein foam, which are defined as the ability of protein to incorporate air in the protein solution and the rate of fading in foam volume, respectively[7]. FC of the thin albumen was higher, whereas the firm part proved to have higher foaming FS. In addition, the EWP are subject to shearing force when it undergo repeated freeze-thaw cycles. The intermolecular forces and protein structure will change to varying degrees, resulting in the loss of some nutrients and functions[8–9]. Therefore, it is particularly important to study the structural and functional changes of EWP during freezing and thawing and find a method to reduce the loss of foaming properties.

The foaming properties of EWP are fundamentally governed by the aggregation state and the adsorbed layer formation at the air-water interface[10]. Various modification strategies, including physical, chemical, and enzymatic methods, have been employed to enhance these properties by manipulating protein structure and interfacial behavior[11]. However, chemical modifications risk introducing residues, while enzymatic processes can be time-consuming and may generate undesirable by-products[12–13]. In pursuit of safer and more efficient approaches, physical techniques like supercritical carbon dioxide (SCCD) have gained attention due to their environmentally benign nature[14]. Our previous work demonstrated that SCCD treatment (9 MPa, 60 min) markedly

improved the FC of EWP, yet it simultaneously and notably impaired FS. This detrimental effect on FS is likely attributed to the acidic environment created by dissolved CO_2, which neutralizes protein surface charges, reduces electrostatic repulsion, and may induce excessive protein structural loosening or partial denaturation, weakening the interfacial film strength[15]. To achieve foams with both high FC and robust FS, further exploration of methods to mitigate this stability loss is necessary.

In many food products formulations, pH variation, salt as well as sucrose, could occur together with proteins to contribute on the final structure, texture and stability[16]. Researches have found that mixture of protein and surcrose can create several structures through their gelation and aggregation properties[17]. According to Yang et al.[18], increasing sucrose concentration increased solution viscosity and decreased foam overrun of EWP and whey protein. Laursen et al.[19] also found that the addition of sucrose can significantly improve the FC of wheat protein and potato protein, and can form a gel-like network at the air-water interface, further stabilizing the foam structure. Although many studies investigated sucrose effects on protein foams and interfaces, most of them were conducted using a single level of sucrose content. Therefore, this study aims to examine the combined effect of SCCD and sucrose treatment on the foaming properties and aggregation behavior of EWP, along with its stability during freeze-thaw cycling.

2 Materials and methods

2.1 Materials

Fresh eggs were purchased from the Poultry Research Centre farm in Wuhan, China. Potassium bromide and sucrose were purchased from Yuanye Biotechnology Co. (Shanghai, China). Other chemical agents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents used in our experiment were of analytical grade.

2.2 Pretreatment of EWP

Eggs were broken manually and the egg white was separated from the yolk. Excess components in egg white such as chalaza were picked out with tweezers and discarded. Preliminary homogenization was carried out using a magnetic stirrer (DF-101S; Shanghai Weikai Instrument Equipment Co., Ltd., Shanghai, China). The stirring speed and time were selected based on pre-experiments to ensure complete dispersion of the firm and thin albumen without causing foam formation. The chosen condition was 100 r/min for 1 h, which yielded a visually uniform, bubble-free mixture.

2.3 SCCD-sucrose treatment

A 100 mL of egg white solution was taken into a 250 mL beaker, 0, 5, 10, 15, 20 g of sucrose was added respectively. Samples were stirred with a magnetic stirrer for 20 min until the sucrose is completely dissolved. Then, each samples of 50 mL were put into the reactor of the SCCD sterilization device structure (HKY-1; Hua'an Petroleum Scientific Research Instrument Co., Ltd., Hai'an, China) according to the method of Ding et al.[15]. The reaction kettle was heated by 45 °C water bath. Samples were treated by SCCD at 9 MPa for 1 h. Samples were coded as SCCD, SS-5 g/100 mL, SS-10 g/100 mL, SS-15 g/100 mL, SS-20 g/100 mL, respectively. Additionally, samples containing sucrose at 10 g/100 mL but not subjected to SCCD treatment were prepared as sucrose-only controls (Sucrose). For each operating condition, the egg white samples were placed at room temperature for 2 h in order to remove CO_2 and subsequently cooled at 4 °C for 24 h.

2.4 Freeze-thaw cycles treatment

Samples were placed in a freezer at –20 °C for 12 h and then thawed at 30 °C for 3 h in a constant temperature incubator, which was one freeze-thaw cycle. The same method was used to prepare 0, 1, 3, and 5 cycles of freeze-thaw treatment of EWP samples. Samples were coded as U-*X* (*X* represents the number of freeze-thaw cycles for untreated EWP) and SS-*X* (*X* represents the number of freeze-thaw cycles for SCCD-sucrose (10 g/100 mL) treated EWP).

2.5 Evaluation of FC and FS

FC and FS were measured according to the methods of Ding et al.[15]. A 20 mL of egg white solution was introduced in a 70 mL measuring cylinder and then homogenized at a speed of 9 500 r/min for 60 s at room temperature by a high-speed homogenizer (XHF-DY; Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China).

FC and FS were calculated with the Eqs. (1) and (2), respectively:

$$\mathrm{FC}\ (\%) = \frac{V_0 - V_\mathrm{L}}{V_\mathrm{L}} \times 100 \tag{1}$$

$$\mathrm{FS}\ (\%) = \frac{V_\mathrm{L}}{V_0 - V_\mathrm{L}} \times 100 \tag{2}$$

Where V_0 and V_L are the total volume (mL) of foam and liquid at 0 and 30 min after homogenization, respectively.

2.6 Zeta potential measurement

Egg white solutions were dissolved in 0.01 mol/L Tris-base solution (pH 7.4) to a concentration of 1% (*V*/*V*) and then injected directly into the zeta potential cuvette by a pipette. The zeta potential of egg white samples was determined in triplicate at 25 °C using a zeta potential analyzer (Zetasizer Nano; Malvern, UK) according to the methods of Ding et al.[15].

2.7 Dynamic surface tension measurement

Dynamic surface tension measurements were performed with a surface tension meter (K100; Krüss GmbH, Germany) according to the Wilhelmy's plate method. A 20 mL of the solution to be tested was introduced in a 50 mL beaker and then transferred into the measurement chamber of the instrument. Measurement was performed at room temperature for 30 min. The drop profile was used by the equipment software for calculating the surface tension based on Laplace's equation.

2.8 Particle size measurement

The measurement was performed by the dynamic light scattering (DLS) using a Zetasizer Nano-ZS instrument (Malvern, UK) at 25 °C according to the method of Zhai et al.[20]. Samples were added to 500 mL of dispersant (deionized water) with a pipette and stirred evenly at 1 800 r/min until the measurement range was reached. The measurement range was set between 0.2% and 10%. The refractive index and absorption parameters were set to 1.450 and 0.001, respectively.

2.9 Fourier transform infrared (FTIR) spectroscopy measurement

Freeze-dried samples were mixed with KBr (spectroscopic grade), followed by mixing and tablet preparation. A FTIR spectrometer system (IS50 FTIR; Nicholas Instruments Co., USA) was used to scan the entire band 3 800–600 cm^{-1}. Analysis of secondary structure was carried out using Resolution Pro software version 2.5.5 (Agilent Technologies, Santa Clara, CA, USA) according to Xu et al.[21].

2.10 Differential scanning calorimeter (DSC) measurement

Thermal analysis was done with DSC (DSC200PC; Netzsch Instrument Co., Germany) instrument to determine energy transitions (exothermic/endothermic) and thermal behaviour. EWP powder (5 mg) placed in 40 μg aluminium crucibles was heated up to 200 °C at a constant heating rate of 10 °C/min. DSC thermograms were analysed for denaturation temperature (T_d) and change in enthalpy (ΔH) with the help of Lab METTLER STAR 9.3 (Mettler-Toledo, Switzerland) and OriginPro 8 software (OriginLab Corp., USA).

2.11 Dynamic surface rheology measurement

Measurement of storage modulus (G') and loss modulus (G'') of samples was carried out in a rheometer (R2000ex; TA Instruments, USA) according to Liang et al.[22]. A sample volume of approximately 3 mL was poured into the sample stage. Dynamic surface rheology was performed using a flat-plate measuring system with a flat horizontal grip of 60 mm and a spacing of 2.4 mm at 25 °C. The scanning frequency was 1 Hz, and measuring scanning stress was 0.1–1 000 Pa, respectively.

2.12 Scanning electron microscope (SEM) observation

An amount of EWP samples were taken and stuck on the stage. Conductive layer was sprayed on the samples. Morphological changes of EWP were observed under high-resolution biological SEM (JSM-6390LV; JEOL Ltd., Japan) at a magnification of 2 000×.

2.13 Statistical analysis

Statistical analysis was performed using the SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA). All experiments sets were executed in triplicate and mentioned as the mean ± standard deviation (SD) for this study. Results were considered statistically significant at $P < 0.05$ (Duncan's new multiple range test).

3 Results and discussion

3.1 Foaming properties and freeze-thaw stability of EWP under SCCD-sucrose treatment

The FC and FS of untreated and SCCD-sucrose treated EWP samples are presented in Figure 1A. Untreated EWP exhibited an FC of 30.33% and an FS of 87.94%. Following SCCD treatment alone, FC increased significantly, whereas FS decreased markedly ($P < 0.05$), consistent with our previous report[23]. The addition of sucrose at 10 g/100 mL (sucrose group) raised FC to 55.33%. When combined with SCCD, increasing sucrose concentration further enhanced FC, reaching a maximum of 139.5% at 10 g/100 mL (SS-10 g/100 mL group). Notably, there was no significant difference between the FS of untreated samples, sucrose treated samples and SCCD-sucrose treated samples when the concentration of sucrose did not exceed 10 g/100 mL ($P > 0.05$);

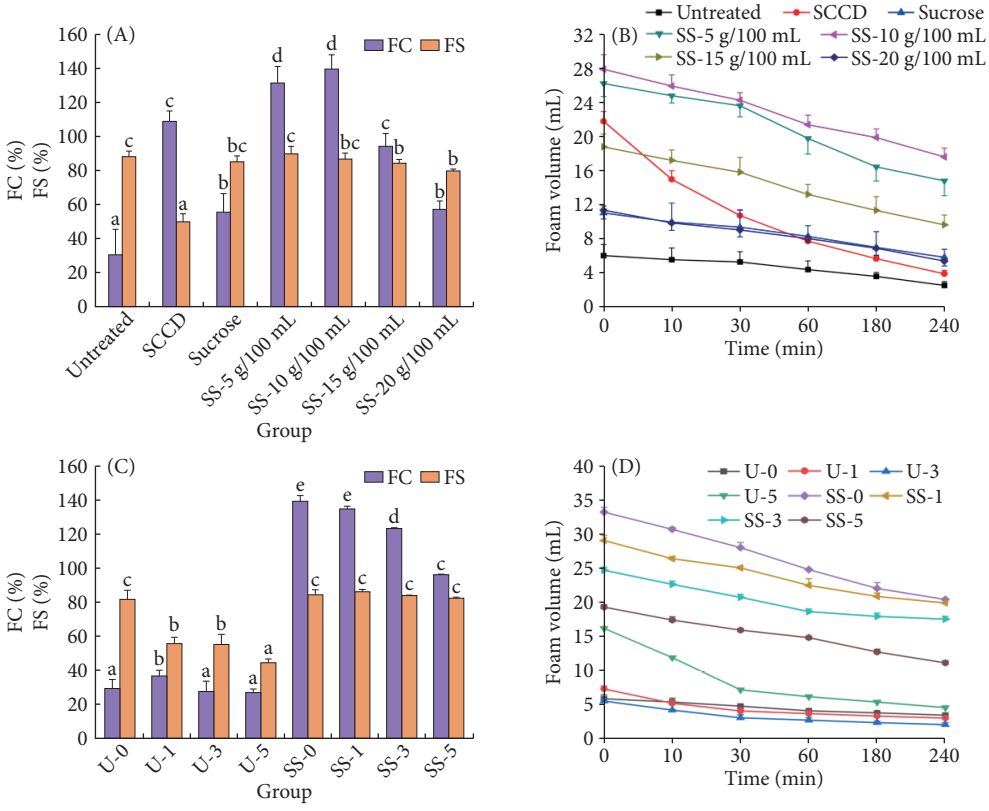

Figure 1 Effect of SCCD-sucrose treatment on (A) FC, FS, and (B) foam volume. (C) FC and FS of samples after freeze-thaw cycles; (D) foam volume with time after freeze-thaw cycles. Values are given as the mean ± SD ($n = 3$). Bars denoted with different lowercase letters are significantly different ($P < 0.05$).

further increases in sucrose led to a decline in FS. The observed stabilization at moderate sucrose levels may be attributed to aggregate formation, which positively influences FS[24–25]. Aggregates can enhance the viscosity of the liquid phase, thereby retarding drainage[18]. Moreover, aggregates can become confined within liquid channels between bubbles, physically obstructing drainage pathways[19]. Sucrose is known to increase system viscosity and promote a more compact protein structure, contributing to foam stability[18]. Foam instability generally arises from drainage, coalescence, and disproportionation[7]. As shown in Figure 1B, sucrose delayed both overrun and drainage in SCCD-treated foams, an effect likely mediated through increased solution viscosity[26].

In order to investigate whether the foam properties of egg white liquids are impaired during frozen storage, samples were separately subjected to a multiple freeze-thaw process (1, 3, 5 times, respectively) for untreated samples and SCCD-sucrose treated samples (10 g/100 mL), and the resulting changes in FC, FS and foam volume were shown in Figures 1C and D. FC of untreated samples increased after 1 freeze-thaw cycle, and then began to decrease with increasing freeze-thaw cycles. FS of untreated samples decreased significantly with the increase of freeze-thaw cycles ($P < 0.05$). Whereas there was no significant difference in FS during SCCD-sucrose treatment. FC of the SCCD-sucrose treated groups decreased with the increase of freeze-thaw cycles, but was always significantly higher than that of the untreated group ($P < 0.05$).

Ma et al.[27] proved that FC of egg white increased after freeze-thaw cycles for 1–5 times, which is different from our results. We speculated that ice crystal formation during freeze-thaw for 1 cycle would disrupt the structure of the EWP. The squeezing effect of ice crystals made the protein unfold and the internal groups were exposed, making it easier to adsorb and unfold at the water-air interface. Thereby the FC of EWP improved. With the increase of freeze-thaw cycles, ice crystals destroyed the ordered structure in the protein, making it more loose and disordered. This resulted in reduced molecular forces between the proteins and was unfavorable for the formation of a strong film to encapsulate the gas. The formation of adsorbed layer and aggregates of proteins is crucial for the foam formation and stabilization[28]. Laursen et al.[19] found that the addition of sucrose could significantly improve the FC of wheat and potato proteins and could form a gel-like network at the air-water interface, thereby further stabilizing the foam structure. Similarly, Simiqueli et al.[29] demonstrated that sucrose can bind to ovalbumin via hydrophobic interactions to form a sugar-protein complex, which increases the viscosity of ovalbumin. Therefore, we speculate that the unaffected FS of the SCCD-sucrose treated group after freeze-thaw cycles may be attributed to sucrose-induced protein aggregation and the consequent increase in viscosity.

3.2 Structural changes of EWP under SCCD-sucrose treatment

The zeta potential, defined as the electrostatic potential at the slipping plane relative to the bulk fluid, is a key indicator of the colloidal stability of protein dispersions[30]. It reflects the magnitude of the charge on protein particles, where a higher absolute value typically signifies greater electrostatic repulsion and thus better stability against aggregation[31]. As shown in Figure 2A, the absolute zeta potential of untreated EWP was –13.36 mV, which decreased

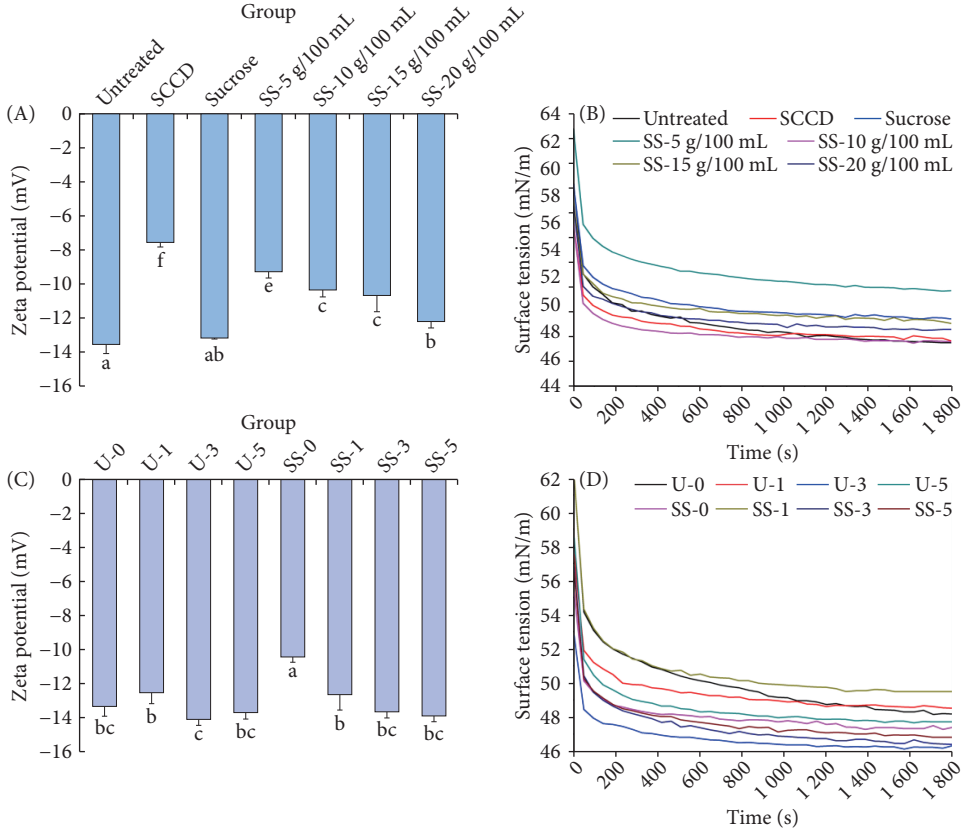

Figure 2 Effect of SCCD-sucrose treatment on (A) zeta potential and (B) surface tension. Effect of freeze-thaw cycles on (C) zeta potential and (D) surface tension. Values are given as the mean ± SD ($n = 3$). Bars denoted with different lowercase letters are significantly different ($P < 0.05$).

significantly ($P < 0.05$) to −7.56 mV after SCCD treatment. This reduction can be attributed to the acidic nature of dissolved CO_2, which neutralizes surface negative charges, bringing the system pH closer to the protein's isoelectric point. The consequent decrease in electrostatic repulsion likely promotes protein dissociation and re-aggregation[32]. Notably, the zeta potential increased again with the addition of sucrose in the SCCD-sucrose treated groups (Figure 2A).

Following freeze-thaw cycles, the zeta potential of untreated EWP increased with cycle number, reaching a maximum of −14.1 mV after 4 cycles (Figure 2C). A similar significant increase was observed for the SCCD-sucrose treated group after freeze-thaw treatment. For EWP—a mixture of proteins with different isoelectric points—the formation of a stable three-dimensional network relies on a balance of electrostatic and hydrophobic forces. The increased zeta potential (greater negative charge) observed after freeze-thaw, particularly in the SCCD-sucrose group, suggests a possible rearrangement of protein molecules or changes in ion distribution at the interface. The enhanced electrostatic repulsion improves dispersion stability, which hinders bubble coalescence and is beneficial for foam stabilization[33]. This mechanism likely contributes to the maintained FS in the sucrose-SCCD group after freeze-thaw stress.

Researches have reported that the dynamic surface tension is the main determining factor which is directly associated to the foaming properties of proteins[30]. Overall, a rapid decrease in the surface tension indicates a fast protein adsorption and stabilization of air bubbles against coalescence[34]. The impact of SCCD-sucrose treatment on EWP has been studied through the evolution of the surface tension as function of time (Figure 2B). Results confirmed that SCCD-sucrose treated group with the concentration of 10 g/100 mL was most efficient to reduce the tension at air water interface, which was in agreement with our previous results of foaming properties. Indeed, proteins have the best foam properties under this condition where they have just perfect amount of compactness and flexibility. It can be observed from Figure 2D that the surface tension of the untreated EWP group decreased with the increase of freeze-thaw times at first, and then reached the minimum after 3 freeze-thawed cycles. The surface tension of SCCD-sucrose treatment group reached the maximum after 1

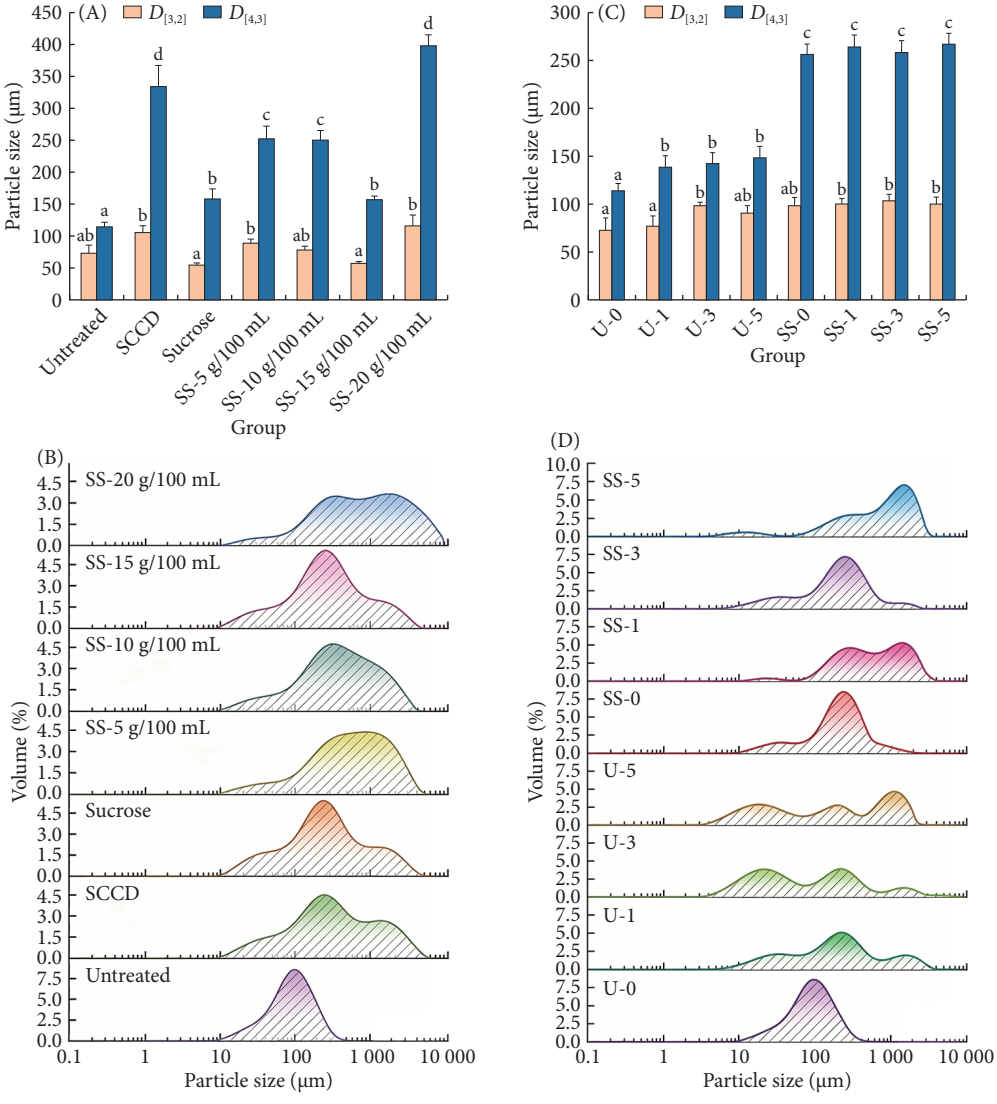

Figure 3 Effect of SCCD-sucrose treatment on (A) particle size and (B) particle size distribution. Effect of freeze-thaw cycles on (C) particle size and (D) particle size distribution after freeze-thaw cycles. Values are given as the mean ± SD ($n = 3$). Bars denoted with different lowercase letters are significantly different ($P < 0.05$).

freeze-thaw cycle. There was no significant difference in surface tension between the non-freeze-thawed, 3 and 5 freeze-thaw cycles treated groups for SCCD-sucrose treated EWP. The decrease of surface tension proved that the molecular activity of EWP increased, and the hydrophobic groups in the molecules were exposed, which made it easier to unfold at the water-air interface and was more conducive to foaming[35]. Based on the results of this experiment, it can be assumed that the ice crystals and shear force generated by the freeze-thaw treatment would unfold the protein and expose the internal groups.

Particle size distributions yield two critical metrics: the surface volume mean diameter $D_{[3,2]}$ and volume moment mean diameter $D_{[4,3]}$. The more closely the shape of the particle resembles a perfect sphere, the more similar the values of $D_{[3,2]}$ and $D_{[4,3]}$ become[36]. As shown in Figure 3A, the particle size distribution range of untreated EWP samples was narrow and the peak was observed at 100 μm. Three dwarf peaks could be observed at 10–1 000 μm in the particle size distribution after SCCD treatment. In addition, the peak range of the sucrose-treated group and the SCCD-sucrose treated group was wider, ranging from 10 to 2 000 μm. The average particle size of EWP was significantly increased after the addition of sucrose (Figure 3B), which was consistent with our previous assumption that EWP and sucrose were bound by hydrophobic bonds and hydrogen bonds to form aggregates, thereby increasing the particle size of protein solution. $D_{[3,2]}$ and $D_{[4,3]}$ of the untreated group increased significantly with the increase of freeze-thaw times, while the average particle size of the SCCD-sucrose treated group had no significant change under different freeze-thaw times (Figure 3C). After 1, 3, and 5 freeze-thaw cycles of untreated EWP, the peak at 100 μm became shorter, and two new shorter peaks appeared at 10 and 1 000 μm (U-1, U-3, U-5), which proved that the ice crystals formed by freezing and thawing caused the protein to dissociate and unfold, and then re-aggregate into macromolecular polymers (Figure 3D). Therefore, the particle size distribution of EWP after freeze-thaw cycles was wider. Freeze-thaw treatment made the EWP of the SCCD-sucrose treated group to form larger polymers, and the peak at 1 445.44 μm increased with the increase of freeze-thaw times. Particle size is important factor in protein diffusion and adsorption to the air-water interface. The diffusion rate correlates negatively with particle size; in fact, diffusion rate can decrease with increasing particle size[37]. On the contrary, the formation of aggregates can increase the viscosity and reduce the flow of drainage, which would have positive effect on FS[31]. According to these results, it could be assumed that sucrose can combine with EWP to form new aggregates, which made up for the protein loosening and overexpansion caused by SCCD, and achieved a good synergistic effect.

3.3 FTIR spectra analysis of EWP under SCCD-sucrose treatment

FTIR spectra of EWP under SCCD-sucrose treatment are presented in Figure 4. It can be observed that the untreated group and the SCCD-treated group have obvious peaks at 930, 1 110, 1 244, 1 377, 1 443, 1 510, 1 627, 1 710, 2 924, 3 320 cm^{-1}. According to previous reports, absorbance at 3 800–3 100 cm^{-1} is attributed to the O–H stretching vibration[38]. Compared with the untreated group, the peak areas of 3 320 cm^{-1} in SCCD-treated group decreased to varying degrees, indicating that the content of O–H bonds in the protein decreased. In addition, the peak area of 930 cm^{-1} decreased after SCCD treatment, while the peak area of the SCCD-sucrose treated group gradually recovered with the increase of sucrose content, and finally was approach to the untreated group. In the protein FTIR image, the peak at 930 cm^{-1} corresponds to the helical structure in the protein, and the helical structure is the most stable structure in the protein. Bands appearing in the range of 930 cm^{-1} in the FTIR spectrum of protein were ascribed to the protein helix structure, which was the most stable structure of protein[39]. Results confirmed that the addition of sucrose and CO_2 constitute a synergistic effect, which restored the content of the helical structure in the protein, thereby improving the protein stability.

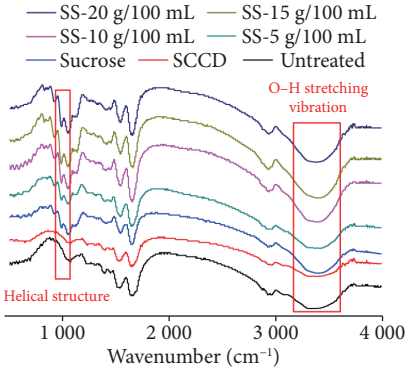

Figure 4 FTIR spectra of EWP after SCCD-sucrose treatment.

The most informative part of the FTIR spectrum with regard to the secondary structure of proteins is the amide I region band (1 600–1 700 cm^{-1})[40]. The changes of secondary structure of EWP calculated according to FTIR spectroscopy are shown in Table 1. Compared to untreated group, the α-helix relative content of EWP was reduced to 12.74% after SCCD treatment, while the relative content of α-helix in the sucrose-treated group was 17.80%, which proved that sucrose treatment could increase the relative content of α-helix in EWP. The highest β-sheet relative content (44.16%) of EWP was found on untreated samples, which then decreased dramatically after SCCD and sucrose treatment ($P < 0.05$). With the increase of sucrose concentration, the β-sheet relative content of SCCD-sucrose treated EWP showed a downward trend, until the minimum (27.19%) was reached when the sucrose concentration was 15 g/100 mL. β-Turn and random content were significantly increased after SCCD and sucrose treatment ($P < 0.05$). Researches indicated that the sugars brought about the change of secondary structure and functional properties of the protein[41]. α-Helix and β-sheet are ordered structures with higher stability in the secondary structure of protein. The reduction of its overall content proved that SCCD-sucrose treatment could disrupt the ordered structure in the protein, thereby making it more flexible. Moreover, the relative content of α-helix in the SCCD-sucrose-treated EWP was significantly higher than that in the SCCD-treated group, which proved that the three-dimensional network structure in the SCCD-sucrose-treated EWP was more stable than that in the SCCD-treated group.

3.4 DSC analysis of EWP under SCCD-sucrose treatment

DSC parameters recorded during DSC analysis of EWP samples are presented in Table 2. The T_d and ΔH are main parameters of DSC

Table 1 Secondary structure relative contents (%) calculated from FTIR spectra after SCCD-sucrose treatment.

Group	α-Helix	β-Sheet	β-Turn	Random
Untreated	13.81 ± 0.23^{a}	44.16 ± 1.02^{d}	27.25 ± 0.67^{a}	14.78 ± 0.21^{a}
SCCD	12.74 ± 0.12^{a}	30.98 ± 0.45^{b}	37.87 ± 0.78^{c}	16.41 ± 0.33^{b}
Sucrose	17.80 ± 0.57^{c}	33.47 ± 0.89^{c}	31.93 ± 0.31^{b}	16.80 ± 0.23^{b}
SS-5 g/100 mL	15.41 ± 0.56^{b}	30.51 ± 0.21^{b}	37.61 ± 0.24^{c}	16.47 ± 0.42^{b}
SS-10 g/100 mL	16.42 ± 0.31^{bc}	28.23 ± 0.20^{a}	39.53 ± 0.12^{d}	15.82 ± 0.51^{ab}
SS-15 g/100 mL	17.10 ± 0.33^{c}	27.19 ± 0.43^{a}	39.30 ± 0.32^{d}	16.42 ± 0.20^{b}
SS-20 g/100 mL	16.92 ± 0.21^{bc}	28.51 ± 0.24^{a}	37.31 ± 0.33^{c}	17.26 ± 0.44^{b}

Note: Different superscript lowercase letters in the column indicate significant differences among groups ($P < 0.05$).

Table 2 Thermal T_d and ΔH of EWP after SCCD-sucrose treatment.

Parameters	Untreated	SCCD	Sucrose	SS-5 g/100 mL	SS-10 g/100 mL	SS-15 g/100 mL	SS-20 g/100 mL
T_d (°C)	98.92 ± 1.02^{b}	90.11 ± 1.01^{a}	116.76 ± 0.98^{c}	97.91 ± 0.50^{b}	113.17 ± 1.16^{c}	124.93 ± 1.23^{d}	118.32 ± 1.00^{c}
ΔH (J/g)	117.36 ± 0.56^{d}	105.41 ± 0.89^{c}	89.64 ± 0.32^{a}	84.94 ± 0.35^{a}	85.73 ± 0.50^{a}	95.20 ± 0.98^{b}	104.72 ± 1.02^{c}

Note: Different superscript lowercase letters in the row indicate significant differences among groups ($P < 0.05$).

profile, which reflects the degree of thermal stability of protein. T_d and ΔH of untreated EWP samples were observed as 98.92 °C and 117.36 J/g, respectively. After SCCD treatment, T_d and ΔH of EWP samples decreased significantly ($P < 0.05$), while sucrose treatment increased the T_d of the sample and decreased the ΔH. Finally, it was notable that the ΔH of SCCD-sucrose treated EWP was lower than that of untreated group and SCCD treated group, which suggested that weaker protein-protein interactions and partly denaturation occurred during the process. The information on protein thermal properties is useful for understanding the structural stability of proteins. Decrease in ΔH was proved to be beneficial to the unfolding of proteins at the water-air interface[42].

3.5 Rheological analysis of EWP under SCCD-sucrose treatment

Since rheological properties are related to strength (elastic structure) and flexibility (rigid or viscoelastic) of a sample, rheological behaviors may be used to correlate with FS[43]. The frequency evolution of G', G'' and sine angle (tanδ) of surface films was shown in Figure 5. There was a significant increase in G' and G'' with the increase of frequency at the interface for the EWP samples treated by sucrose, whilst the G' of untreated sample and SCCD-treated sample decreased with the increase of frequency after 10 Hz, indicating that sucrose treatment changed the elastic structure and flexibility of EWP. Tanδ is defined as the ratio between viscous and elastic modulus (G''/G'). When $G' > G''$ (tan$\delta < 1$), the material may be considered solid-like whereas; when $G' < G''$ (tan$\delta > 1$), it may be considered as a fluid. Untreated and SCCD-treated samples presented viscous behaviour (tan$\delta > 1$), which implied a lower resistance to rupture of the film formed[44]. Conversely, samples treated by sucrose presented elastic-dominant rheological properties. Sadahira et al.[45] proved that viscous behaviour led to lower FS than elastic behaviour, which was consistent with our experimental results of foam properties and rheological properties. It could be assumed that the presence of sucrose contributed to the stability of the aerated system by binding to proteins and forming aggregates of macromolecules.

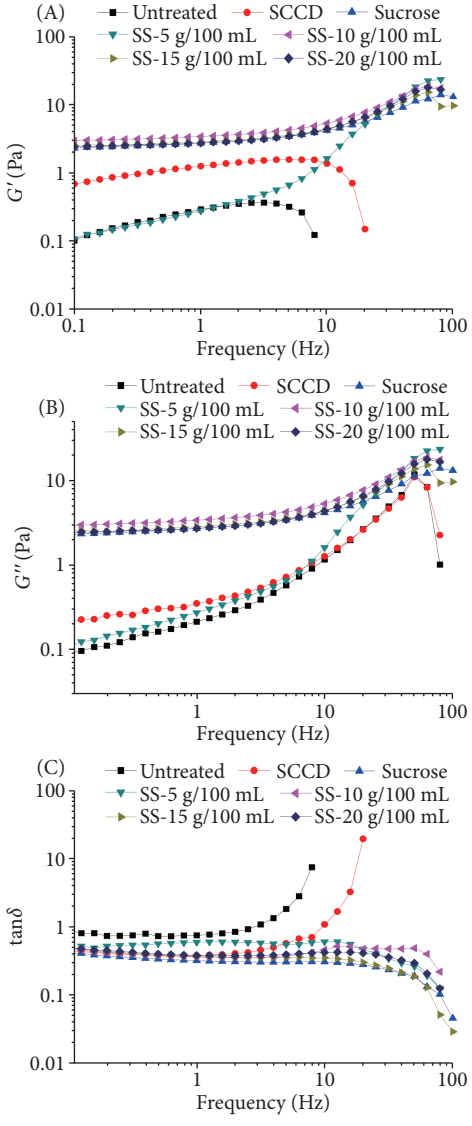

Figure 5 Rheological behavior of EWP. (A) G', (B) G'' and (C) tanδ of EWP at the air/water interface.

3.6 Morphology of EWP under SCCD-sucrose treatment

To have tangible evidence of the transformation of EWP microstructure under SCCD-sucrose treatment, we employed SEM (Figure 6). The untreated EWP sample showed a rough, folded microstructure with abundant irregular protrusions and pits. Samples treated by SCCD developed spongy structure and had abundant pores. In our previous study, it has been proved that the intervention of CO_2 developed loose and flexible structure of EWP by changing the intermolecular electrostatic force and viscoelasticity[15]. It could be assumed that the spongy structure was formed because of the intervention of CO_2 fluid between protein molecules and water phase, causing the dissociation and re-aggregation of the protein. After sucrose treatment, proteins collapsed into irregular shapes and aggregated into larger structures, while the cross section of the sample was observed to be smooth, which suggested that partial rearrangement and denaturation and aggregation occurred.

FS of EWP are generally influenced by viscoelasticity and protein structures[2]. The process of formation of foam implies the creation of surface area that should be rapidly covered by protein to prevent rupture of the film[44]. Combined with above-mentioned results, the addition of sucrose increased the electrostatic repulsion of EWP and caused particle size more dispersed. This indicated that sucrose interacted with EWP to form macromolecular aggregates, which reduced the damaging effect of SCCD on the system and obtained a stable protein structure.

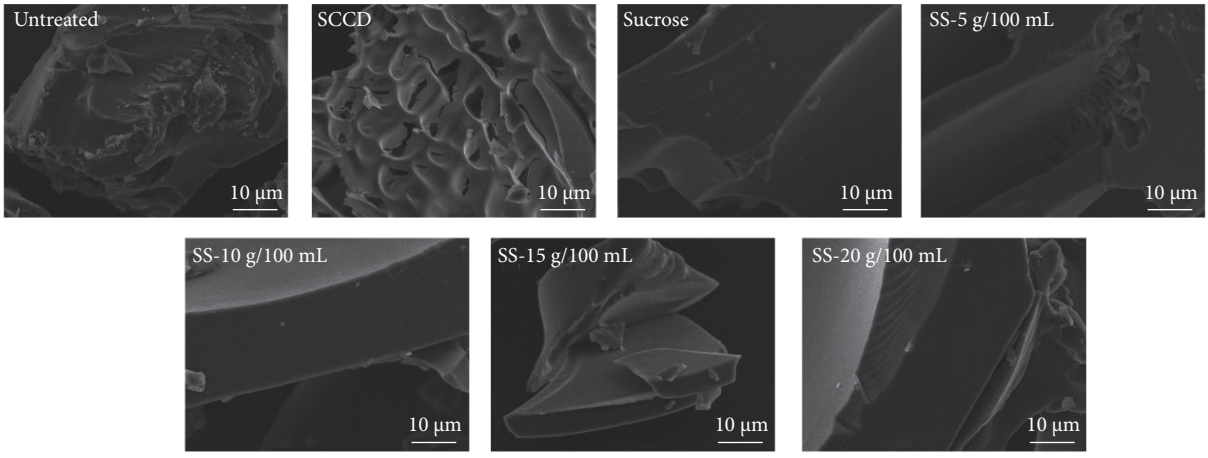

Figure 6 SEM images of EWP after SCCD-sucrose treatment at magnification of 2 000×.

4 Conclusion

In this study, the foaming properties and freeze-thaw stability of EWP treated with a combined SCCD and sucrose process were systematically evaluated. The results demonstrated that SCCD-sucrose treatment effectively enhanced the functional performance of EWP, notably improving FC, FS and freeze-thaw stability. Mechanistic investigations revealed that sucrose contributes to foam stabilization primarily by increasing the viscosity of the continuous phase and modulating protein conformational arrangements. Specifically, sucrose compensates for the structural loosening and over-expansion induced by SCCD, yielding a synergistic stabilization effect. Furthermore, sucrose interacts with EWP through hydrogen bonding and hydrophobic interactions, promoting the formation of intermolecular aggregates and increasing the α-helix relative content in the protein's secondary structure. This structural reinforcement facilitates the establishment of a robust three-dimensional network at the air-water interface, which is crucial for maintaining foam integrity under repeated freeze-thaw cycles. This study presents a promising physical modification strategy. However, it is primarily focused on model systems. Future research should validate the effectiveness of this SCCD-sucrose synergy in complex food matrices and explore the long-term storage stability of the treated egg white liquid under industrial conditions.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Lixian Ding: Formal analysis, investigation, data curation, visualization, writing-original draft, writing-review & editing. Minquan Xia: Formal analysis, writing-review & editing. Xinyue Zhang: Writing-review & editing. Dewei Shu: Funding acquisition. Zhaoxia Cai: Conceptualization, supervision, resources, writing-review & editing, project administration, funding acquisition.

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