

Differential mechanisms of saccharides in mitigating heat-induced aggregation of liquid egg yolk: a comparative study on rheological, functional, and structural properties

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Abstract: The quality deterioration of liquid egg yolks caused by increased sterilization temperature was alleviated. This study investigated the effects of sucrose, glucose, trehalose, and arabinose on the heat aggregation behavior of egg yolks. The results showed that after heat treatment, saccharides significantly increased the thermal denaturation temperature, effectively inhibited heat-induced thickening, and slowed the decrease in soluble protein content and emulsifying activity of the liquid egg yolk. Moreover, the Fourier transform infrared spectrometry and surface hydrophobicity data indicated that saccharides could alleviate the changes in the degree of structuration and the exposure of hydrophobic residues to egg yolk protein molecules. It was also found that the aggregation degree of sugared egg yolks was relatively low according to the microstructure and particle size. In summary, sucrose, glucose, trehalose, and arabinose could alleviate the heat aggregation behavior of egg yolks and could be applied to produce liquid egg yolks with enhanced heat stability.

Keywords: liquid egg yolk; saccharides; heat stability; rheological property; functional property; protein structure

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1 Introduction

Liquid eggs are the egg products with the closest quality to fresh eggs. Compared to shell eggs, liquid eggs can effectively solve the problems of fragility and difficulties in transportation. This has become one of the main development directions of egg products. Liquid egg yolk has extremely high nutritional value; in addition to being rich in protein and lipids, it also contains various trace elements and vitamins^[1-3]. As a natural and complex protein-lipid emulsifier system, it is often used as a natural food emulsifier in the food industry and is widely used in various industries, such as medicine, cosmetics, and health products^[4].

At present, the main sterilization method used in the processing of liquid egg products is pasteurization. The commonly used sterilization conditions for egg yolk liquid are 60–68 °C and 3–4 min^[5]. With the pursuit of a higher quality liquid egg, egg yolk manufacturers are trying to use higher temperatures to sterilize the liquid egg yolk. However, liquid egg yolk is a thermosensitive substance, and a thermal sterilization temperature higher than 65 °C easily causes aggregation and denaturation of liquid egg yolk, leading to deterioration of its quality and performance^[6], such as a decrease in the emulsification and fluidity of liquid egg yolk. An increase in the viscosity of liquid egg yolks after heat treatment can also have adverse effects on processing equipment, such as scaling and blockage of egg yolk transportation pipelines^[7]. Due to the impact of thermal sterilization on the functional properties of egg yolks, their processing and application in the food industry are limited. Therefore, it is necessary to develop a method to improve the heat stability of liquid egg yolks. Barbiroli et al.^[8] reported that saccharides are beneficial for the folding of proteins and can

improve the heat resistance of β -lactoglobulin. Nafchi et al.^[9] reported that sugars can strengthen hydrophobic interactions to stabilize proteins and improve heat resistance by changing the binding form of water. Several studies have reported that saccharides have a positive effect on the heat stability of proteins. However, further research is needed to investigate the effects of sugars on the thermal stability of egg yolks, including their beneficial effects, the underlying mechanisms, and comparisons among different sugars.

Therefore, in this study, we investigated the influence of sucrose, glucose, trehalose, and arabinose on the thermal stability of liquid egg yolks, using fresh liquid egg yolks (FEY) as controls. This study elucidated the interrelationships between sugars and the rheological, functional, and structural properties of egg yolk liquid under different temperature treatments, aiming to provide insights into the mechanisms and key factors involved in sugar-induced changes in the structural properties of egg yolk proteins. We hope that our research can provide a theoretical basis for inhibiting the aggregation of egg yolk proteins, decreasing the sensitivity of egg yolk liquid to higher pasteurization temperatures.

2 Materials and methods

2.1 Materials

Fresh eggs were obtained from the Haoyouduo supermarket, and soybean oil was obtained from Jiusan Oils & Grains Industries Group Co., Ltd. Sucrose, glucose, trehalose and arabinose were purchased from Jiangsu Caiwei Biotechnology Co., Ltd. Sodium dodecyl sulfate (SDS) and 1-anilino-8-naphthalene sulfonic acid

(ANS) were purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, USA). The water used in the experiment was deionized water.

2.2 Preparation of samples

After the fresh eggs were cleaned, the egg white and yolk were separated. Tweezers were used to remove the tie, and absorbent paper was used to remove excess egg white. The yolk film was broken and stirred slowly for 5 min with an electric mixer to obtain a uniform liquid egg yolk. Then, the samples were divided evenly into 5 parts, and 5% (*m/m*; relative to final formulation) sucrose, glucose, trehalose, and arabinose were added, which were designated as EY_{sucrose}, EY_{glucose}, EY_{trehalose}, and EY_{arabinose}, respectively. The liquid egg yolk was stirred at low speed with a magnetic stirrer at room temperature for 20 min until the sugar was completely dissolved and mixed evenly. The thermal treatment used the water bath method, with temperatures of 68, 72, and 76 °C and a treatment time of 4 min. Different sugar-treated liquid egg yolks were used as the experimental group, and FEY was used as the control group.

2.3 Characterization of the rheological properties of the liquid egg yolk samples

2.3.1 Temperature sweep

Yolk rheological measurements were determined using a rotary rheometer (HAAKE MARS6 Modular Rotational Rheometer, Thermo Fisher Scientific Co., Ltd., China) with a 35-plate system and a 0.3 mm flat gap for measurement. Then, a 1 mL sample was placed on the center of the circular plate of the transducer. Immediately after the probe drops, the sealed cover is put on, and a layer of silicon oil is applied around and above the seam to prevent the moisture from evaporating. Prior to heating, the temperature of the sample was balanced at 25 °C for 1 min, and the temperature was increased from 25 to 90 °C at a heating rate of 3 °C/min^[10].

2.3.2 Variable-frequency scanning

With a fixed strain value of 1% and a plate clearance of 0.3 mm, the dynamic oscillatory frequency sweep was determined for dynamic viscoelastic measurements over a frequency range of 0.1–15.0 Hz at (25 ± 1) °C^[11].

2.3.3 Apparent viscosity

Apparent viscosity measurements were determined using a rotary rheometer (HAAKE MARS6 Modular Rotational Rheometer, Thermo Fisher Scientific Co., Ltd., China). The fixed strain force was 1%, and the shear rate range from 0 to 100 s⁻¹. The measured apparent viscosity and shear rate were fitted using a power-law model^[12] with the following Eq. (1):

$$\eta = K \times \dot{\epsilon}^{n-1} \quad (1)$$

Where η is the viscosity (Pa·s), K is the consistency index (Pa·s^{*n*}), $\dot{\epsilon}$ is the shear rate (s⁻¹), and n is the flow behavior index.

2.4 Determination of differential scanning calorimetry (DSC)

Approximately 10 mg of the sample was placed in an aluminum pot, accurately weighed, and then sealed on an aluminum plate. In the range of 25–95 °C, the heating rate was set to 5 °C/min, and the thermal denaturation temperature and denaturation enthalpy value (ΔH) were calculated based on a heatmap^[13].

2.5 Characterization of the functional properties of the liquid egg yolk samples

2.5.1 Determination of emulsifying activity index (EAI) and emulsifying stability index (ESI)

The egg yolk sample was diluted with deionized water to a concentration of 5 mg/mL protein-water solution, and 20 mL of the diluted liquid was added to 5 mL (20%, V/V) of soybean oil with a high-speed homogenizer at a speed of 12 000 r/min for 1 min. After the emulsion was prepared, 50 μ L of emulsion was immediately extracted from the bottom, and 5 mL of 0.1 g/100 mL SDS solution was added for dilution. The absorption value of the emulsion after dilution was measured with an ultraviolet visible spectrometer at 500 nm. After 30 min, 50 μ L of emulsion was extracted again from the bottom, and the light absorption was measured according to the above method. The EAI of the sample is expressed by the absorbance (A_0) at time zero, indicated as m²/g, ESI is calculated using the following formula (2)^[14]:

$$\text{ESI (\%)} = \frac{A_0 \times 30}{A_0 - A_t} \times 100 \quad (2)$$

Where A_0 is the absorbance at time 0, and A_t is the absorbance after 30 min.

2.5.2 Determination of soluble protein content

Based on the standard curve of bovine serum protein, the concentration of protein was measured using the BCA method^[15].

2.6 Characterization of the structural changes in liquid egg yolk samples

2.6.1 Determination of zeta potential

The sample was diluted 500 times with deionized water and stirred magnetically at room temperature for 2 h. The zeta potential was measured using a zeta potential analyzer (ZS90, Malvern Instruments Ltd., UK).

2.6.2 Determination of surface hydrophobicity

According to Yan et al.^[16], ANS was used as a fluorescent probe. The sample stock solution was diluted with 0.01 mol/L phosphate buffer (pH 7.0) to five protein concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL. Four millilitres of each diluted liquid was added to 20 μ L of ANS solution (phosphate buffer configuration of 0.01 mol/L with pH 7.0 and a concentration of 8 mmol/L) and subjected to vortex oscillation and reaction in the dark for 15 min. The measurement parameters were as follows: excitation wavelength of 390 nm, emission wavelength of 470 nm, and slit width of 2.5 nm. The surface hydrophobicity index is expressed as the slope of the curve of protein concentration versus fluorescence intensity.

2.6.3 Determination of Fourier transform infrared (FTIR) spectrometry

A freeze-drying machine was used to freeze-dry the egg yolk sample, and the freeze-dried sample was subsequently placed in the attenuated total reflectance method plug-in of the Fourier transform infrared spectrometer for determination, with a curve ranging from 400 to 4 000 cm⁻¹. Spectral data analysis was carried out using Omnic 6.0 and PeakFit v4.12 for spectral data processing^[17].

2.6.4 Determination of ultraviolet-visible (UV-Vis) spectroscopy

The egg yolk was dissolved in 0.01 mol/L sodium phosphate buffer (pH 7.0) to prepare a protein solution with a concentration of 1 mg/mL. Egg yolk samples were scanned with a spectrophotometer (UV-2600, Shimadzu, Tokyo, and Japan) at a rate of 100 nm/min between 250 and 310 nm.

2.6.5 Determination of water distribution

The transverse relaxation time (T_2) was determined by performing low-field nuclear magnetic resonance (LF-NMR) spectroscopy (Suzhou Niumag Analytical Instrument Corp., Suzhou, China) using the procedure described by Wu et al.^[18]. Call-Purcell-Meiboom-Gill measurements of T_2 were also conducted. The operating parameters for the relaxation measurements performed at 22 °C were as follows: main frequency 21 MHz, radiofrequency delay 0.002 ms; echo time 0.4 ms; and scan repetitions 4, with a specified number of iterations of 100 000 echoes.

2.6.6 Determination of particle size distribution

The samples were diluted to 5 mg/mL with double-distilled water. A laser particle size distribution analyzer (SYNC, MacChick, Inc., USA) was used to determine the particle size distribution. The refractive index of the sample was 1.46, the refractive index of the medium was 1.33, and the measurement temperature was 25 °C.

2.6.7 Scanning electron microscopy observation

A freeze-drying machine was used to freeze-dry the egg yolk sample. Under vacuum conditions, the sample was sprayed with gold and placed under a scanning electron microscope (SU8010, Hitachi, Tokyo, Japan) at a magnification of 200× to observe and take photos.

2.7 Statistical analysis

The significance and related analysis of the data were carried out using SPSS 26 software, and the Origin 2021 software was used for three repetitions of each set of experiments.

3 Results and discussion

3.1 Rheological property changes in liquid egg yolk as affected by saccharides

3.1.1 Thermal stability analysis

Egg yolk proteins are very sensitive to heat, and heat treatment causes protein denaturation and structural changes. The change of

egg yolk storage modulus (G') can reflect the formation of gel, and the point where G' rises rapidly can reflect the change of the sample's behavior from liquid to solid, that is, the change of the sample when it establishes the gel network structure with the increase of temperature, which can be used to determine the transition point for the polymer to form a continuous molecular structure^[19]. As the temperature increased, the inflection points of the G' gradually increased, indicating that the sugar addition treatment increased the denaturation temperature of the egg yolk protein. The inflection points of each curve in Figure 1A showed that the gel formation temperature of FEY was approximately 72.46 °C, and EY_{sucrose} and EY_{trehalose} also provided significant protection, with gel formation temperatures of 76.78 and 76.89 °C, respectively; the effects of the two were similar. EY_{arabinose} exhibited a relatively weaker protective effect, with a gel formation temperature of 76.20 °C. The results showed that the addition of the above sugars could improve the heat resistance of liquid egg yolk and delay the temperature of gel formation. This could be because the oxhydryl group in the sugar molecule interferes with the interaction between proteins and promotes the flow of protein molecules, and it can also bind with protein-polar amino acids, further inhibiting the aggregation of the liquid egg yolk^[20]. Differences among various sugars in molecular size, the number of hydroxyl groups, and spatial conformation may further explain the subtle differences in efficacy observed in enhancing the heat stability of egg yolks. The superior effects observed for sucrose, trehalose, and glucose may be attributed to their larger molecular sizes and higher hydroxyl group densities, which might enhance their ability to form protective hydration layers and stabilize the native conformation of the protein. In contrast, the pentose sugar arabinose, due to its smaller molecular structure, likely provides weaker coverage and interactions, thereby exhibiting a relatively lower stabilization effect.

Frequency scanning can reveal subtle changes in the internal interactions of molecules, reflecting the structural information of the sample. The G' , also known as the elastic modulus, represents the solid or elastic properties of the material, while the loss modulus (G''), also known as the viscous modulus, represents the liquid or viscous properties. As shown in Figure 1B, with increasing temperature, the values of G' and G'' in the egg yolk increased, indicating that heat treatment promoted additional interactions between egg yolk proteins. The reason may be that heat causes the expansion and aggregation of protein molecules in egg yolk, leading to the formation of a gel, resulting in increased G' and G'' in egg yolk. It is worth noting that within the frequency scanning range, the G' and G'' of the sugared EY were significantly lower than those of the FEY group, indicating that sugar substances affect the molecular conformation and intermolecular interactions of proteins

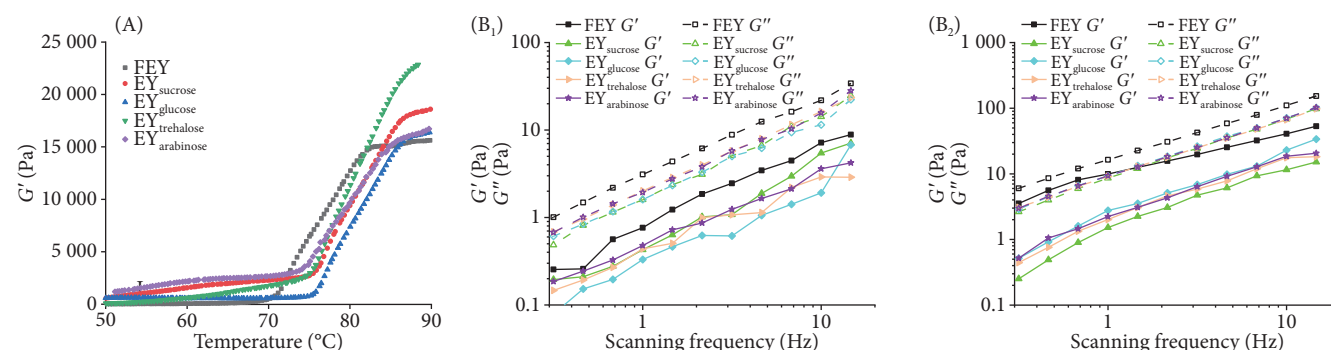


Figure 1 (A) Temperature sweep, (B) variable-frequency sweep, (C) apparent viscosity of egg yolk and sugared egg yolk at different temperatures. Subscript 1–4 represent 25, 68, 72, and 76 °C, respectively.

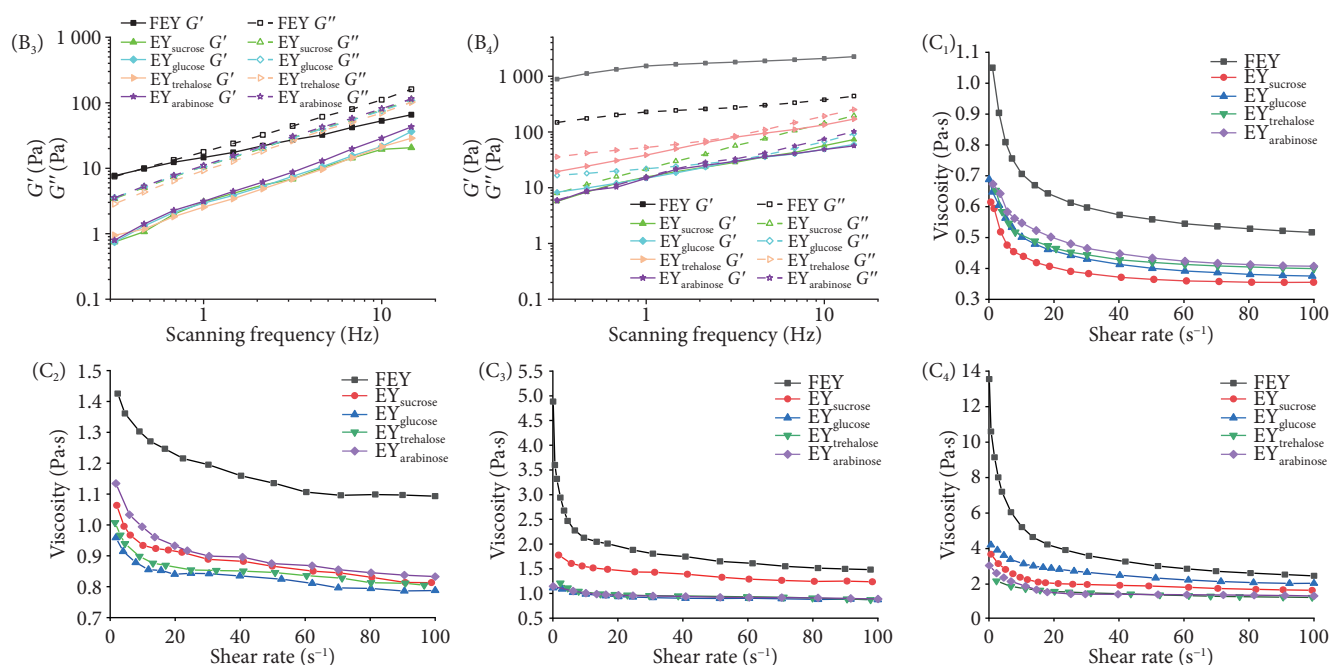


Figure 1 (Continued)

in egg yolks. During the heating process of liquid egg yolk, the infiltration of sugars can hinder the unfolding of protein molecules, helping to maintain the integrity of the egg yolk particle structure^[21]. On the other hand, the entry of sugars into proteins can delay the denaturation of proteins, thereby improve heat tolerance and slow protein recombination and aggregation. Sugars exhibit selective affinity for acidic and basic polar residues on lysozyme via hydrogen bonding. This approach competitively binds to the same residues involved in early protein-protein interactions, thereby reducing the tendency to aggregate.

3.1.2 Apparent viscosity and power-law model fitting

Rheological properties, which are related to protein structure and molecular properties, reflect the degree of deformation of materials under different shear stresses. These parameters are usually characterized by the relationship between the shear rate and apparent viscosity^[23]. Figure 1C shows the change in apparent viscosity of the liquid egg yolk samples with increasing shear rate during the heating process, liquid egg yolk exhibited shear thinning behavior with increasing shear rate, and the viscosity decreased at a low shear rate but remained relatively constant at a high shear rate, indicating that the egg yolk sample was a pseudoplastic fluid. Moreover, the apparent viscosity of the liquid egg yolk gradually increased as the temperature increased, possibly because of the participation of additional branch chains in aggregation. Zero-shear viscosity is a key rheological parameter that defines the bulk physical properties of food materials. This parameter is related to several sensory properties, including adhesion and cohesion^[23]. As the temperature increased, the zero-shear viscosity of FEY significantly increased, and the values increased to (1.426 ± 0.220) , (4.882 ± 0.100) and (13.547 ± 0.250) Pa·s at 68, 72, and 76 °C, respectively. In contrast, the increase in zero-shear viscosity of the sugar-added EY group at the same temperature was smaller than that of the control group. These results indicate that all sugars systematically reduce the apparent viscosity of the samples at various heating temperatures. Zero-shear viscosity data further

quantified this inhibitory effect: at 76 °C, the zero-shear viscosity of the blank control group (FEY) rose sharply to 13.547 Pa·s, while all sugar-added groups were significantly lower than this value, confirming that sugars can effectively mitigate the increase in intrinsic viscosity caused by high temperatures.

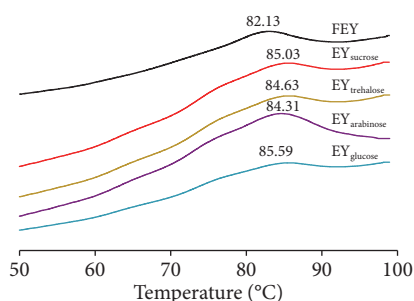
To further elucidate the effect of heat treatment on the apparent viscosity of liquid egg yolks, the power-law model was used to visually analyze the consistency index (K) and flow behavior index (n), as shown in Table 1. K is commonly used to indicate the degree of viscosity of a sample, while n represents the extent of structurization of the sample^[24]. The lower the K value is, the lower the viscosity of the sample, and the higher the n value is, the lower the degree of structurization. As shown in Table 1, the K of FEY (25 °C) increased by 9.06 times after heat treatment at 76 °C, which indicated that heating led to a significant increase in the viscosity of the yolk. Moreover, the n of all the liquid egg yolks were between 0 and 1, once again indicating that the egg yolks are typical non-Newtonian pseudoplastic fluids. As the temperature increased, the n of the sugared EY group was greater than that of the control group, indicating that the sugar substances can effectively reduce the degree of structurization of the heat-induced egg yolk. In general, heat can enhance the hydrophobic interactions of proteins, induce protein aggregation, and increase the attraction between protein molecules beyond electrostatic repulsion, resulting in increased viscosity. The viscosity of the liquid egg yolk in the sugared EY group was lower than that in the control group at the different heating temperatures, indicating that sugars could accelerate shear thinning behavior by increasing the polymer decomposition rate. In addition, the hydration of sugar can disrupt protein-water interactions, resulting in a decrease in the viscosity of liquid egg yolk, which is also a reason for its low viscosity after heat treatment. Taken together, sugars may compete for water through hydration, disrupt protein-water interactions, and potentially accelerate the dissociation of macromolecular aggregates under shear stress, thereby resulting in a macroscopic decrease in viscosity and an increase in pseudoplasticity.

Table 1 Power-law equation of sugared egg yolk at different temperatures.

Temperature (°C)	Sample	K (Pa·s ⁿ)	n	R^2
25	FEY	0.980 ± 0.010	0.887 ± 0.000	0.934
	EY _{sucrose}	0.592 ± 0.010	0.985 ± 0.000	0.976
	EY _{glucose}	0.640 ± 0.010	0.981 ± 0.010	0.940
	EY _{trehalose}	0.680 ± 0.010	0.979 ± 0.000	0.982
	EY _{arabinose}	0.690 ± 0.010	0.978 ± 0.000	0.949
68	FEY	1.483 ± 0.010	0.841 ± 0.000	0.941
	EY _{sucrose}	1.082 ± 0.010	0.848 ± 0.000	0.959
	EY _{glucose}	1.005 ± 0.030	0.863 ± 0.000	0.958
	EY _{trehalose}	0.943 ± 0.070	0.853 ± 0.010	0.963
	EY _{arabinose}	1.121 ± 0.040	0.842 ± 0.000	0.982
72	FEY	3.282 ± 0.020	0.810 ± 0.000	0.994
	EY _{sucrose}	1.893 ± 0.020	0.815 ± 0.000	0.955
	EY _{glucose}	1.100 ± 0.010	0.850 ± 0.000	0.918
	EY _{trehalose}	1.201 ± 0.020	0.835 ± 0.000	0.937
	EY _{arabinose}	1.141 ± 0.010	0.826 ± 0.000	0.920
76	FEY	8.876 ± 0.200	0.729 ± 0.010	0.939
	EY _{sucrose}	3.356 ± 0.060	0.732 ± 0.010	0.971
	EY _{glucose}	4.572 ± 0.100	0.731 ± 0.010	0.958
	EY _{trehalose}	2.496 ± 0.010	0.750 ± 0.000	0.997
	EY _{arabinose}	2.604 ± 0.080	0.747 ± 0.010	0.974

3.2 DSC analysis

DSC is often used to study the thermal transformation process of proteins and can provide information on the transition from the original state to the thermal denaturation state. As shown in Figure 2, an endothermic peak appeared between 80 and 90 °C, representing the thermal denaturation temperature, which may be associated with the formation of new molecular bonds between protein molecules^[25]. The glucose group exhibited the highest denaturation temperature (85.59 °C), followed by the EY_{sucrose} (85.03 °C), while the stabilizing effects of trehalose (84.63 °C) and arabinose (84.31 °C) were relatively lower. This suggests that while sugars generally resist thermal denaturation by stabilizing the natural conformation of proteins, their stabilizing efficacy varies depending on molecular structure (such as the number of hydroxyl groups and spatial configuration).

**Figure 2** DSC maps of egg yolk and sugared egg yolk during heating from 50 to 100 °C.

The enthalpies of denaturation for EY_{sucrose}, EY_{glucose}, EY_{trehalose} and EY_{arabinose} were 0.28, 0.40, 0.25 and 0.25 J/g, respectively. In addition to variations in denaturation temperatures, the enthalpies of denaturation (ΔH , i.e., the energy required for the denaturation process) also exhibited systematic differences. The ΔH for thermal

denaturation of all sugar-containing egg yolk samples were lower than those of the blank control group. This phenomenon suggests that, in the presence of sugars, the energy barrier that must be overcome and/or the intermolecular interactions involved in the transition of the protein system from its native state to a denatured state have changed, which may indicate alterations in the denaturation process or aggregation patterns. A study also analyzed the effect of sugar on protein thermal stability through DSC. Sugars (including glucose) increase the thermal transition temperature of serum proteins as measured by DSC^[26]. A lower ΔH indicates that less energy is required for the transition from the native state to the denatured state. This may be because the presence of sugar molecules alters the denaturation pathway or aggregate conformation of the protein, resulting in different rearrangements of intermolecular bonds (such as hydrogen bonds and hydrophobic interactions) involved in the denaturation process. The glucose group exhibits the relatively highest ΔH (0.40 J/g), which, combined with its highest denaturation temperature, suggests that its stabilization mechanism may differ slightly from that of other sugars.

3.3 Changes in the functional properties of liquid egg yolks by saccharides

3.3.1 Emulsifying properties analysis

Liquid egg yolk has great emulsifying properties, among which EAI reflects the ability of emulsifiers to form emulsions, while ESI reflects the ability of emulsifiers to maintain the stability of emulsion systems^[27]. As shown in Figure 3A, as the temperature of the heat treatment increased, the EAI of each processing group decreased. When heated to 76 °C, the EAI of the control group decreased from the initial 1.62 to 1.29 m²/g; the EAI of EY_{glucose} decreased by 0.38 m²/g; and those of EY_{sucrose}, EY_{trehalose} and EY_{arabinose} decreased by 0.46, 0.48 and 0.54 m²/g, respectively. This phenomenon indicates that several saccharides can inhibit the negative impact of heat treatment on the emulsifying properties of egg yolks, allowing them to maintain great emulsifying properties even after being treated at higher temperatures. The possible reason may be that saccharides can weaken the interaction between egg yolk proteins, interfere with the thermal aggregation behavior of egg yolk proteins, and cause relatively small aggregates to form during heat treatment, which is beneficial for maintaining the adsorption capacity of egg yolk proteins and slowing the decrease in emulsifying properties.

As shown in Figure 3B, the ESI of FEY and sugared FEY gradually increased with increasing temperature. Compared to that of the control group, the emulsification stability of the sugared EY after heat treatment was lower. Heat treatment of egg yolk can significantly increase the protein concentration at the interface protein membrane, and the protein concentration increases with the degree of egg yolk protein denaturation. Within a certain temperature range, an increase in the protein content of the interface protein membrane has a positive impact on emulsification stability^[28]. Due to the low degree of denaturation, the ESI of sugared EY was lower than that of the control group at the same temperature.

3.3.2 Soluble protein content analysis

To some extent, protein solubility can reflect the degree of protein aggregation. As shown in Figure 3C, as the temperature increased

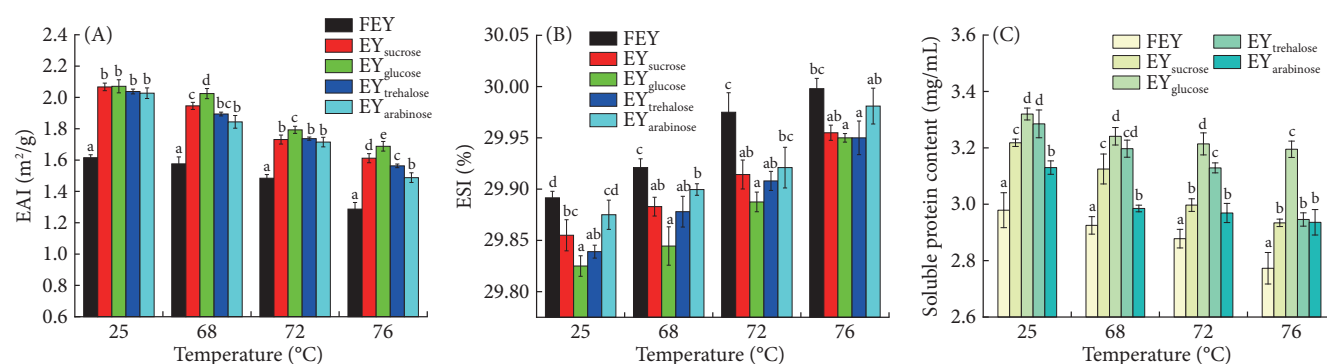


Figure 3 (A) EAI, (B) ESI, and (C) soluble protein content of egg yolk and sugared egg yolk at different temperatures. Different lowercase letters within the same heating temperature indicate statistically significant differences between treatment groups ($P < 0.05$).

to 76 °C, the soluble protein content of each group of liquid egg yolks continuously decreased. By comparing the soluble protein content of sugared EY at different temperatures, we found that under the same temperature conditions, the soluble protein content was significantly greater in the sugared EY than in the FEY ($P < 0.05$). The decrease in solubility of egg yolk is mainly due to the possibility of aggregation between denatured proteins through hydrophobic interactions, which can lead to the formation of insoluble polymers^[39]. The decrease in soluble protein content was lower mainly because saccharides can increase the thermal denaturation temperature of yolks and inhibit cross-linking and aggregation between egg yolk proteins. Another reason is that saccharides can easily increase the surface charge of proteins and enhance the interaction between proteins and water molecules. Moreover, sugar molecules contain a large amount of free hydroxyl groups, which can bind to protein molecules, thereby maintaining the relative stability of the spatial structure of egg yolk proteins through the stabilizing effect of sugar molecules.

3.4 Structural changes in liquid egg yolk as affected by saccharides

3.4.1 Zeta potential analysis

The zeta potential can measure the strength of mutual repulsion or attraction between particles in a solution. Usually, the higher the absolute value of the zeta potential is, the more stable the system is, which means that dissolution or dispersion can prevent aggregation. Conversely, the lower the absolute value of the zeta potential is, the more likely the material is to coagulate or agglomerate. As shown in Figure 4, the absolute zeta potential value of FEY was 7.10 mV, and the absolute values in each group slightly changed at 68 °C but decreased sharply above 76 °C. The absolute zeta potential value of FEY decreased to 5.39 mV at 76 °C, possibly because heat treatment caused aggregation between proteins or other substances, inducing adverse changes in protein conformation^[30]. Under the same heat treatment conditions, the absolute zeta potential value of the sugared EY was significantly greater than that of the control group, which can reflect the high electrostatic repulsion between protein particles, leading to a decrease in the frequency of contact and collision during heat treatment and an increase in the resistance of protein interactions^[31], which also explains the improvement effect of saccharides on the thermal stability of egg yolks.

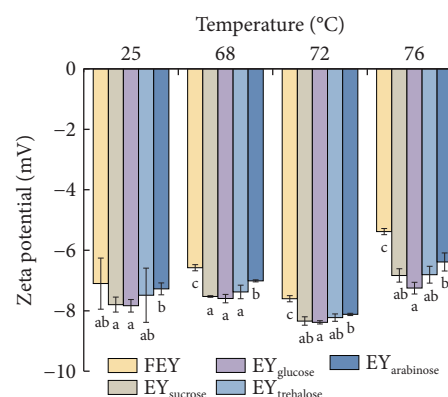


Figure 4 Changes in zeta potential of egg yolk and sugared egg yolk at different temperatures. Different lowercase letters within the same heating temperature indicate statistically significant differences between treatment groups ($P < 0.05$).

3.4.2 Surface hydrophobicity analysis

The surface hydrophobicity of proteins reflects the amount of hydrophobic amino acid residues exposed on the protein surface, which can indirectly indicate the degree of protein denaturation^[32]. In Figure 5, as the heating temperature increased, the surface hydrophobicity of FEY (72 and 76 °C) gradually increased, which may be due to the change in the spatial structure of the original protein molecules caused by heat treatment, which disrupted the hydrophobic interactions within the protein molecules and generated and exposed the hydrophobic groups originally embedded in the molecules, thus increasing the surface hydrophobicity of the protein. The surface hydrophobicity of the sugared EYs exhibited irregular changes with increasing temperature, but overall, the surface hydrophobicity was significantly lower than that of the control group ($P < 0.05$), indicating that these sugars inhibited the exposure of hydrophobic residues in protein molecules. This may be because saccharide molecules bind to egg yolk proteins through hydrophilic interactions, reducing the exposure of nonpolar amino acid side chain groups in the protein peptide chain, thereby preventing excessive aggregation of protein molecules caused by hydrophobic interactions and maintaining the stability and functionality of the protein structure^[33]. In addition, saccharides can also protect proteins from the influence of hydrophobic ANS, limit their ability to interact with proteins, and lead to a decrease in surface hydrophobicity.

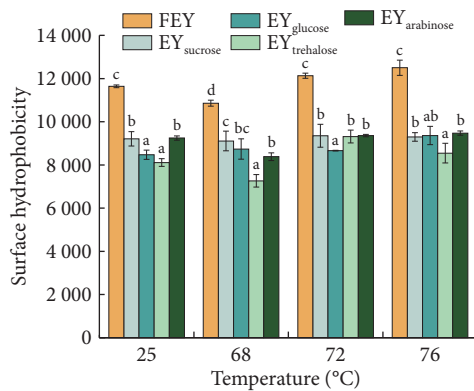


Figure 5 Changes in surface hydrophobicity of egg yolk and sugared egg yolk. Different lowercase letters within the same heating temperature indicate statistically significant differences between treatment groups ($P < 0.05$).

3.4.3 FTIR spectral analysis

The amide I band ($1\,700\text{--}1\,600\text{ cm}^{-1}$) is an important region in the infrared spectrum for determining the secondary spatial composition of proteins (α -helix, β -sheet, β -turn, random coil, etc.). The second derivative method is used to solve the overlapping secondary structure elements, which can easily reveal changes in the secondary structure. As shown in Figure 6A, at different heat treatment temperatures, the control group exhibited absorption peaks at $1\,629$, $1\,654$, $1\,677$ and $1\,693\text{ cm}^{-1}$, which is consistent with the findings of Blume et al.^[34]. Generally, $1\,626\text{--}1\,639\text{ cm}^{-1}$ corresponds to intramolecular β -sheet, α -helix between $1\,650\text{--}1\,660\text{ cm}^{-1}$, and β -turn between $1\,661\text{--}1\,690\text{ cm}^{-1}$ ^[35]. Under the same temperature conditions (25 and $76\text{ }^{\circ}\text{C}$), the α -helix relative content of sugared egg yolk was significantly greater than that of the

control group, while the β -sheet relative content was significantly lower than that of the control group, indicating that sugar can inhibit the transformation of α -helix (Figure 6B). Studies have shown that heating can alter the secondary structure of egg white protein, leading to a decrease in α -helix and an increase in β -sheet. A higher relative content of β -sheet indicates increased protein aggregation. The addition of various types of sugars can prevent the reduction in α -helix and the increase in β -sheet. Biter et al.^[36] reported that secondary structural strength is closely related to the structure of the gel network, and the transformation of α -helix to β -sheet is one of the reasons for the promotion of gel structure formation. This also explains why several sugars delay the formation temperature of the gel and alleviate changes in the fluidity and other characteristics of the liquid egg yolk.

3.4.4 UV-Vis spectroscopy analyses

UV-Vis spectroscopy is a simple and practical method for exploring the tertiary conformational changes of proteins and is based on the contributions of protein side chains containing phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) to different UV absorption spectra due to their different chromophore groups^[37]. The absorption peak at 280 nm is attributed to the π - π transition of the conjugated double bonds in the Trp and Tyr residues. Figure 7 shows that all the samples have peaks at approximately 280 nm , indicating that the UV band of egg yolk was mainly attributed to Trp and Tyr. Specifically, as the temperature increases, the intensity of the UV absorption peak near 280 nm in FEY gradually increases, indicating that temperature changes cause changes in the molecular conformation of egg yolk protein. An increase in peak intensity indicates that heat treatment induces partial unfolding of the protein structure^[37] and that chromophores are transferred to the protein surface, thereby increasing UV absorption and leading to an

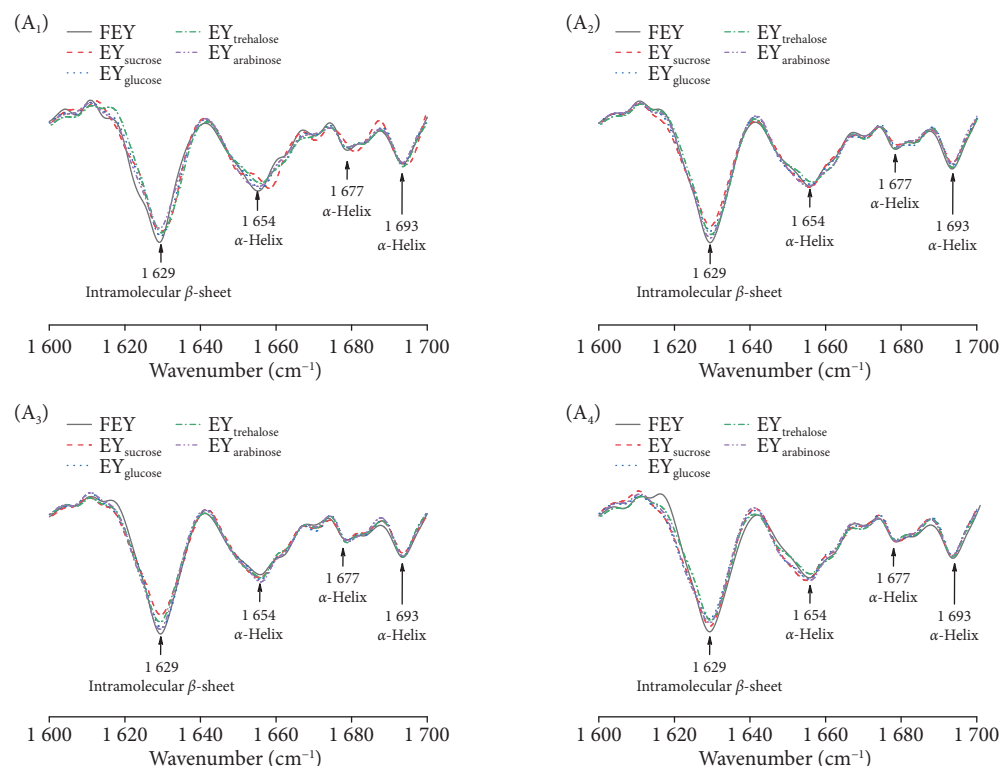


Figure 6 Changes in (A) FTIR spectrum and (B) secondary structure relative content of egg yolk and sugared egg yolk at different temperatures. Subscript 1–4 represent 25, 68, 72, and $76\text{ }^{\circ}\text{C}$, respectively.

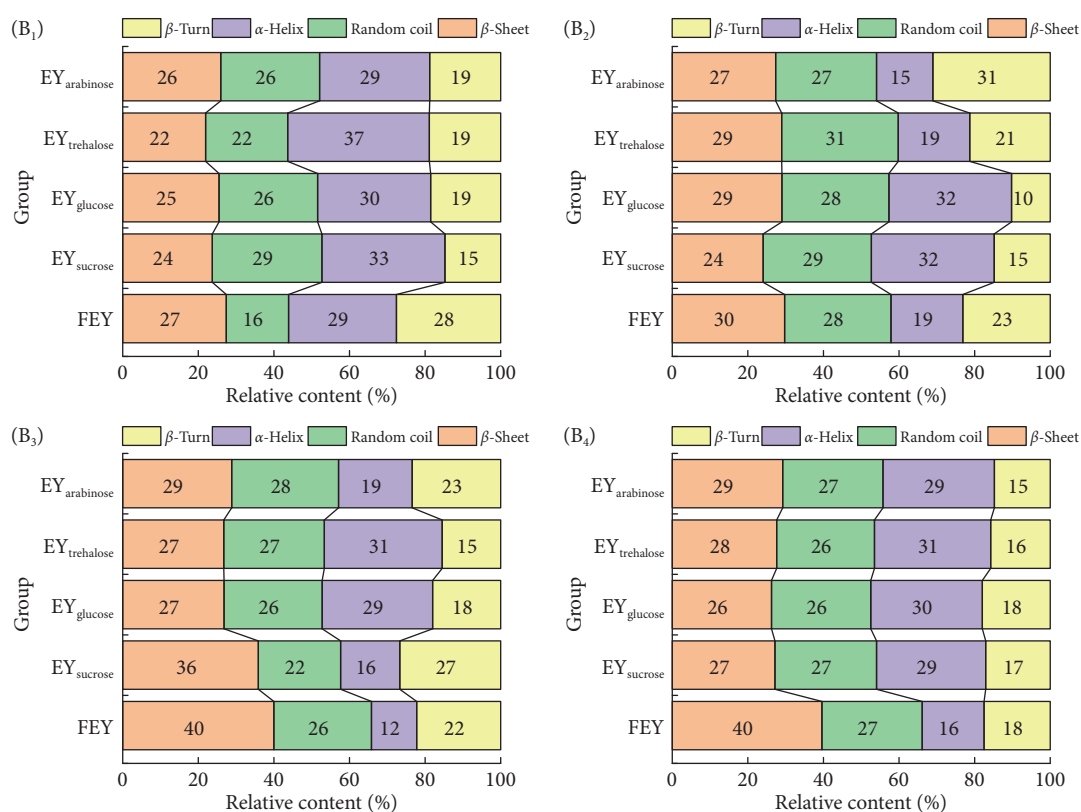


Figure 6 (Continued)

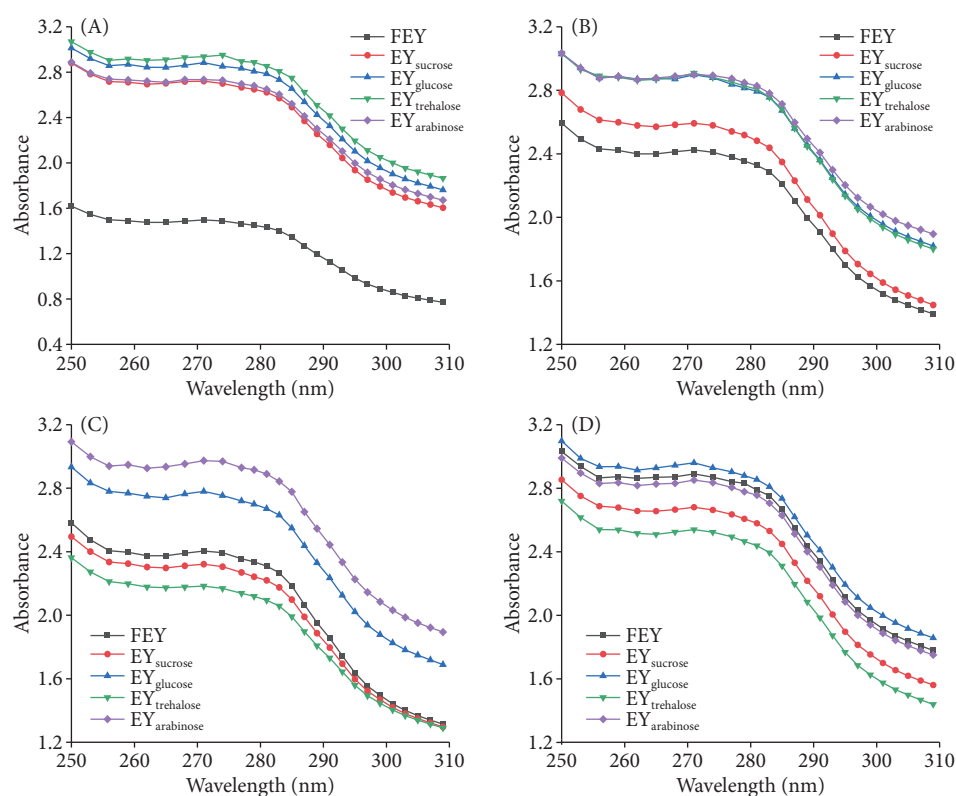


Figure 7 Changes in the UV-Vis spectrum of egg yolk and sugared egg yolk at different temperatures. (A–D) 25, 68, 72, and 76 °C, respectively.

increase in the absorbance. As sugar was gradually added, the absorption peaks of proteins in all egg yolk samples shifted toward smaller wavelengths, and during the initial heating period

(25–68 °C), the intensity of the absorption peaks in the sugared EY group was greater than that in the FEY group. The increase in absorption peak intensities indicates that, compared to those of

FEY, the presence of sugar substances is more likely to cause the protein molecular structure to tend to unfold, resulting in the buried exposed chromophores within the molecules on the surface of the protein molecules^[38]. Additionally, due to the enhanced colorimetric effect of the protein solution at ultraviolet wavelengths, the absorption peak intensity increases. This further confirmed that there may be differences in the aggregation mode of proteins between the sugared EY group and the FEY group. As the temperature increased during the later stage of heating (72–76 °C), the absorption peak intensity of some of the sugared EY groups decreased compared to that of the FEY group. This difference may be due to the change in protein energy level after mixing with sugar, which causes changes in the UV-Vis spectrum of the protein. This difference may also be caused by protein aggregation. When proteins aggregate, some chromogenic groups are hidden inside the protein molecules and cannot play a chromogenic role, resulting in a decrease in intensity.

3.4.5 Water distribution analysis

LF-NMR is a highly efficient and nondestructive analytical method that can be applied to analyze the moisture distribution and degree of combination with other components. As shown in Figure 8A, the T_2 spectrum has three peaks: T_{21} (approximately 1 ms) represents bound water, T_{22} (approximately 10–100 ms) represents immobilized water, and T_{23} (approximately 100–1 000 ms) represents unbound water. The shorter the relaxation time T_{21} is, the stronger the water binding capacity of the sample^[39]. It can also be seen that the T_{22} and T_{23} of all the samples are continuous, which may be due to the complex high viscosity system of liquid egg yolk, where macromolecules crosslink to form a dense network, hindering the migration of lipids and water. Among them, the T_{21} peak of FEY treated at 76 °C shifted to the left to the greatest extent. These phenomena are related to the interaction force between

proteins and water caused by heat treatment. Heat treatment increases the interaction between lipids, proteins, and water, which reduces the movement of water molecules and the fluidity of egg yolks and promotes an increase in the consistency coefficient of liquid egg yolks. The LF-NMR curves of the sugared EYs were on the right of the egg yolk, which indicated that the presence of saccharides can inhibit the structural unfolding of proteins, slow the interaction between proteins and water, and slow the heat-induced conversion of unbound and immobilized water to bound water in the system, thereby reducing the degree of thermal aggregation.

As shown in Figure 8B, after heating at 76 °C, the proportion of bound water in the FEY was significantly higher than that in EY_{sucrose}, EY_{glucose}, EY_{trehalose}, and EY_{arabinose} ($P < 0.05$), indicating that the addition of these sugars effectively hindered the conversion of bound water. This may be because the abundant hydroxyl groups in these sugars can bind with water molecules to form a stable hydration layer^[40], which reduces direct contact between protein molecules and impedes the process of heat-induced aggregation and gel network formation in egg yolk. Macroscopically, this manifests as an inhibition of the increase in viscosity and K -value after heating, as well as the suppression of reduced protein solubility caused by the formation and aggregation of denatured proteins.

3.4.6 Particle size and scanning electron microscopy analysis

To elucidate the effects of heat treatment temperature and sugar content on the heat resistance of liquid egg yolks according to the degree of aggregation, the particle size distribution of liquid egg yolks treated under different conditions was measured. As shown in Figure 9A, with increasing temperature, the proportion of particles in the range of 0.1–100 μm in the liquid egg yolk gradually decreased, while the proportion of particles in the 100–1 000 μm range increased, indicating an increasing trend in the particle size of

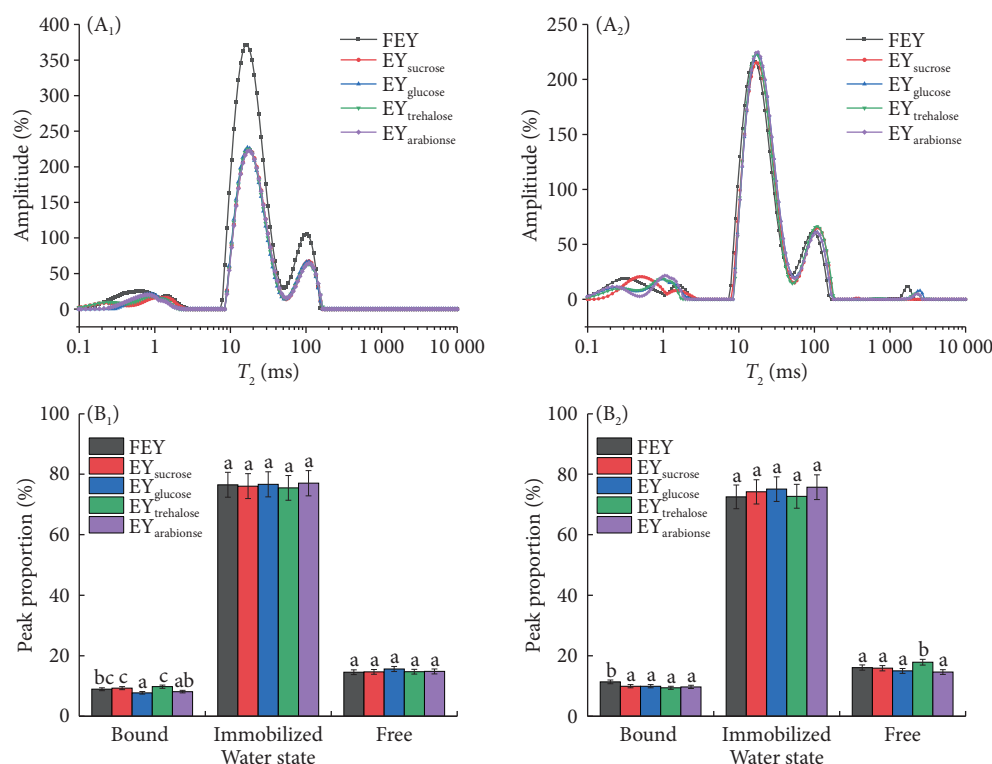


Figure 8 Changes in the (A) LF-NMR curves and (B) peak proportions of egg yolk and sugared egg yolk at different temperatures. Subscript 1–2 represent 25 and 76 °C, respectively. Different lowercase letters indicate significant differences between groups ($P < 0.05$).

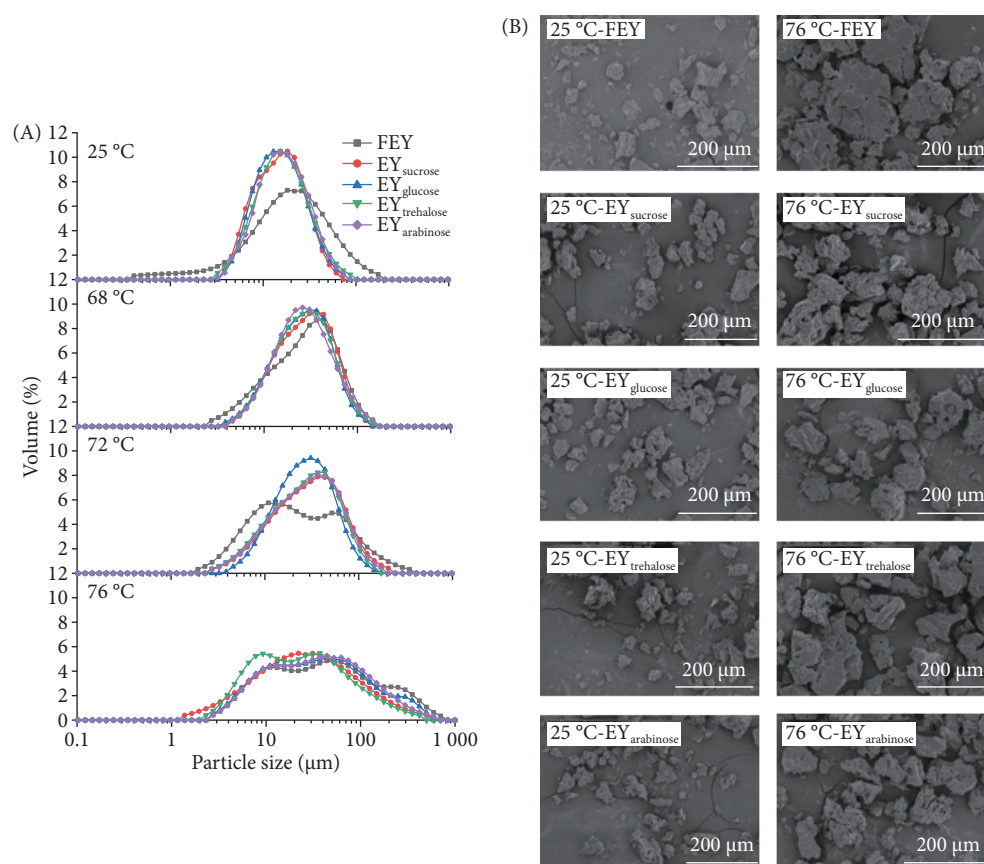


Figure 9 Changes in (A) particle size distribution and (B) the microstructure of egg yolk and sugared egg yolk at different temperature.

the liquid egg yolk. The increase in the particle size of the liquid egg yolk further indicated aggregation of egg yolk proteins. Compared with those in the control group, the egg yolk samples treated with various sugars were distributed on the left side of the control group, ranging from 0.1 to 700 μm ; however, overall, they were still smaller than the FEY. The above results indicate that sugar has an inhibitory effect on egg yolk protein aggregation and the increase in particle size caused by the external environment.

The microstructure of proteins reflects the aggregation state of protein molecules. Figure 9B shows that the egg yolk samples not subjected to heat treatment had uniformly distributed and smaller block-shaped particles. When the temperature increased to 76 °C, larger particles were observed in the microstructure of the egg yolk, which was consistent with the particle size distribution results shown in Figure 9A. The increase in particle size is related to the unfolding and subsequent aggregation of proteins caused by heat treatment^[41]. Under the same heat treatment, compared with those in the control group, the egg yolk samples treated with sugar exhibited a relatively low degree of aggregation at the macroscale. The possible reason for this difference may be that sugars can effectively improve the deterioration of the egg yolk protein structure, thereby maintaining the various forces required for its spatial structure. Wang et al.^[42] also through scanning electron microscopy, reported that sugar inhibits aggregation by enhancing the stability of proteins.

4 Conclusion

The addition of saccharides (sucrose, glucose, trehalose, and arabinose) has a significant impact on the thermal stability of liquid

egg yolks. Saccharides can effectively inhibit the degree of thermal denaturation of egg yolk protein. It was also found that the saccharides alleviated the decreases in soluble protein content and emulsifying activity caused by heat treatment. In addition, saccharides alleviated the changes in the surface hydrophobicity, β -sheet structure relative content, and growth amplitude of the aggregates, and the transformation from unbound and immobilized water to bound water was delayed. Overall, this study provides insight into the mechanism by which saccharines affects the rheological properties, function, and structure of heat-treated liquid egg yolks and provides theoretical guidance for the further development of heat-resistant specialized liquid egg yolks.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Jingnan Zang: Software. Mingmin Qing: Investigation. Qian Yu: Writing-original draft, software. Yujie Chi: Writing-review & editing, funding acquisition, supervision.

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