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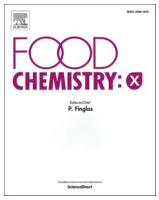

Study on the Mechanism of Modified Cellulose Improve the Properties of Egg Yolk gel

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ABSTRACT

Natural fiber is not suitable for modifying yolk gel as a modifier because of its large size and high compactness. In this study, two kinds of modified cellulose were selected to improve the thermal gel properties of yolk. The results showed that the two kinds of cellulose promoted the formation of ordered structure in yolk gel. The ordered gel network not only improved the texture properties and rheological properties, but also improved the water retention of yolk gel significantly. CMC and CNFC at the same concentration, the modification effect of CMC on yolk gel was better than CNFC because of its excellent dispersion. However, high concentration of CNFC (1.20–1.60%) disrupted the cross-linking and ordered structure formation of yolk protein, and the quality of gel was significantly reduced.

1. Introduction

Abundant and various protein in yolk provide a prerequisite for the formation of its gel. Essentially, the formation of yolk gel is a process in which denatured protein molecules gather together and form an orderly protein network structure (Miao, Peng, Wang, Cao, & Li, 2021). The formation of yolk gel can be induced by physical, chemical and biological methods. The effect of modifiers on yolk gel mainly focuses on the spatial structure and intermolecular forces of protein (Totosaus, Montejano & Salazar, 2002). On the one hand, these modification methods can alter the primary or spatial structure of yolk protein, and the spatial structures can also undergo interconversion, which has an important effect on the unfolding and aggregation of yolk protein molecules. On the other hand, the unfolding of protein structure is conducive to the exposure of polar groups or action sites, and promotes the interaction between protein molecules. In this process, the main forces (including disulfide bond, hydrophobic interaction, ionic bond and hydrogen bond) that form and maintain yolk gel will change accordingly (Felix, Romero, Rustad & Guerrero, 2017). During induction process, yolk solution gradually gelation by forming three-dimensional network structures, which have unique texture characteristics (Yang, Zhao, Xu, Yao, & Wu, 2020). At the same time, these network structure provide enough space for water, lipids, flavor substances and other food ingredients, which make yolk gel widely used in dried eggs, salted eggs, preserved egg, etc (Yang, Zhao, Xu, Wu, & Yao, 2019).

Macromolecular biomaterials such as polysaccharides, especially some insoluble plant fibers, have the advantages of low cost, good availability, and low concentration effectiveness (Choi, Choi, Han, Kim, & Lee, 2011). New gel structures can be formed by physical entanglement or electrostatic interaction, which can be used to improve the gel properties of protein. Generally, natural plant fiber is insoluble in water and its particles are too large. Adding it directly to protein food will increase the hardness of food gel and cause unpleasant roughness (Eim, Simal, Rossello & Femenia, 2008). Natural fiber can be modified by breaking the hydrogen bond or replacing some groups, and converted into water-soluble fiber, which can expand its application range. At present, common modified cellulose such as nanocellulose, carboxymethyl cellulose (CMC), methyl cellulose, ethyl cellulose, and cellulose

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acetate have all achieved industrial production, CMC and nano cellulose have been widely used to improve the gel properties of meat products (Zhou, Zhang & Wang, 2022). Research has shown that the addition of CMC can improve the water retention ability of meat products (Gibis & Weiss, 2017); Nanocellulose has the characteristics of high purity, large specific surface area, high crystallinity, high hydrophilicity, high elastic modulus, low coefficient of thermal expansion, etc. It can be used as a food additive to improve product quality (Siro & Plackett, 2010). For example, the addition of carboxylated cellulose nanofibers (CNFC) significantly improved the water retention and texture of chicken protein gel (Zhang, Wang, Wang, Wang, & Wang, 2018). However, the effect of modified cellulose on yolk gel properties has not been reported. According to the molecular characteristics of CMC and CNFC, they both contain hydroxyl group, which can form intermolecular hydrogen bond with yolk protein, and enhance the strength of yolk gel. The molecular difference between CMC and CNFC is reflected in size, CNFC is a nano-sized modified fiber with a diameter of 50 nm and a length of 1–3 μm, the size of CNFC is smaller than CMC, which can compare the modification effect of different size fibers on yolk gel.

In order to compare the effects of different sizes of cellulose with similar molecular structure on the gel properties of yolk, and to clarify the effects of different amounts of modified cellulose on the gel properties of yolk, CMC and CNFC were selected as two kinds of modified cellulose, and the effects of different addition levels (0–1.60%) on yolk gel properties were discussed; The texture, moisture distribution, rheological properties and color of the composite gel were systematically investigated, and the effect of modified cellulose on the macro properties of yolk gel was clarified; the changes of yolk gel structure were analyzed by SDS-PAGE, infrared spectrum, endogenous fluorescence spectrum and laser confocal analysis, and the internal mechanism of modified cellulose enhanced yolk gel was revealed. It is of great significance to extend the application field of modified cellulose, improve the gel properties of yolk protein and enhance its nutritional value. This paper is of great significance in broadening the application field of modified cellulose, improving the gel properties of yolk protein and enhancing its nutritional value.

2. Materials and methods

2.1. Materials

Eggs purchased from Fujian Guangyang Egg Industry Co., Ltd.. Carboxymethyl cellulose (CMC, M.W.2500, DS = 0.91500–3100 mpa.s), carboxymethyl cellulose nanofibers (CNFC, diameter = 50 nm, length = 1–3 μm), potassium bromide (chromatographic grade), Nile Blue A (AR, dye content ≥75%) were purchased from Macklin Biochemical Technology Co., Ltd. All other chemical reagents were analytical pure and purchased from Shanghai Guoyao Group Chemical Reagent Co., Ltd.

2.2. Methods

2.2.1. Sample preparation

A certain amount of CNFC and CMC powder were respectively dissolved in ultrapure water, stirred evenly by magnetic force at room temperature, and then hydrated overnight at 4℃, hydrogel with different concentrations (1.20%, 2.40%, 3.60%, 4.80% w/w) were prepared. Fresh egg was broken and yolk was separated manually, the tie and excess egg white were removed with filter paper, the yolk membrane was broken and stirred evenly by hand to obtain fresh yolk liquid. The fresh yolk solution was mixed with CNFC and CMC hydrogel of different concentrations at 2:1 (w/w) at room temperature, the mixture was stirred continuously for 1 h. The final concentration of modified cellulose was 0.40%, 0.80%, 1.20%, 1.60%, respectively. The composite solution obtained was used for the determination of rheological properties, gel electrophoresis, and fluorescence spectrum.

The mixed composite solutions were transferred to cylindrical weighting bottle (60 × 30 mm), heated in 90 °C water bath for 30 min, refrigerated at 4 °C overnight. The prepared gel was used for the determination of other indexes.

2.2.2. Texture characteristics

Texture analyzer (Stable Micro System, TA.TX-plus, UK) was used to detect the hardness of sample. Cylindrical probe of P/36R was used. The speed before and after the test was 2 mm/s, the test speed was 1 mm/s, the compression deformation was 45%, and the actuating force was 5 g. Each group of experiments was repeated three times (Wang, Huang, Zhou, Xu, & Xiang, 2021).

2.2.3. Water holding capacity

The water holding capacity of yolk gel was determined by high-speed centrifugation (Wu, Xiang, Liu, An, & Geng, 2022). At room temperature, yolk gel was cut into thin pieces of the same thickness, and its mass was accurately weighed and recorded as M_1. Then, sample was centrifuged at 6000 r/min for 10 min at 4 °C, weighed and recorded the mass as M_2. Each sample was measured three times in parallel, and the results were averaged. Water holding capacity was calculate according to formula (2).

$$\text{Water holding capacity}(\%)=\frac{M1}{M2}\times 100\backslash\% \quad (2)$$

2.2.4. Low field nuclear magnetic resonance (LF-NMR) analysis

The determination of low field nuclear magnetic resonance (MesoMR23-060V-I, Suzhou Niumai, China) was based on the method of Sun et al. (Sun, Wang, Jin, Li & Sheng, 2021) with appropriate modifications. The gel samples were balanced at room temperature. 2 g gel was weighed and placed in a 15 mm nuclear magnetic tube. The relaxation time T_2 was determined by CPMF pulse sequence. The test conditions were as follows: temperature was 32℃, sampling frequency was 200 kHz, resonance frequency was 21 MHz, sampling interval was 150 μs, number of echoes was 18,000, repeated scanning was 4 times, repeated sampling wait time was 5500 ms, RF delay was 2 μs, analog gain was 10.0 dB, digital gain was 3 dB. Each group of samples should be repeated at least 3 times and the data recorded.

2.2.5. Rheological properties

The Rheometer (MCR301, Anton Paar, Austria) was used to test the rheology properties of the sample. PP50 (diameter 50 mm) was selected as probe, and 1 mm was gap between the probe and the instrument. The static rheological testing conditions were as follows: shear rate 0.1–100 s^{-1}, temperature 25℃. The obtained curve of apparent viscosity with shear rate was fitted by power law equation:

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

In the equation: η was the apparent viscosity (pa.s), K was the viscosity coefficient (pa.s^n), γ was the shear rate (s^{-1}), and n was the flow index.

The sample was subjected to amplitude scanning at 25℃, strain force was 1.0%, and angular frequency was 0.1–100 rad/s, storage modulus (G') and loss modulus (G'') were obtained. The sample was subjected to temperature scanning at a frequency of 1 Hz and a strain of 1%. The sample was gradually heated from 20℃ to 90℃ at a heating rate of 2℃/min, and the storage modulus (G') and loss modulus (G'') were recorded (Huang, Tu, Song, Dong, & Geng, 2022).

2.2.6. Color

The brightness (L*), red/green value (a*) and yellow/blue value (b*) of yolk gel were measured with an automatic colorimeter (Wang, Huang, Zhou, Xu, & Xiang, 2021).

2.2.7. SDS-PAGE analysis

The effect of modified cellulose on yolk protein composition was analyzed by SDS-PAGE gel electrophoresis. The composite solution was

diluted 70 times with deionized water, then the diluted sample solution (60 uL) was mixed with the sample buffer (20 μL), heated in boiling water bath for 10 min, cooled to room temperature, centrifuged for 10 min at 4℃ and 10,000 rpm, 5 uL of supernatant was injected into the gel hole. The concentration gel and separation gel are 5% and 12%, respectively, with a constant voltage of 100 V. After electrophoresis, the electrophoresis strip was first decolorized with distilled water for 30 min, then dyed with Coomassie Brilliant Blue G-250 solution for 12 h, and finally decolorized with distilled water until the background was clear. The electrophoresis image was scanned and saved, and the electrophoresis strip was analyzed by protein standard (Huang, Liu, Wu, Huang, & Wang, 2022).

2.2.8. Fourier transform infrared spectroscopy (FTIR) analysis

The gel sample was freeze-dried, and the freeze-dried sample and potassium bromide were grinded and tableted at a ratio of 1:100 for standby. Infrared spectral scanning (VERTEX70, Brooker, Germany) was performed in the frequency range of 4000–400 cm^{-1} (Wu, Xiang, Liu, An, & Geng, 2022).

2.2.9. Fluorescence spectrum

The Fluorescence spectrum of yolk protein was measured using RF-5301 fluorescence spectrophotometer. The test conditions were as follows: excitation wavelength was 295 nm, slit width was 5 nm, and emission wavelength range was 250–350 nm.

2.2.10. Confocal laser scanning microscope (CLSM) analysis

Yolk gel was cut into 5 mm × 5 mm × 1 mm thin slice, yolk protein was stained with 0.2 g/100 g Nile Blue A fluorescent dye. The sample was imaged on Confocal Laser Scanning Microscope (TCS SP8X DLS, Leica, Germany).

2.3. Data analysis

Each group of experiments should be repeated in parallel at least 3 times, with data expressed as mean ± standard deviation. Duncan analysis, variance analysis, and significance analysis were applied through SPSS 25.0, with $P < 0.05$ as the significant level. Origin 2018 was used to plot the chart.

Results and discussion

3.1. Appearance and texture properties

As shown in Fig. 1(A), the yolk gel in the control group (0%) was uneven, porous, and obviously had poor water holding capacity. With the addition of CNFC or CMC, the gel surface gradually became smooth and dense, indicating that the addition of CNFC or CMC had an improvement effect on the quality of yolk gel. However, there were many small holes on the surface of yolk gelwhen the addition of CNFC was 1.6%, it looks loose in appearance, and the quality of gel became worse. Therefore, the highest concentration of modified cellulose was set as 1.6%.

TPA can directly reflect the quality of gel by simulating the masticatory mechanical process of two extrusion deformation cycles (Yu, Li, Xue, Wang, & Song, 2020). The influence of CNFC or CMC on the texture characteristics of yolk gel is shown in Table 1A. The hardness of CNFC samples were increased first and then decreased, it reaching the maximum value at the additive amount of 1.2%. CNFC of appropriate concentration (≤1.2%) promoted the aggregation and cross-linking of yolk protein molecules, which was conducive to the formation of a stable gel network structure. In addition, CNFC could be filled in the gaps of gel network through water absorption expansion, resulting in an increase in its hardness (Pereira, Sathuvan, Lorenzo, Boateng, & Brohi, 2021). However, the excessive CNFC (1.6%) promoted the excessive aggregation of yolk protein, and the formation of larger aggregates tended to hinder the cross-linking of yolk protein, resulting in loose structure and lower hardness (Yin & Park, 2014). With the increase of CMC concentration, the hardness of yolk gel decreased significantly ($P < 0.05$), but it was still higher than that of the control group, and tended to be relatively stable when the concentration was 1.2%. The reason for this phenomenon might be that CMC was conducive to the expansion of yolk protein structure, and the interaction between protein and water was enhanced, which weakened the interaction between protein and protein to a certain extent, resulting in the decrease of gel hardness (Luo, Wang, Wu, Duan, & Zhang, 2021). At the same concentration, the hardness of CNFC sample was significantly higher than that of CMC sample, which was closely related to the two cellulose structures. The particle size of CNFC-yolk gel was smaller, and it was easier to aggregate with yolk

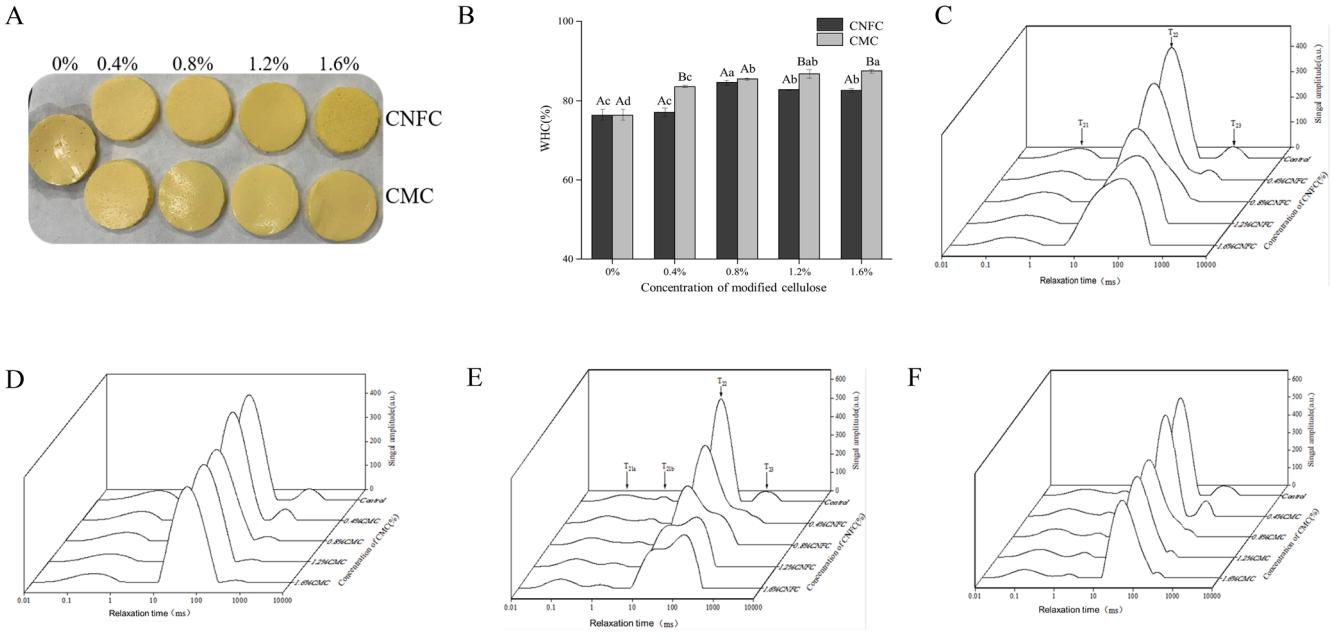

Fig. 1. (A) Appearance of CNFC or CMC modified yolk gel; (B) Water holding capacity of CNFC or CMC modified yolk gel; (C-F) Low field nuclear magnetic resonance (LF-NMR) analysis of CNFC (C, E) or CMC (D, F) modified yolk gel, (the iterations of C, D is 10,000, the iterations of E, F is 100,000).

Table 1A
Textual properties of CNFC or CMC modified yolk gel.

Samples	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness (g)
Control	248.62 ± 14.56^{eD}	0.95 ± 0.00^{abC}	0.70 ± 0.01^{bB}	172.85 ± 10.55^{eD}	163.99 ± 10.42^{eD}
0.4 % CNFC	398.44 ± 19.01^{d}	0.95 ± 0.01^{ab}	0.66 ± 0.01^{c}	264.10 ± 18.34^{d}	251.28 ± 19.51^{d}
0.8 % CNFC	691.40 ± 38.66^{b}	0.96 ± 0.00^{a}	0.71 ± 0.01^{b}	487.62 ± 23.14^{b}	466.72 ± 22.52^{b}
1.2 % CNFC	742.32 ± 10.51^{a}	0.94 ± 0.00^{b}	0.73 ± 0.00^{a}	539.26 ± 7.35^{a}	509.25 ± 5.16^{a}
1.6 % CNFC	574.02 ± 20.14^{c}	0.92 ± 0.01^{c}	0.73 ± 0.01^{a}	417.05 ± 8.42^{c}	383.56 ± 7.78^{c}
0.4 % CMC	455.08 ± 8.31^{A}	0.95 ± 0.01^{BC}	0.70 ± 0.01^{AB}	319.47 ± 3.69^{A}	304.15 ± 6.19^{A}
0.8 % CMC	360.32 ± 9.09^{B}	0.96 ± 0.00^{AB}	0.71 ± 0.02^{AB}	257.51 ± 5.24^{B}	247.39 ± 5.42^{B}
1.2 % CMC	312.83 ± 15.24^{C}	0.96 ± 0.00^{ABC}	0.72 ± 0.01^{A}	224.66 ± 11.26^{C}	215.33 ± 11.19^{C}
1.6 % CMC	310.58 ± 7.44^{C}	0.97 ± 0.01^{A}	0.72 ± 0.01^{A}	223.00 ± 7.14^{C}	213.19 ± 2.92^{C}

Note: different lowercase letters indicate significant differences between CNFC samples; different uppercase letters indicate significant differences between CMC samples ($p < 0.05$).

protein.

Springiness refers to the ability of gel to recover to its original shape and size after being compressed (Wu, Xiang, Liu, An, & Geng, 2022). With the increase of CNFC concentration, the elasticity of yolk gel first increased and then decreased, reaching the maximum value of 0.96 when the concentration was 0.8%; The elasticity of CMC-yolk gel increased in a concentration dependent way, it was significantly higher than that of CNFC-yolk gel, indicating that the addition of CMC or CNFC with appropriate concentration could effectively improve the flexible structure of yolk gel. Cohesiveness represents the compression resistance of gel, it is an important indicator to evaluate the integrity of gel network. With the increase of CNFC or CMC, the cohesiveness of yolk gel showed an overall upward trend and remained stable at the concentration of 1.2%. These two kinds of cellulose were beneficial to the construction of yolk gel network. Gumminess is an indirect index related to hardness and cohesiveness (Gumminess = hardness × cohesiveness); chewiness is a comprehensive reflection of stickiness and elasticity (chewiness = hardness × cohesiveness × springiness) (Li, Tu, Sha, Li, & Li, 2022). The trend of these two indicators was similar, science the hardness had a large order of magnitude (Liu, Wu, Duan, Song, & Geng, 2023). In general, CNFC with proper concentration (≤1.2%) improved the texture of yolk gel, and the addition of CMC was conducive to the formation of yolk gel.

3.2. Water holding capacity

Water retention is one of the important indicators to measure the quality of gel, which to some extent reflects the binding condition between protein and water molecules (Rawdkuen & Benjakul, 2008). As shown in Fig. 1(B), with the increase of CNFC, the water holding capacity of CNFC-yolk gel first increased and then decreased, reaching the maximum value. The increase of the water holding capacity was attributed to the highly carboxylated CNFC and nano size. Fiber carried anions, intermolecular aggregation was reduced, interaction between fibers and water molecules was inhanced through surface carboxylation treatment; CNFC was easy to bind to water molecules because its nanoscale size, it was more conducive to the exposure of hydrophilic groups. If the concentration of CNFC was greater than 0.8%, the steric hindrance effect and water absorption of CNFC-yolk gel woull be enhanced. In the process of gel formation, CNFC and yolk protein would absorb water competitively, resulting in a decrease in the water retention of gel.

The water holding capacity of the composite gel increased significantly with the increase of CMC concentration ($P < 0.05$). In addition, CMC, as a food colloid, contained rich hydrophilic groups and interacted with water molecules through hydrogen bonds, resulting in poor mobility of free water, thus improving the water retention of gel (Kasiri & Fathi, 2018). At the same concentration, the water holding capacity of CMC-yolk gel was higher than that of CNFC-yolk gel, which was attributed to the fact that CMC was superior to CNFC on the ability of absorb water.

Combined with the texture characteristics and water holding capacity, it could be inferred that adding CNFC (≤0.8%) of appropriate concentration could significantly improve yolk gel performance. When the concentration of CNFC was 1.2%, the hardness of yolk gel reached its maximum value, but the water holding capacity reached minimum value, indicating that the addition of 1.2% CNFC only enhanced the rigid structure of gel, but did not improve the density of gel network. However, when the concentration of CNFC reached 1.6%, the formation of larger aggregates destroyed the integrity of gel matrix, and the texture and water holding capacity of gel became worse. For the addition of CMC, with the increase of its concentration, the hardness of yolk gel decreased, but the elasticity, cohesion and water retention of gel significantly increased, indicating that the addition of CMC improved the flexible structure of yolk gel, which was conducive to the formation of a more compact gel network structure.

3.3. LF-NMR analysis

LF-NMR was used to analyze the water distribution in yolk gel. The sample relaxation time T_2 and its corresponding peak area ratio are shown in Fig. 1 (C-F) and Table 1B. The T_2 relaxation time diagram of pure yolk gel mainly included three peaks. T_{21} represented bound water at about 0.25 ms, T_{22} (10–200 ms) represented immobile water, and T_{23} peak greater than 700 ms represented free water (Sun, Wang, Jin, Li & Sheng, 2021). The distribution area of T_{22} accounted for 85.73%, indicating that the immobile water was the main water in yolk gel. T_{21} and P_{21} of gel did not change significantly due to the addition of CNFC ($P > 0.05$), indicating that the bound water was not affected by CNFC. When the concentration of CNFC was 0.4%–0.8%, the relaxation peaks of T_{22} and T_{23} moved to the lower relaxation time, indicating that the water in the yolk gel binded more tightly to the proteins, and the fluidity of water was weakened (Shao, Deng, Song, Batur, & Jia, 2016). This change was

Table 1B
Relaxation time T_2 and peak area proportion of CNFC or CMC modified yolk gel.

Samples	T_{21}/ms	T_{22}/ms	T_{23}/ms	P_{21}/%	P_{22}/%	P_{23}/%
Control	0.25 ± 0.02^{aA}	31.44 ± 0.00^{bB}	784.71 ± 31.81^{aA}	8.98 ± 0.16^{aA}	85.73 ± 0.27^{cD}	5.29 ± 0.31^{aA}
0.4 % CNFC	0.25 ± 0.02^{a}	30.74 ± 1.22^{b}	554.57 ± 22.48^{b}	8.98 ± 0.07^{a}	87.73 ± 0.23^{b}	3.29 ± 0.25^{b}
0.8 % CNFC	0.25 ± 0.02^{a}	30.03 ± 1.22^{b}		8.90 ± 0.81^{a}	91.10 ± 0.81^{a}	
1.2 % CNFC	0.23 ± 0.00^{a}	75.92 ± 6.22^{a}		8.71 ± 0.25^{a}	91.29 ± 0.25^{a}	
1.6 % CNFC	0.23 ± 0.00^{a}	79.38 ± 3.22^{a}		8.69 ± 0.07^{a}	91.31 ± 0.07^{a}	
0.4 % CMC	0.24 ± 0.02^{A}	31.44 ± 0.00^{B}	529.48 ± 20.97^{B}	8.81 ± 0.45^{A}	86.79 ± 0.59^{C}	4.40 ± 0.30^{B}
0.8 % CMC	0.26 ± 0.00^{A}	31.44 ± 0.00^{B}	506.08 ± 35.11^{B}	8.94 ± 0.12^{A}	89.21 ± 0.23^{B}	1.85 ± 0.14^{C}
1.2 % CMC	0.26 ± 0.00^{A}	40.58 ± 1.61^{A}	517.37 ± 20.97^{B}	8.60 ± 0.14^{A}	90.50 ± 0.11^{A}	0.90 ± 0.03^{D}
1.6 % CMC	0.26 ± 0.00^{A}	39.65 ± 1.61^{A}	541.59 ± 0.00^{B}	9.15 ± 0.53^{A}	89.92 ± 0.42^{A}	0.93 ± 0.11^{D}

Note: different lowercase letters indicate significant differences between CNFC samples; different uppercase letters indicate significant differences between CMC samples ($p < 0.05$).

most significant in yolk with 0.8% CNFC, the number of peaks in yolk gel changed from three to two, and almost all free water in the system was converted to bound water, and the water retention of gel also reached the maximum value at this time. However, when the concentration of CNFC was greater than 0.8%, T_{22} increased significantly ($P < 0.05$), indicating that the binding force between gel protein and immobile water was weakened. The over saturated immobile water began to transform into free water (Fig. 1(E)), and the water holding capacity of gel decreased.

Compared with yolk gel added with CNFC, yolk gel added with CMC did not change the peak number of yolk gel, and the proportion of immobile water was the highest. As the concentration of CMC increased, there was no significant change in T_{21} and P_{21}, but T_{22} moved towards to the higher relaxation time. There was no significant difference in T_{23} between CNFC-yolk gel and CMC-yolk gel, and T_{23} in CNFC-yolk gel and CMC-yolk gel were smaller than the control group. This indicated that the addition of CMC weakened the binding force between yolk gel and immobile water, while enhanced the binding force between yolk gel and free water. In this process, the free water was continuously transformed into immobile water, and the water holding capacity of gel was increased. Based on the above analysis, the addition of CNFC (≤0.8%) with appropriate concentration promoted the combination of protein and water, increased the content of immobile water in gel, this change was conducive to the formation of a dense network. However, excessive CNFC (≥0.8%) acted as an "active dehydrating agent", and water was constantly being transferred out of the protein matrix. The water content of gel was significantly reduced, especially the proportion of immobile water was reduced, which reduced the water holding capacity of gel. In addition, the addition of CMC significantly increased the content of immobile water in yolk gel, and enhanced the interaction between CMC and yolk gel, which was conducive to stabilizing the water distribution of the mixed system.

2.4. Rheological properties

2.4.1. Apparent viscosity

As is shown in Fig. 2(A) (B), the apparent viscosity of all samples decreased with the increase of shear rate, indicating that the modified cellulose-yolk composite solution had shear thinning properties and belonged to a pseudoplastic fluid. Moreover, its apparent viscosity increased with the increase of CNFC or CMC. CNFC and CMC were anionic polysaccharides, they could crosslink with the cationic region of yolk protein, formed a network structure, resulting in a significant increase in the apparent viscosity of yolk liquid (Alberto Masuelli, 2010). In order to clarify the rheological properties of modified cellulose yolk composite solution, the Power law model was used to fit the relationship curve between shear rate and apparent viscosity to obtain the viscosity coefficient (K) and flow index (n), the results are shown in Table 1C. The n values of all samples were between 0 and 1, verifying the fact of pseudoplastic fluid. As the concentration of CNFC or CMC increased, the K value of all samples significantly increased ($P < 0.05$), while the n value gradually decreased. At the same concentration, the K value of the CNFC-yolk gel was always higher than that of the CMC-yolk gel, while the change in n value was opposite to the change in K value. The higher the concentration, the more significant the difference between the CNFC-yolk gel and the CNC-yolk gel. The reason for this phenomenon might be that the nanostructure of CNFC was more prone to aggregation and cross-linking with yolk protein than the CMC structure. As its concentration increased, the interaction inside the CNFC-yolk gel was more significant, which was conducive to the formation of a stronger network structure, resulting in an increase in fluid viscosity, a decrease in fluidity, and an enhancement in shear thinning behavior at the macro level.

2.4.2. Temperature scanning

The storage modulus (G') was used to evaluate the energy storage

Table 1C
Rheological parameters of CNFC or CMC modified yolk gel.

	Concentration (%, w/w)	CNFC	CMC
K/(Pa.s)	0%	0.0240 ± 0.003^{d}	0.024 ± 0.003^{e}
	0.4%	1.882 ± 0.039^{d}	0.543 ± 0.011^{d}
	0.8%	11.854 ± 0.191^{c}	1.192 ± 0.044^{c}
	1.2%	27.441 ± 0.079^{b}	2.428 ± 0.007^{b}
	1.6%	64.175 ± 3.329^{a}	5.156 ± 0.167^{a}
n	0%	0.902 ± 0.045^{a}	0.902 ± 0.045^{a}
	0.4%	0.761 ± 0.004^{b}	0.806 ± 0.006^{b}
	0.8%	0.673 ± 0.001^{c}	0.776 ± 0.010^{bc}
	1.2%	0.646 ± 0.002^{cd}	0.768 ± 0.002^{bc}
	1.6%	0.601 ± 0.015^{d}	0.744 ± 0.001^{c}

Note: Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

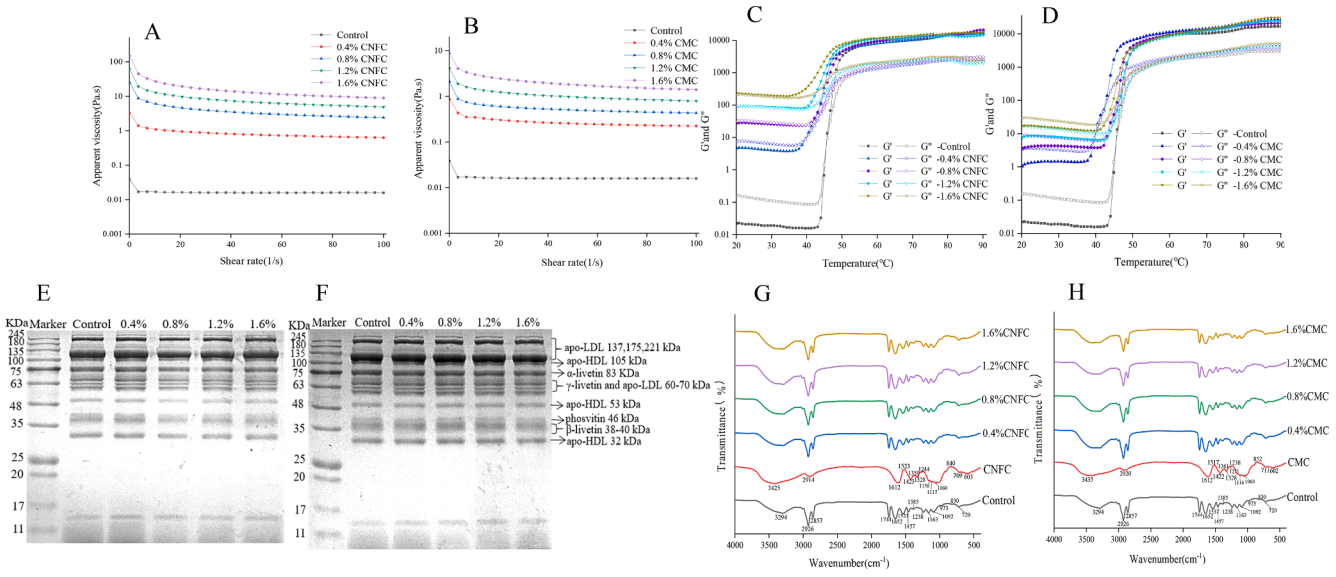

Fig. 2. (A) Apparent viscosity of CNFC modified yolk gel; (B) Apparent viscosity of CMC modified yolk gel; (C) Storage modulus (G') and loss modulusc (G'') of CNFC modified yolk gel; (D) Storage modulus (G') and loss modulusc (G'') of CMC modified yolk gel; (E) SDS-PAGE of CNFC modified yolk gel; (F) SDS-PAGE of CMC modified yolk gel; (G) FTIR analysis of CNFC modified yolk gel; (H) FTIR analysis of CMC modified yolk gel.

caused by elastic changes, representing the elastic properties of the sample; The loss modulus (G''), also known as the viscosity modulus, represented the viscosity properties of the sample. The effect of CNFC or CMC on G'and G''values is exhibited in Fig. 2(C) (D). From the graph, it could be seen that the concentrations of CNFC and CMC were in the range of 0–1.6%, the G'and G'' values of all samples had similar trends with temperature. In the range of 20-40℃, G''>G', the viscosity played a dominant role in the system, and the G' and G'' values showed an increasing trend as the increase of CNFC or CMC concentration, indicating that both modified celluloses effectively improved the viscosity of yolk-gel. When the temperature rised from 40 to 50℃, the change track of G'and G''crossed, and the liquid system gradually transformed into the solid system, which was the beginning of the gel process. With the further increase of temperature (>50℃), the values of G'and G''presented a relatively stable curve and G'>G''. At this time, the yolk gel system had been completely formed, and the gel network structure had been solidified at high temperature.

It was worth noting that when the concentration of CNFC $\geq$ 1.2%, G'>G'' was maintained throughout the temperature scanning range, indicating that the high viscosity of CNFC-yolk gel presented a weak gel state of low fluidity when CNFC was added at a high amount, resulting in no cross between G' and G '' values in the whole heating process. In addition, the study found that when the temperature exceeded 75℃, the G' and G'' values of CMC-yolk gel were significantly higher than those of the control group, and increased in a concentration dependent manner. However, with the increase of the concentration of CNFC, the values of G' and G'' increased first and then decreased. CMC or CNFC within an appropriate concentration range was conducive to the construction of yolk gel network, and significantly improved the viscosity of composite gel. While the addition of excessive CNFC had the opposite effect, which was consistent with the results of TPA analysis.

2.5. Colour

Color was an important indicator to evaluate the appearance of gel products, and it was also one of the important factors affecting consumers' purchase. As is shown in Table 1D, as the concentration of CNFC or CMC increased, the color of yolk gel showed a similar trend, and the redness and yellowness increased while the brightness decreased (consistent with the color change in Fig. (A)). This might be related to the morphology of the two cellulose themselves. Through surface carboxylation, the anions attached to the cellulose would combine with carotenoids in the yolk, so that the absorption peak of the chromophore moved to the long wave direction, resulting in the overall color of gel became darker (Anstoter, Dean & Verlet, 2017). In addition, the aggregation during the formation of gel led to the color changes (Wang, Zhang, Teng & Liu, 2017). Compared with CMC, CNFC had a smaller particle size, which effectively promoted the mutual aggregation of yolk protein. Therefore, at the same concentration, the trend of changes in CNFC-yolk gel was more significant than that in the CMC-yolk gel.

Table 1D
Color of CNFC or CMC modified yolk gel.

Concentration (g/100 g)	CNFC			CMC		
	L*	a*	b*	L*	a*	b*
0%	84.84 ± 0.16^{a}	14.65 ± 0.73^{b}	40.46 ± 0.27^{d}	84.84 ± 0.16^{a}	14.65 ± 0.73^{a}	40.46 ± 0.27^{d}
0.4%	84.51 ± 0.12^{b}	15.23 ± 0.30^{ab}	42.28 ± 0.26^{c}	83.44 ± 0.21^{c}	15.55 ± 0.49^{a}	45.56 ± 0.35^{a}
0.8%	83.56 ± 0.18^{c}	16.13 ± 0.23^{a}	44.69 ± 0.46^{b}	83.92 ± 0.19^{b}	15.74 ± 0.58^{a}	44.36 ± 0.34^{b}
1.2%	82.25 ± 0.16^{d}	16.42 ± 1.24^{a}	45.16 ± 0.37^{b}	84.00 ± 0.08^{b}	15.47 ± 0.97^{a}	43.70 ± 0.54^{b}
1.6%	81.28 ± 0.16^{e}	15.96 ± 0.69^{ab}	47.44 ± 0.64^{a}	83.90 ± 0.11^{b}	15.59 ± 0.17^{a}	42.88 ± 0.41^{c}

Note: Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

2.6. SDS-PAGE analysis

The effect of CNFC or CMC on yolk protein bands was analyzed by SDS-PAGE gel electrophoresis, as is presented in Fig. 2(E)(F). The protein in yolk included low-density lipoprotein (LDL, 137, 175, 221 KDa), high-density lipoprotein (HDL, 32, 53, 105 KDa), vitellin (livetin, 83, 68–40, 60–70 KDa) and vitellin (PV, 46 KDa) (Ren, Liu, Zhang, Zhang, & Zhao, 2020). The LDL band were narrow and sparse, it was the main protein involved in the gelation of yolk. Compared with the control group, the strength of LDL bands in 0.4% CNFC-yolk gel and 0.8% CNFC-yolk gel decreased to a certain extent, indicating aggregation and cross-linking of LDL. When the concentration of CNFC was greater than 0.8%, the band strength and abundance of LDL gradually increased, indicating that excessive addition of CNFC would affect LDL cross-linking. In addition, the increase in CMC concentration did not cause significant changes in the bands of yolk protein. Some studies had pointed out that there was no covalent cross-linking between cellulose and protein, and their combination was mainly relied on physical entanglement and non-ovalent bond (such as hydrogen bond and van der waals force), these combination force were weak force (Guo, Zhang, Hao, Xie, & Chen, 2018). It was speculated that the two kinds of cellulose might be partially separated from yolk protein during denaturation electrophoresis, and because the aggregation degree of CMC was far less than CNFC, the addition of CMC did not cause changes in the peptide chain structure of yolk protein. The infrared spectrum analysis showed that CMC and CNFC contained a large number of hydroxyl groups, which could form intermolecular hydrogen bonds with yolk protein, and enhanced the interaction between cellulose and protein, which was also the reason for the increase in the hardness and viscosity of yolk gel.

2.7. FTIR analysis

Protein had many characteristic absorption peaks in the FTIR region, which was of great significance for studying changes in the secondary structure of protein. When the functional groups of biological macromolecules interacted at the molecular level, new absorption peaks or changes in the intensity and position of absorption peaks appeared in the infrared spectrum (Pirestani, Nasirpour, Keramat, Desobry & Jasniewski, 2018). The infrared spectra of yolk gel, modified cellulose and their composite gel are shown in Fig. 2(G)(H). In general, the characteristic absorption peak displayed by the composite gel was almost the same as that displayed by the single yolk gel, indicating that there was no new covalent bond between CNFC/CMC and yolk protein, which confirmed the conclusion of SDS-PAGE gel electrophoresis that modified cellulose and protein molecules interacted through non-covalent bond. It was worth noting that individual CNFC-yolk gel and CMC-yolk gel exhibited significant absorption peaks at 3425 cm^{-1} and 3435 cm^{-1} respectively, corresponding to the stretching vibration of –OH (Li, Sha, Yang, Ren & Tu, 2023). With the continuous addition of the two celluloses, the stretching vibration of –OH at 3294 cm^{-1} in yolk gel tended to move to the high wave region, the type of the peak became widen and the intensity of the peak slightly increased, indicating that hydrogen bond association occured between CNFC or CMC and yolk protein.

In order to understand the changes of CNFC or CMC on the conformation of yolk gel, the amide I band (1600–1700 cm^{-1}) was further analyzed. Through fitting calculation, the yolk protein change of the secondary structure is shown in Table 1E. As the concentration of CNFC increased, the relative content of α-helix and β-turn first decreased and then increased, while the relative content of β-folding gradually increased and then decreased. In addition, the addition of CMC

Table 1E
Protein secondary structure of CNFC or CMC modified yolk gel.

	α-helixe(%)	β-folding(%)	β-turn(%)	Irregular curls (%)
Control	32.34 ± 0.09^{aA}	17.58 ± 0.08^{dD}	19.55 ± 0.29^{aA}	30.53 ± 0.29^{bA}
0.4 % CNFC	32.06 ± 0.10^{b}	18.08 ± 0.15^{c}	19.05 ± 0.08^{b}	30.81 ± 0.01^{ab}
0.8 % CNFC	30.80 ± 0.05^{d}	19.98 ± 0.13^{a}	18.28 ± 0.08^{c}	30.94 ± 0.04^{a}
1.2 % CNFC	31.33 ± 0.12^{c}	19.17 ± 0.23^{b}	19.41 ± 0.06^{a}	30.09 ± 0.17^{c}
1.6 % CNFC	32.00 ± 0.08^{b}	17.89 ± 0.09^{c}	19.35 ± 0.11^{a}	30.76 ± 0.11^{ab}
0.4 %CMC	31.11 ± 0.08^{B}	19.76 ± 0.08^{C}	18.51 ± 0.12^{B}	30.62 ± 0.15^{A}
0.8 %CMC	30.99 ± 0.04^{B}	19.95 ± 0.29^{BC}	18.61 ± 0.27^{B}	30.46 ± 0.03^{A}
1.2 %CMC	30.86 ± 0.14^{BC}	20.17 ± 0.33^{B}	18.54 ± 0.51^{B}	30.43 ± 0.36^{A}
1.6 %CMC	30.59 ± 0.29^{C}	20.56 ± 0.06^{A}	18.58 ± 0.08^{B}	30.27 ± 0.27^{A}

Note: different lowercase letters indicate significant differences between CNFC samples; different uppercase letters indicate significant differences between CMC samples ($p < 0.05$).

significantly reduced the relative content of the α-helix and increased the relative content of β-folding ($P < 0.05$), There was no significant difference in β-turn and irregular curls ($P > 0.05$).

α-helixe achieved stable structures primarily through hydrogen bonds between carbonyl oxygen (–CO) and amino hydrogen (–NH) in peptide chains, while β- folding was stabilized by hydrogen bonds between peptide chains, which promoted the formation of protein gel (Zhu, Wang, Li, Li, & Teng, 2017). Therefore, modified cellulose interacted with yolk protein and rearranged intramolecular hydrogen bonds and electrostatic interactions, resulting in the conformation change of the protein from α-helixe to β-folding. These results indicated that the addition of CMC or appropriate CNFC (≤0.8%) was conducive to the unfolding of the molecular structure of yolk protein, these additions induced the formation of more β-folding, promoted mutual aggregation and cross-linking between protein. However, excessively high concentrations of CNFC (≥1.2%) promoted excessive aggregation of yolk protein, The relative content of β-folding decreased along with the relative content of α-helical structure increased, the cross-linking of yolk protein was blocked, and the gel network structure gradually became loose (Miao, Peng, Wang, Cao, & Li, 2021).

2.8. Fluorescence spectrometry analysis

The fluorescence spectra of yolk protein and modified cellulose yolk protein composite solution are shown in Fig. 3(A)(B). After the addition of modified cellulose, the fluorescence intensity of yolk protein significantly decreased, and with the increased concentration of modified cellulose, the fluorescence intensity of CNFC-yolk gel first decreased and then increased, while the CMC-yolk gel had been in a fluorescence quenching state. This was attributed to the unfolding of the spatial structure of yolk protein, and the exposure of internal tryptophan residues. On the other hand, CNFC or CMC interacted with yolk protein, forming a more stable tertiary conformation inside the protein, thereby shielding tryptophan residues, resulting in a decrease in fluorescence intensity (Tao, Jiang, Chen, Zhang, & Pan, 2018). Compared to CMC, nanosized CNFC was more prone to aggregation in yolk gel, thus CNFC-yolk gel had a greater fluorescence quenching effect. However, the addition of high concentrations of CNFC promoted excessive

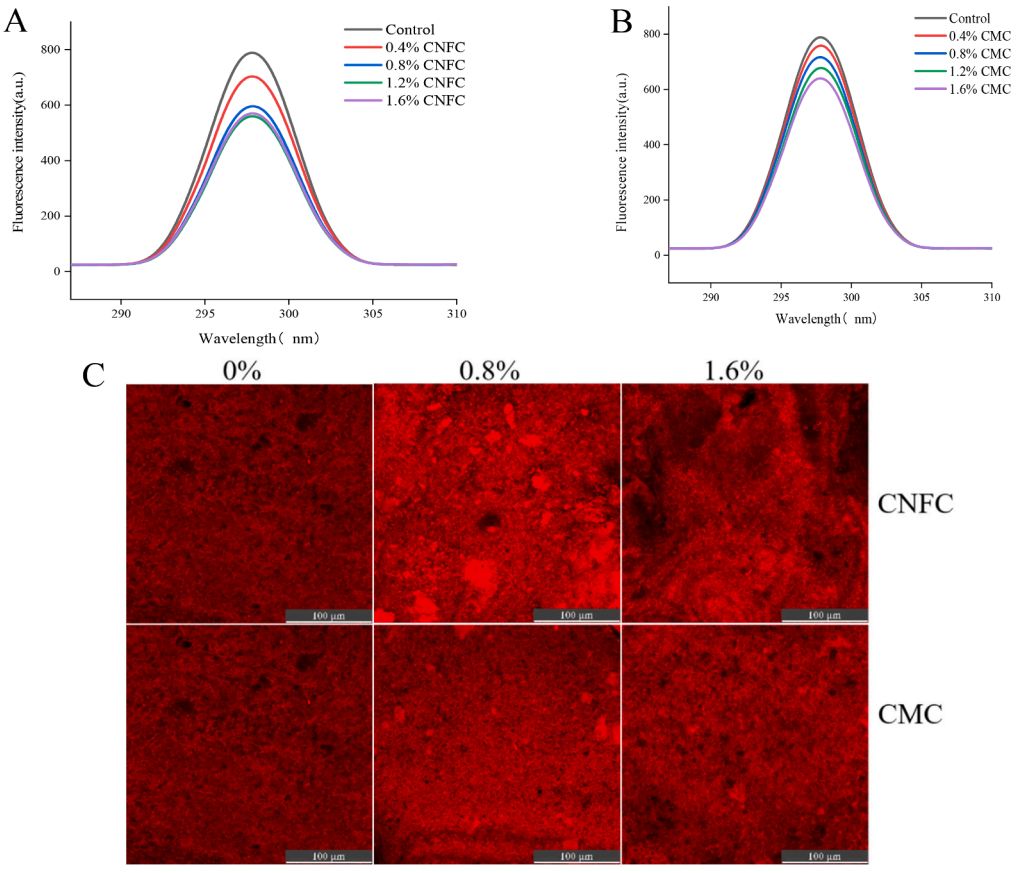

Fig. 3. (A) Fluorescence spectroscopy analysis of CNFC modified yolk gel; (B) Fluorescence spectroscopy analysis of CMC modified yolk gel; (C) CLSM images of CNFC or CMC modified yolk gel.

aggregation of yolk protein, resulting in tryptophan residues to be embedded within hydrophobic molecules, and the fluorescence intensity of the composite solution was slightly enhanced (Xu, Wang, Zhao, Yin, & Li, 2020).

2.9. CLSM analysis

CLSM was used to stack the samples and observe the effect of different concentrations of CNFC or CMC on the microstructure of yolk gel, in order to reveal the differences in the microstructure of protein matrix. As is shown in Fig. 3(C), the microstructure of the control group was rough and uneven, with large pores, this might be because the protein molecules of yolk gel were not fully unfolded during the heat induction process, and they did not form a good cross- link with each other, which led to the poor quality of yolk gel (Xiao, Liu, Wang, Jin, & Guo, 2020). When CMC or 0.8% CNFC was added, the yolk gel network became denser and uniformly. This was because the addition of CMC or CNFC with proper concentration formed a more compact structure, which was conducive to the stability of gel structure. This highly regular and dense network structure had stronger resistance to external stress, this structure could intercept more water through mutual entanglement (Wang, Li, Zhou, Ma, & Li, 2019), thus improving the texture and water retention of yolk gel.

At the same concentration, due to the smaller nano size of CNFC, the self aggregation effect of CNFC-yolk gel was more significant than that of CMC-yolk gel, its dispersity in composite gel system was relatively poor, resulting in that the mesh size of gel was not as uniform as CMC (Zhao, Zhou & Zhang, 2019), so a small number of large holes appeared in 0.8% CNFC-yolk gel images. With the increase of CNFC concentration, the gel network structure of 1.6% CNFC-yolk gel became loose, and more large pores could be observed. In addition, it was worth noting that the mesh of the 1.6% CMC-yolk gel was more and larger than that of the 0.8% CMC-yolk gel. This was because the high concentration CMC trapped more water molecules in the gel network, forming more water channels (Zhuang, Han, Bai, Liu & Xing, 2017), which was also the reason for better water retention.

4. Conclusion

The interaction between CNFC or CMC and yolk protein changed the structure of the protein, thus affecting the quality of the cellulose-yolk gel. Specifically, adding CMC or an appropriate concentration of CNFC ($\leq$0.8%) could induce the unfolding of yolk protein, promoting α-helix direction β-folding transformation. In this process, the exposure of hydrophobic groups was conducive to the orderly aggregation of yolk protein, forming a more uniform and dense gel network. The ordered gel network not only improved the texture, elastic modulus (G') and viscous modulus (G'') of gel, but also promoted the conversion of free water to non-flowing water, and the water retention of gel was significantly improved. At the same concentration, the enhancement effect was more significant with the addition of CMC. Excessed CNFC ($\geq$0.8%) promoted the excessived aggregation of yolk gel. The formation of larger aggregates hindered the cross-linking of yolk gel. The integrity of gel matrix was also destroyed, and the performance of gel became worse. SDS-PAGE gel electrophoresis analysis showed that the celluloses and yolk protein mainly combined through non-covalent bond, and the force was weak, FT-IR conclusions confirmed this conclusion. Therefore, CMC or low concentration CNFC could be used as exogenous additives to improve yolk thermal gel. In addition, compared with CNFC, CMC was more suitable for practical production to improve the thermal gel performance of yolk gel because of its better dispersion.

Declaration of Competing 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.

Data availability

No data was used for the research described in the article.

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