



Rheological properties and microstructure of rennet-induced casein micelle/ κ -carrageenan composite gels

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ABSTRACT

This study aimed to investigate effects of kappa-carrageenan (κ -carrageenan) concentration and storage temperature on the rheological and texture properties of casein micelle/ κ -carrageenan composite gels. The particle size, zeta potential, Fourier transform infrared spectroscopy of the mixture, and microstructure of the composite gels were measured. The movement of κ -carrageenan characteristic bond showed that the casein micelle and κ -carrageenan formed a complex with larger particle size through electrostatic adsorption. The electrostatic repulsion between molecules increased with the increase of κ -carrageenan concentration, which stabilized the mixed system. It was found that both κ -carrageenan concentration and storage conditions affected the gel's rheological properties and microstructure. As the concentration of κ -carrageenan increased, the storage modulus (G') of the composite gels increased, the rennet coagulation time (RCT) decreased, and the water holding capacity (WHC) increased at 25 °C. Microstructure results showed that a more uniform and denser gel network structure with smaller pore sizes was formed after the addition of κ -carrageenan compared with the control. G' and RCT were reduced and microscopic phase separation occurred after storage at 4 °C, resulting in a porous structure with large pore sizes. Thus, the addition of κ -carrageenan could enrich the diversity of food gel structure to obtain an ideal gel.

1. Introduction

The gel is an elastic semi-solid with a three-dimensional network structure. It has attracted extensive attention in many subjects, especially in the food, pharmaceutical, and cosmetic fields (Cao & Mezzenga, 2020). Polysaccharides and proteins are macromolecules and can be used as materials for preparing gels in the food system with low cost. They provide the body with energy and nutrients and enhance satiety. The mixture of polysaccharides and proteins has a variety of phase behaviors because of their unique chemical structures, thus providing different gel microstructures that make it possible to design gel products with specified tastes and textures (Kasapis & Stefan, 2008). Therefore, it is necessary to investigate the compatibility between the proteins and polysaccharides and control their interaction to obtain desired gels with good function and performance.

The rennet-induced gel is essential for cheese making, which directly

affects the texture and taste of cheese (Bönisch, Heidebach, & Kulozik, 2008). The mechanism of the rennet-induced gel has been widely studied. It can be divided into two stages: *i*) the peptide bond between Phe105 and Met106 of κ -casein is hydrolyzed by rennet to form para- κ -casein micelle and caseinomacropeptide (CMP); *ii*) after a sufficient amount of κ -casein is hydrolyzed, the steric stabilization and electrostatic interaction of casein micelles are destroyed, resulting in the aggregation of para- κ -casein micelle to form a three-dimensional network (Lucey, 2002). Several studies have shown that the texture and structure of glucono- δ -lactone induced casein gel is affected by polysaccharides (Li, Guo, Li, Li, et al., 2021; Li, Guo, Li, Sun, et al., 2021; Sun et al., 2018). However, there are few studies on the cross-linking network of rennet-induced casein micelle/polysaccharides composite gels.

Carrageenan is a linear sulfated anionic polysaccharide extracted from red seaweed and consists of galactose and 3,6-dehydrated galactose, which is widely used in the food industry as a thickener, stabilizer,

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and gelling agent. Kappa-carrageenan (κ -carrageenan) has a sulfate group in each disaccharide repeating unit and can transform from a coiled structure to a helical structure and aggregate to form a gel under specific ionic environments and temperature conditions (Derkach, Ilyin, Maklakova, Kulichikhin, & Malkin, 2015; Wang et al., 2014; Yuguchi, Thuy, Urakawa, & Kajiwarra, 2002; Zia et al., 2017). The stability of the milk system is influenced after adding κ -carrageenan. Because the κ -carrageenan could bind with casein micelles through electrostatic interaction at neutral pH (Arltoft, Ipsen, Madsen, & Vries, 2007; Perrechil & Cunha, 2013; Snoeren, Both, & Schmidt, 1976). Moreover, the adsorption of κ -carrageenan on the surface of a casein micelle and helix aggregation of κ -carrageenan are effective ways to prevent macroscopic phase separation of the casein micelles and polysaccharides (Spagnuolo, Dalglish, Goff, & Morris, 2005). The addition of κ -carrageenan to skim milk affected the formation of the rennet-induced gel. The κ -carrageenan (0.15 g/kg) could reduce the release rate of CMP, which was mainly due to the increase in the incorporation of proteins through calcium bridges and hydrophobic interactions (Wang, Zhang, & Ren, 2017).

However, the effect of κ -carrageenan on the texture and structure of rennet-induced casein micelle gel is still lacking. Especially, the effect of storage conditions on rennet-induced casein micelle/carrageenan composite gels is unclear. This study analyzed the microstructure, texture, and water holding capacity (WHC) of rennet-induced casein micelle/ κ -carrageenan composite gels. Moreover, the network structures of composite gels adjusted by the storage conditions of the mixture were further explored. We aimed to investigate the gelation mechanism of the casein micelle/ κ -carrageenan mixture to improve the understanding of the gel texture, which will provide a theoretical foundation for the polysaccharide/protein mixed gels.

2. Materials and methods

2.1. Materials

Micelle casein concentrate (MCC) (72.5 g/100 g protein and 4.5 g/100 g moisture) was obtained from cow milk by membrane separation according to our previously reported method (Zhang, Zhang, Song, Wang, & Lv, 2021). The κ -carrageenan (10.4 g/100 g moisture and 21.7 g/100 g ash) was provided by Zhejiang Shangfang Biotechnology Co., Ltd (Zhejiang, China).

2.2. Sample preparation

The casein micelle solution (12 g/100 mL) was prepared by dispersing 36 g MCC in 300 mL deionized water. After stirring for 2 h, it was hydrated overnight at 4 °C. The κ -carrageenan solution (0.75 g/100 mL) was prepared by dispersing 0.3 g κ -carrageenan powder in 40 mL deionized water and stirring at room temperature for 2 h. The casein micelle solution was sonicated (40 kHz, 30 min) and broad spectrum microtabs II was added to prevent microbial growth. Then, different volumes of the solution were mixed as listed in Table 1. The final

mixtures included 8 g/100 mL casein micelle and different concentrations of κ -carrageenan (0.00, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, and 0.10 g/100 mL). They were incubated at 60 °C for 30 min and placed at room temperature for 30 min. The mixtures were stored at 25 °C for 2 h, 25 °C for 12 h, and 4 °C for 12 h, respectively. When the concentration of κ -carrageenan was higher than 0.04 g/100 mL, the casein micelle/ κ -carrageenan solution underwent gel inversion after being stored at 4 °C for 12 h. Therefore, the results of the casein micelle/ κ -carrageenan solution were not detected when κ -carrageenan concentration was higher than 0.04 g/100 mL.

2.3. Particle size and zeta potential measurements

The particle size and zeta potential were measured by a Zetasizer analyzer (Malvern Instruments, Worcestershire, UK). All measurements were carried out at room temperature. Particle size was measured using 1 mL of the solution at 25 °C with a refractive index of 1.46. Zeta potential was measured after diluting the samples 100-fold with deionized water at 25 °C. The refractive indices of protein and water were 1.46 and 1.33, respectively. Each sample was tested in triplicate.

2.4. Fourier transforms infrared (FTIR) spectroscopic analysis

The casein micelle solution (8 g/100 mL), κ -carrageenan solution (0.10 g/100 mL), and casein micelle (8 g/100 mL)/ κ -carrageenan (0.10 g/100 mL) mixtures were detected by FTIR spectrum instrument (Model Tensor 27, Bruker Corporation, Bremen, Germany). The freeze-dried samples were mixed with KBr and ground evenly, this mixture was then pressed into tablets. The measurement parameters were set as follows: the resolution was 4 cm⁻¹, the scanning range was 4000–400 cm⁻¹ and an average of 64 scans was recorded. Each sample was tested in triplicate.

2.5. Rheological properties

The gelation process was observed by a rheometer (MCR 502, Anton Paar, Austria) (Ragab et al., 2020). The composite solution (13 mL) was added into the cylinder and kept at 33 °C to equilibrate, then rennet (2235 IMCU/g) was added to the samples at 0.04 g/100 mL. The monitoring time was 30 min, the oscillation frequency was 1 Hz and the strain was 1%. The storage modulus (G') and loss modulus (G'') were monitored during the formation of gels. Each sample was tested in triplicate.

2.6. Confocal laser scanning microscope (CLSM) observations

The rennet (40 μ L, 2 g/100 mL) was added to casein micelle/ κ -carrageenan mixtures (2 mL). The fast green (40 μ L, 1.6 mg/mL) was used to label the caseins. Next, the mixtures (20 μ L) were loaded onto the slides, covered with cover slips and incubated at 33 °C for 30 min. The microstructures of gels were observed by Leica TCS SP8 CLSM (Leica Microsystems, Heidelberg, Germany) at a magnification of 20 \times . The excitation wavelength was set at 633 nm and the emission wavelength was set at 655–755 nm. The resolution of the images was 1024 \times 1024.

2.7. Texture measurement

Adding rennet to the mixed solution, the samples (7 mL) were poured into 10 mL beakers and incubated at 33 °C for 30 min to form gels. The texture of gels was measured by a texture analyzer (TA.HDplus, Stable Micro Systems, Surrey, UK) with a probe (P/0.5R). The measurement parameters were set as follows: the pre-test speed, test speed, and post-test speed were 1 mm/s, the trigger force was 5 g and the deformation was 40%. Each sample was repeated three times.

Table 1
Components of the mixed casein micelle/ κ -carrageenan solutions.

κ -carrageenan concentration (g/100 mL)	12 g/100 mL Casein micelle solution (mL)	0.75 g/100 mL κ -carrageenan solution (mL)	Deionized water (mL)
0.00	30.00	0.00	15.00
0.01	30.00	0.60	14.40
0.02	30.00	1.20	13.80
0.03	30.00	1.80	13.20
0.04	30.00	2.40	12.60
0.06	30.00	3.60	11.40
0.08	30.00	4.80	10.20
0.10	30.00	6.00	9.00

2.8. Water holding capacity (WHC) measurement

The rennet was added to the mixed solution ($m_0 = 4$ g) in the centrifuge tubes. Subsequently, they were held at 33 °C for 30 min for gel formation. After centrifuging the gels at 4000 rpm for 15 min, the water was removed and the remaining samples were weighed (m_1). The WHC was defined as follows: $WHC (\%) = 100 \times (m_1/4)$. Each sample was repeated three times.

2.9. In vitro gastric digestion

Prepared simulated gastric fluid according to previous work (Yang, Wei, et al., 2020). Dissolved 0.7 mL concentrated hydrochloric acid, 0.2 g sodium chloride, and 3.0 g pepsin (activity >250 U/mg) in deionized water and adjusted pH to 1.2 to volume 100 mL. Eight mL rennet-induced composite gels (at 25 °C for 12 h) were added into 16 mL simulated gastric fluid which was pre-heated at 37 °C for 10 min. The mixtures were incubated at 37 °C with shaking (150 rpm) for 2 h. The digested mixtures were heated at 90 °C for 5 min at 0, 15, 30, 60, 90, and 120 min, respectively. Then, they were centrifuged at 4000 rpm for 15 min at 25 °C and the content of free amino groups was measured according to the method (Wang, Yang, Yang, & Ji, 2019). Each sample was repeated three times.

2.10. Statistical analysis

All measurements were repeated three times. The results were expressed as mean \pm standard deviation. SPSS version 20.0 (Chicago, IL, USA) was used for one-way analysis of variance (ANOVA). Differences were regarded as significant if $p < 0.05$.

3. Results and discussion

3.1. Zeta potential measurements

Zeta potential represents the number of molecular surface charges, which reflects the electrostatic interaction between proteins and polysaccharides. It is connected to the stability of colloidal dispersion. The strength of electrostatic interaction depends on the number and distribution of proteins and polysaccharides, as well as the total charge of proteins (Azarikia & Abbasi, 2016). The results of the zeta potential of

the mixed solution under different storage conditions are shown in Fig. 1. The surface charge number of proteins was related to pH. The pI of casein is about 4.6 and the pH of the solution was close to neutral. Therefore, the zeta potential of each solution was negative. The zeta potential values of casein micelle solution stored at 25 °C for 2 h, 25 °C for 12 h, and 4 °C for 12 h were −20.57 mV, −20.20 mV, and −20.87 mV, respectively. There was a similar result that the zeta potential value of casein was −35.40 mV at pH 6.7 (Sun, Liang, Yu, Tan, & Cui, 2016). The zeta potential values decreased significantly ($p < 0.05$) with the increase of κ -carrageenan concentration. This was due to the neutralization of the positively charged amino group in casein micelles and the negatively charged ester sulfate group of κ -carrageenan, which increased negative charges (Li, Wang, et al., 2018). The κ -carrageenan was adsorbed to the casein micelle surface by electrostatic attraction. A similar study on other anionic polysaccharides with proteins formed negatively charged mixtures by electrostatic interaction between casein micelles and pectin (Kim & Wicker, 2011). According to the classical DLVO theory, the system is unstable and prone to flocculation when the intermolecular repulsion force is small (the zeta potential absolute value was lower). The repulsion force increased (the zeta potential absolute value was higher) with the addition of κ -carrageenan, thus preventing protein precipitation and stabilizing the system.

3.2. FTIR measurements

The FTIR was determined to characterize the interaction between casein micelles and κ -carrageenan as shown in Fig. 2. Changes in the spectra bands indicate the vibrational characteristics of functional groups and changes of polar bonds in proteins. There were several characteristic absorption peaks in the FTIR spectrum of casein micelles, including Amide A (3500–3000 cm^{-1} , O–H or N–H stretching), Amide B ($=\text{C}-\text{H}$ and $-\text{NH}_3^+$ stretching), Amide I (1700–1600 cm^{-1} , $\text{C}=\text{O}$ and $\text{C}=\text{N}$ stretching), Amide II (1550–1500 cm^{-1} , N–H deformation), and Amide III (1300–1200 cm^{-1} , C–H stretching and N–H deformation) (Li, Wang, et al., 2018; Li, Zhang, Sun, Wang, & Lin, 2018). In the FTIR spectrum of κ -carrageenan, the characteristic peaks were observed at 1259 cm^{-1} (ester sulfate group $\text{O}=\text{S}=\text{O}$), 931 cm^{-1} (3,6-anhydride-galactose group), and 847 cm^{-1} (β -galactose group) (Sinthumran, Benjakul, Swedlund, & Hemar, 2017).

The shape of the casein micelle bonded with κ -carrageenan was similar to that of the casein micelle. The ester sulfate group of

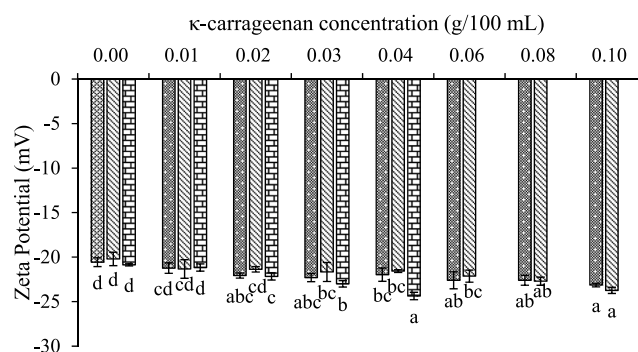


Fig. 1. Effect of concentration of κ -carrageenan on zeta potential under different storage conditions (▨: at 25 °C for 2 h; ▨: at 25 °C for 12 h; ▩: at 4 °C for 12 h). Different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

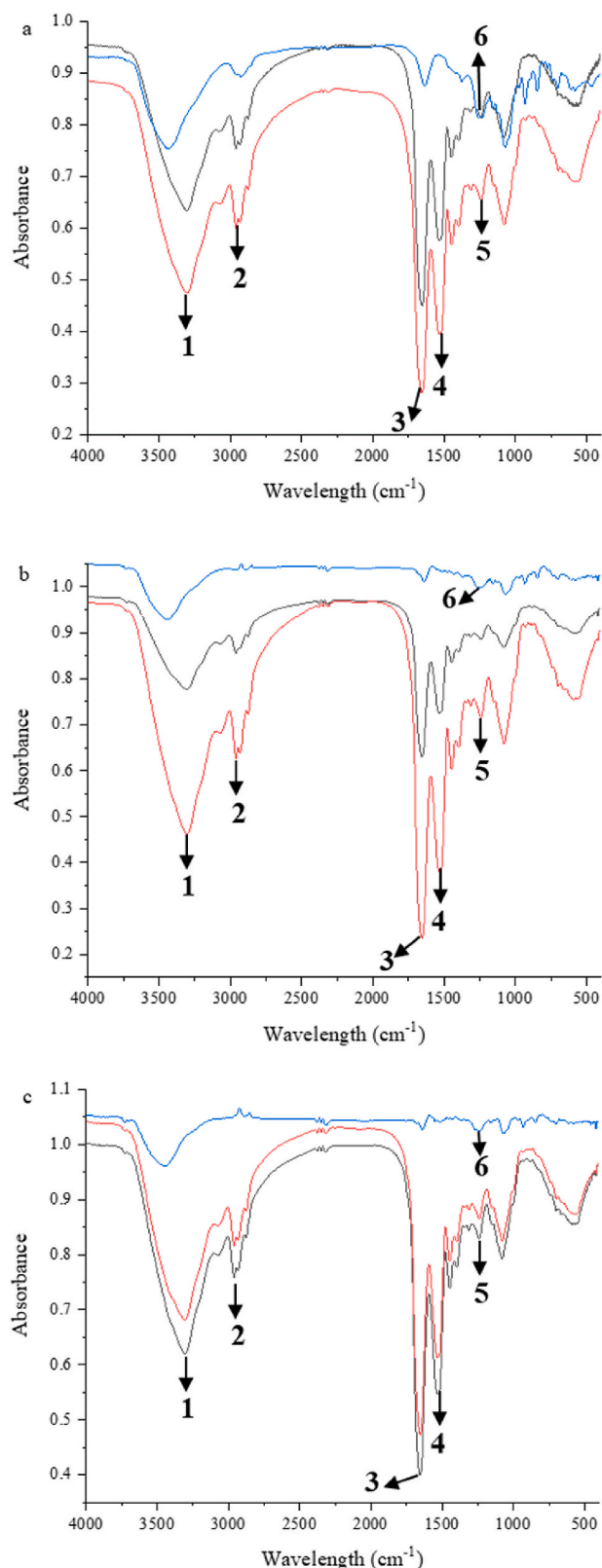


Fig. 2. FTIR of casein micelle, κ -carrageenan, casein micelle/ κ -carrageenan (—: casein micelle; —: casein micelle/ κ -carrageenan; —: κ -carrageenan; a: at 25 °C for 2 h; b: at 25 °C for 12 h; c: at 4 °C for 12 h; 1: Amide A; 2: Amide B; 3: Amide I; 4: Amide II; 5: Amide III; 6: ester sulfate group). Results are shown as averages of three replicates.

κ -carrageenan all shifted from 1259 cm^{-1} , 1267 cm^{-1} , and 1230 cm^{-1} to 1240 cm^{-1} at 25 °C for 2 h, 25 °C for 12 h, and 4 °C for 12 h, respectively, indicating the interaction between casein micelles and κ -carrageenan. The interaction was electrostatic adsorption between the $-\text{NH}_3^+$ of casein micelles and the $-\text{OSO}_3^-$ of κ -carrageenan (Tang, Lei, Wang, Li, & Wang, 2021). An observation suggested that Amide I and Amide II bands shifted in the mixed system of casein and κ -carrageenan, indicating that there were electrostatic interactions and hydrogen bonding between casein and κ -carrageenan molecules (Tang, Zhu, Li, Adhikari, & Wang, 2019). However, the hydrogen bonding mentioned above was not found in this study.

3.3. Particle size measurements

The particle size of the mixed system increased significantly ($p < 0.05$) from 336.65 nm to 428.03 nm with the κ -carrageenan concentration increasing from 0.00 g/100 mL to 0.04 g/100 mL at 25 °C for 2 h. As the κ -carrageenan concentration increased from 0.00 g/100 mL to 0.04 g/100 mL, the particle size of the samples increased significantly ($p < 0.05$) from 332.13 to 353.48 nm to 486.68 and 1203.75 nm at 25 °C and 4 °C for 12 h, respectively. A previous study also showed a similar result after adding different types of carrageenan to casein solution (26 mg/mL) (Wang et al., 2014). The results indicated the complexes were formed by the interaction between casein micelles and κ -carrageenan. The binding force was mainly electrostatic adsorption according to the results in Figs. 2 and 3. The particle size and negative charges of the mixed solution increased with the increase of κ -carrageenan concentration, indicating that the casein micelle surface could bind more κ -carrageenan. In addition, the particle size was the largest and absolute negative charges were the highest at 4 °C for 12 h when the κ -carrageenan concentration was the same. This may be due to the disordered coil structure of κ -carrageenan being transformed to the double helix structure during the cooling process, then the κ -carrageenan aggregated to form a weak gel network. It caused an increase in particle size of the mixed system and affected the electrostatic binding between casein micelles and κ -carrageenan (Yang, Li, et al., 2020).

3.4. Rheological measurements

The gelation process of rennet-induced casein micelle/ κ -carrageenan composite gels was monitored by rheological. As shown in Fig. 4, G' and G'' of all samples increased rapidly within 20 min, indicating that the gel was gradually formed in mixed system. The growth rate of G' and G'' became slow until it reached a plateau with time prolonged. At 25 °C for 30 min, G' increased with the increasing κ -carrageenan concentration. The highest value of G' in all samples was showed in 0.10 g/100 mL of κ -carrageenan concentration. At 4 °C, the G' of composite gels was higher than that of the control when the concentration of κ -carrageenan was 0.01 g/100 mL or 0.02 g/100 mL. Furthermore, the G' of composite gels was lower than that of the control when the concentration of κ -carrageenan increased to 0.03 g/100 mL or 0.04 g/100 mL. The G' was the highest when adding 0.01 g/100 mL concentration of κ -carrageenan. These results indicated that the addition of κ -carrageenan could lead to the rearrangement of gel network structure. The rennet coagulation time (RCT) was defined as the time when G' was greater than 1 Pa, which is important for evaluating the gelation properties of casein micelles. It was shortened with the addition of κ -carrageenan, which showed that κ -carrageenan could promote the formation of gels. This could be due to the concentration of total polymer increased by the addition of κ -carrageenan, leading to a gel network of the mixed system being formed earlier than the control. In addition, the higher concentration of κ -carrageenan caused stronger repulsion and promoted casein aggregation to form a gel network (Li, Guo, Li, Sun, et al., 2021).

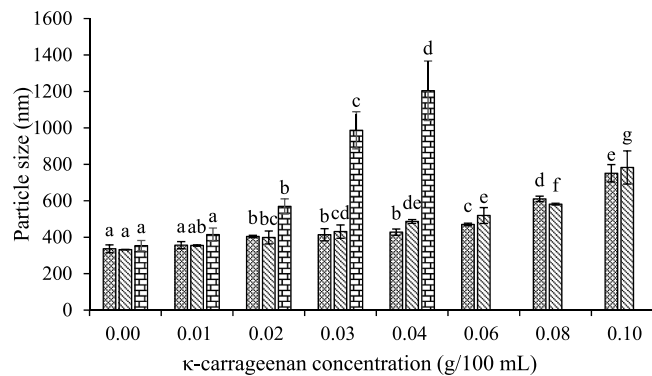


Fig. 3. Effect of concentration of κ -carrageenan on particle size under different storage conditions (▨: at 25 °C for 2 h; ▤: at 25 °C for 12 h; ▩: at 4 °C for 12 h). Different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

3.5. Microstructure of composite gels

To observe the microstructure of composite gels, Fast Green FCF was used to label casein micelles (Fig. 5). Without κ -carrageenan addition, the casein micelle gel showed a unique network structure with large pore sizes. At 25 °C, the composite gels showed a denser network structure with smaller pore sizes than the casein micelle gel when κ -carrageenan concentration increased from 0.00 g/100 mL to 0.03 g/100 mL. With a further increase of κ -carrageenan concentration, the network structure changed with fewer connections among different proteins. The structure was changed from a strip-like network to a flocculent network with a uniform structure and small pore sizes. However, the network structure of composite gels became loose at 4 °C with 0.01 g/100 mL of κ -carrageenan concentration. As the κ -carrageenan concentration further increased, the structure was transformed from a homogeneous strip-like network structure to a loose and inhomogeneous structure with large pore sizes. The results were similar to a previous micrograph of rennet-induced gels (Corredig, Sharabafi, & Kristo, 2011).

3.6. Texture and WHC of composite gels

To explore the influence of κ -carrageenan concentration on the organization state of composite gels, the texture properties were measured. As shown in Tables 2–4, the addition of high concentration κ -carrageenan had a significant influence on the texture properties of gels. At 25 °C for 2 h, the hardness of rennet-induced casein micelle gel was 49.24 g, whereas the gel hardness decreased to 46.97 g at 0.06 g/100 mL of κ -carrageenan addition ($p > 0.05$). As the κ -carrageenan concentration further increased, the hardness of composite gels decreased significantly ($p < 0.05$) and reached the minimum value (36.18 g) at 0.10 g/100 mL of κ -carrageenan addition. The adhesion and resilience of the composite gels decreased significantly ($p < 0.05$) with the addition of κ -carrageenan. The texture trends of rennet-induced composite gels with the addition of κ -carrageenan after incubated for 12 h at 25 °C and 4 °C were similar to those for 2 h at 25 °C. The hardness of composite gels decreased with the increase of κ -carrageenan concentration, which may be due to the reduction of protein-protein linkages in the gel structure. Compared with 25 °C for 2 h, the hardness of composite gels was slightly increased at 25 °C for 12 h, indicating that the prolongation of the reaction time could promote the cross-linking between protein-protein.

The WHC refers to the interaction between the protein and water and is an important indicator for evaluating protein gel systems. The addition of κ -carrageenan had a significant effect on the WHC of composite gels and the effect was different under different storage conditions (Fig. 6). The WHC of composite gels increased significantly ($p < 0.05$)

compared with the casein micelle gel at 25 °C. When κ -carrageenan concentration increased from 0.00 g/100 mL to 0.10 g/100 mL, WHC of composite gels was increased significantly ($p < 0.05$) from 54% to 60%–57% and 71% at 25 °C for 2 h and 12 h, respectively. The WHC of composite gels was increased with the increase in reaction time at 25 °C, indicating that the binding of proteins or polysaccharides with water was promoted by the increase in reaction time. A previous study had shown that agar could help to retain unbound water and improve the WHC of casein-agar gels (Sun et al., 2018). At 4 °C, the WHC of composite gels was decreased significantly ($p < 0.05$) from 60% to 32% when κ -carrageenan concentration increased from 0.00 g/100 mL to 0.04 g/100 mL. The appearance of composite gels at 4 °C clearly showed water exudation. This may be due to the conformation of κ -carrageenan being changed at 4 °C, leading to a rough network with large pores and reduced water accommodation space.

3.7. In vitro digestibility

The content of free amino groups characterized the simulated gastric digestion of composite gels. The content of free amino groups increased with the prolonged digestion time, indicating that the composite gels were continuously digested in the gastric digestion solution (Fig. 7). There were no significant differences ($p > 0.05$) in gastric digestibility between the casein micelle gel and composite gels at 0 min. However, the digestibility of gels was affected by the κ -carrageenan within the time range of 15–120 min. The content of free amino groups was decreased significantly ($p < 0.05$) with increasing κ -carrageenan addition, indicating that the presence of κ -carrageenan reduced the digestibility of rennet-induced gel. The results showed that κ -carrageenan had a protective effect on the gastric digestion of casein micelles. A related study was consistent with this result, which explored protein digestion of casein- κ -carrageenan gels *in vitro* gastric digestion (Fontes-Candia et al., 2022). The SDS-PAGE results showed that casein's characteristic bands were not present in casein while present in the composite gels, indicating κ -carrageenan had the effect on the protecting casein and delaying hydrolysis of protein during gastric digestion.

3.8. Discussion

A schematic diagram of the formation mechanism of casein micelle/ κ -carrageenan gel is shown in Fig. 8. Polysaccharides and protein mixtures had a wide range of phase behavior based on their significant structural differences. The casein micelle and κ -carrageenan could form complexes through electrostatic interaction, resulting in macro or macroscopic phase separation (Bourriot, Garnier, & Doublier, 1999; Turgeon, 2003). Electrostatic repulsion was existed in casein micelle/ κ -carrageenan complexes (Fig. 1). There was no macroscopic phase

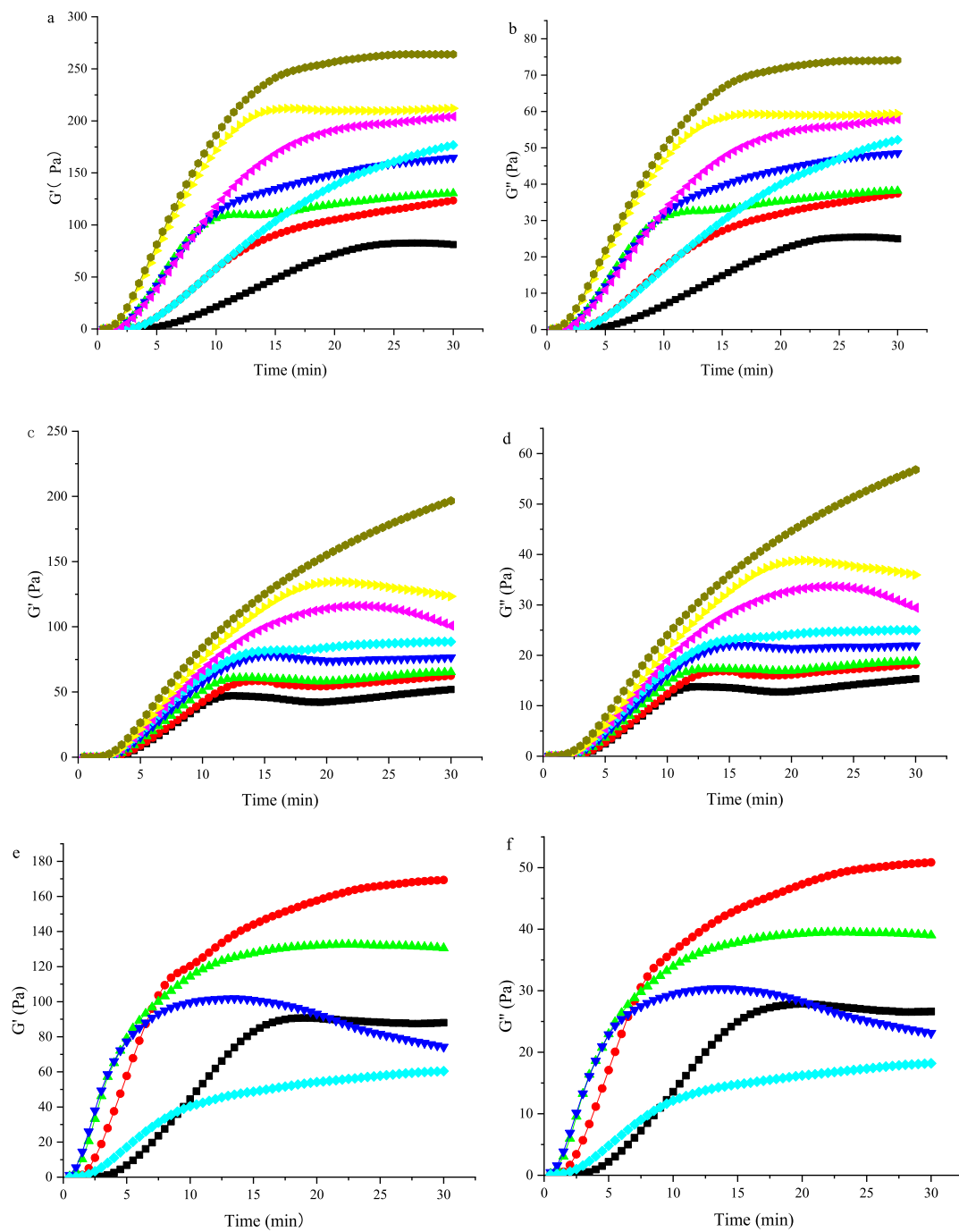


Fig. 4. Time sweep rheology of casein micelle/ κ -carrageenan rennet-induced gels (—■: 0.00 g/100 mL; —●: 0.01 g/100 mL; —▲: 0.02 g/100 mL; —▼: 0.03 g/100 mL; —◆: 0.04 g/100 mL; —◀: 0.06 g/100 mL; —▶: 0.08 g/100 mL; —◈: 0.10 g/100 mL; a, b: at 25 °C for 2 h; c, d: at 25 °C for 12 h; e, f: at 4 °C for 12 h; a, c, e: G' ; b, d, f: G''). Results are shown as averages of three replicates.

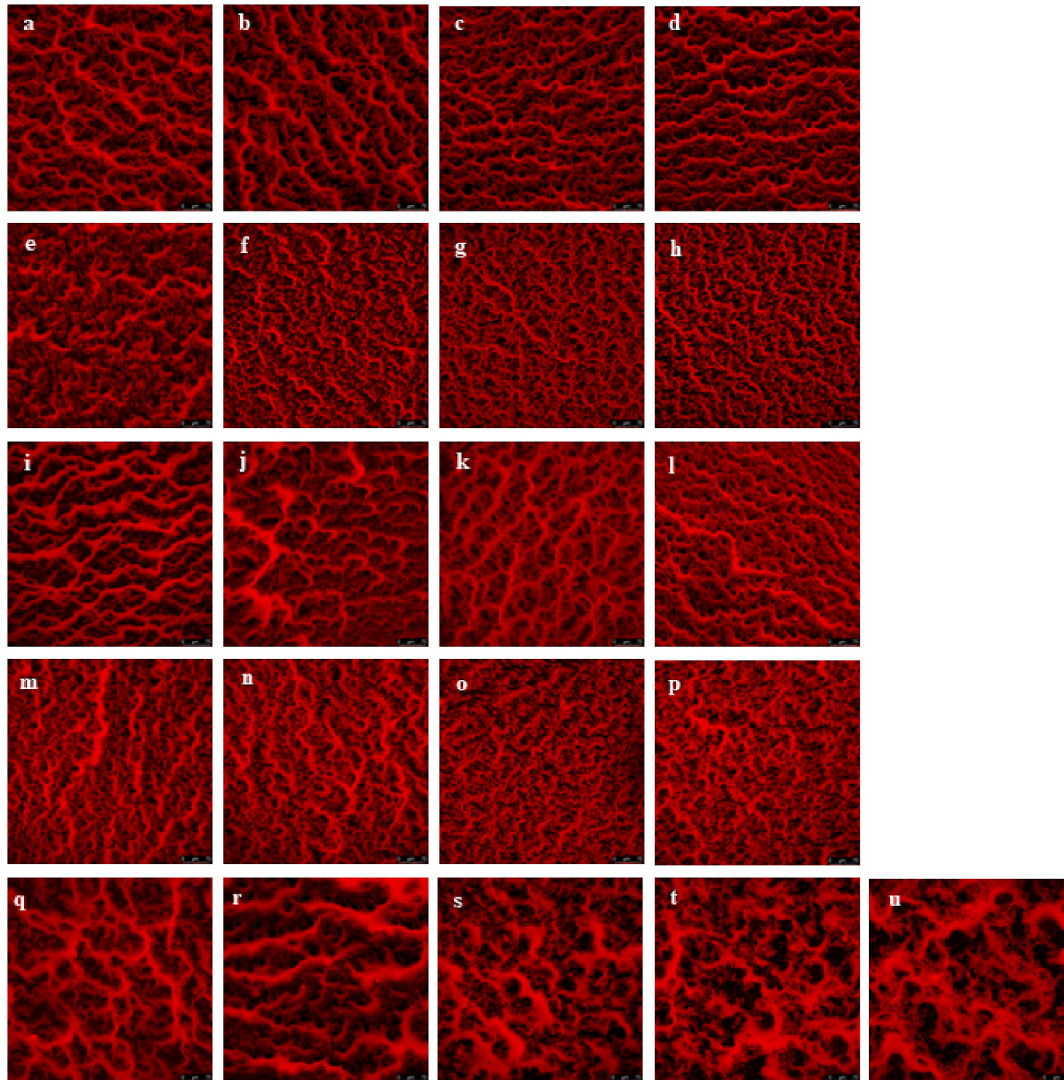


Fig. 5. Micrographs of casein micelle/ κ -carrageenan gels (a–h: at 25 °C for 2 h; i–p: at 25 °C for 12 h; q–u: at 4 °C for 12 h; a, i, q: 0.00 g/100 mL; b, j, r: 0.01 g/100 mL; c, k, s: 0.02 g/100 mL; d, l, t: 0.03 g/100 mL; e, m, u: 0.04 g/100 mL; f, n: 0.06 g/100 mL; g, o: 0.08 g/100 mL; h, p: 0.10 g/100 mL).

Table 2

Effect of concentration of κ -carrageenan on the texture of composite gels (at 25 °C for 2 h).

κ -carrageenan concentration (g/100 mL)	Hardness (g)	Adhesiveness	Resilience	Cohesion	Springiness	Gumminess	Chewiness
0.00	49.24 \pm 2.72 ^{cde}	−13.90 \pm 1.74 ^c	13.82 \pm 1.34 ^b	0.39 \pm 0.04 ^{ab}	91.64 \pm 8.49 ^a	18.95 \pm 1.07 ^{bc}	17.38 \pm 2.08 ^{ab}
0.01	49.92 \pm 5.72 ^{cde}	−17.39 \pm 4.57 ^{bc}	10.73 \pm 3.56 ^a	0.37 \pm 0.04 ^{ab}	96.05 \pm 0.42 ^a	18.60 \pm 3.52 ^{bc}	17.87 \pm 3.44 ^{ab}
0.02	57.57 \pm 4.41 ^e	−20.69 \pm 1.29 ^{abc}	9.41 \pm 0.71 ^a	0.36 \pm 0.01 ^a	95.58 \pm 0.52 ^a	20.92 \pm 2.01 ^c	20.00 \pm 1.98 ^c
0.03	56.26 \pm 2.17 ^{de}	−25.41 \pm 8.75 ^{ab}	8.15 \pm 1.13 ^a	0.36 \pm 0.01 ^a	96.37 \pm 1.12 ^a	20.09 \pm 1.32 ^{bc}	19.37 \pm 1.47 ^b
0.04	47.85 \pm 2.45 ^{cd}	−28.68 \pm 12.12 ^a	8.36 \pm 1.06 ^a	0.38 \pm 0.01 ^{ab}	96.12 \pm 1.02 ^a	18.02 \pm 0.41 ^{bc}	17.31 \pm 0.57 ^{ab}
0.06	46.97 \pm 9.81 ^{bc}	−24.85 \pm 3.88 ^{abc}	10.00 \pm 1.40 ^a	0.41 \pm 0.02 ^b	95.96 \pm 0.78 ^a	19.23 \pm 3.22 ^{bc}	18.47 \pm 3.21 ^{ab}
0.08	39.11 \pm 3.74 ^{ab}	−29.12 \pm 1.27 ^a	8.64 \pm 1.58 ^a	0.41 \pm 0.03 ^b	95.84 \pm 1.10 ^a	16.21 \pm 1.79 ^{ab}	15.54 \pm 1.70 ^a
0.10	36.18 \pm 1.15 ^a	−20.29 \pm 2.34 ^{abc}	7.87 \pm 0.85 ^a	0.37 \pm 0.00 ^{ab}	95.28 \pm 0.57 ^a	13.40 \pm 0.53 ^a	12.76 \pm 0.54 ^a

Note: different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are expressed as averages of three replicates \pm one standard deviation.

Table 3Effect of concentration of κ -carrageenan on texture of composite gels (at 25 °C for 12 h).

κ -carrageenan concentration (g/100 mL)	Hardness (g)	Adhesiveness	Resilience	Cohesion	Springiness	Gumminess	Chewiness
0.00	56.89 \pm 2.91 ^b	-17.95 \pm 1.50 ^c	14.54 \pm 2.28 ^c	0.40 \pm 0.03 ^{ab}	96.95 \pm 0.37 ^{ab}	22.52 \pm 0.69 ^c	21.83 \pm 0.59 ^b
0.01	59.45 \pm 1.92 ^b	-21.69 \pm 3.06 ^{bc}	12.77 \pm 1.99 ^{bc}	0.36 \pm 0.02 ^a	96.80 \pm 0.41 ^{ab}	21.59 \pm 1.82 ^{bc}	20.90 \pm 1.83 ^b
0.02	55.66 \pm 2.35 ^b	-21.40 \pm 2.31 ^{bc}	13.79 \pm 2.22 ^c	0.39 \pm 0.02 ^{ab}	97.31 \pm 0.12 ^b	21.65 \pm 0.44 ^{bc}	21.06 \pm 0.40 ^b
0.03	55.56 \pm 1.10 ^b	-22.47 \pm 3.10 ^{bc}	9.09 \pm 0.51 ^a	0.36 \pm 0.02 ^a	97.36 \pm 0.26 ^b	20.00 \pm 1.28 ^{ab}	19.48 \pm 1.25 ^{ab}
0.04	54.07 \pm 7.94 ^b	-20.70 \pm 2.28 ^{bc}	13.72 \pm 0.60 ^c	0.40 \pm 0.02 ^{ab}	96.56 \pm 0.67 ^a	21.60 \pm 2.25 ^{bc}	20.85 \pm 2.18 ^b
0.06	44.75 \pm 4.38 ^a	-27.94 \pm 10.15 ^{ab}	9.32 \pm 3.59 ^{ab}	0.42 \pm 0.05 ^b	96.84 \pm 0.45 ^{ab}	18.86 \pm 1.00 ^a	18.26 \pm 0.92 ^a
0.08	46.94 \pm 3.84 ^a	-32.19 \pm 2.05 ^a	6.24 \pm 0.57 ^a	0.38 \pm 0.02 ^{ab}	97.21 \pm 0.02 ^{ab}	17.93 \pm 0.57 ^a	17.43 \pm 0.55 ^a
0.10	44.07 \pm 4.29 ^a	-24.05 \pm 4.33 ^{bc}	9.88 \pm 1.61 ^{ab}	0.41 \pm 0.02 ^{ab}	97.30 \pm 0.11 ^b	17.94 \pm 1.26 ^a	17.46 \pm 1.22 ^a

Note: different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

Table 4Effect of concentration of κ -carrageenan on texture of composite gels (at 4 °C for 12 h).

κ -carrageenan concentration (g/100 mL)	Hardness (g)	Adhesiveness	Resilience	Cohesion	Springiness	Gumminess	Chewiness
0.00	51.80 \pm 1.23 ^c	-18.57 \pm 4.26 ^a	11.61 \pm 2.02 ^a	0.38 \pm 0.01 ^a	96.29 \pm 0.89 ^b	19.67 \pm 1.09 ^c	18.94 \pm 1.05 ^c
0.01	52.71 \pm 6.05 ^c	-19.55 \pm 0.76 ^a	13.50 \pm 3.20 ^a	0.40 \pm 0.02 ^a	96.25 \pm 0.80 ^b	20.86 \pm 1.80 ^c	20.09 \pm 1.88 ^c
0.02	42.03 \pm 1.24 ^b	-20.30 \pm 5.10 ^a	10.85 \pm 1.37 ^a	0.39 \pm 0.03 ^a	95.96 \pm 0.41 ^b	16.56 \pm 1.33 ^b	15.90 \pm 1.31 ^b
0.03	17.41 \pm 4.23 ^a	2.48 \pm 0.55 ^b	10.02 \pm 2.71 ^a	0.36 \pm 0.01 ^a	88.88 \pm 7.64 ^a	6.16 \pm 1.33 ^a	5.54 \pm 1.59 ^a
0.04	11.36 \pm 2.99 ^a	2.97 \pm 0.25 ^b	12.43 \pm 3.92 ^a	0.40 \pm 0.06 ^a	96.51 \pm 0.48 ^b	4.67 \pm 1.92 ^a	4.50 \pm 1.84 ^a

Note: different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

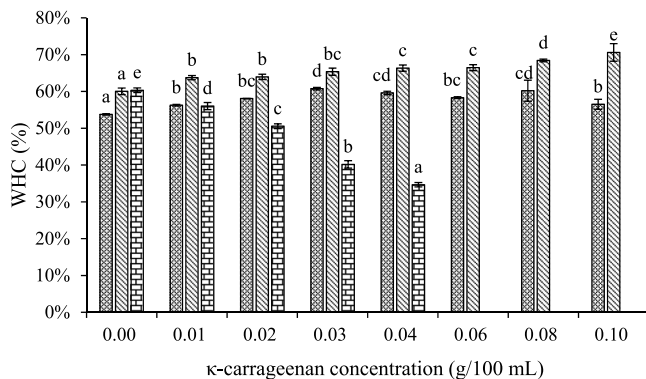


Fig. 6. Effect of concentration of κ -carrageenan on WHC of gels (□: at 25 °C for 2 h; ▨: at 25 °C for 12 h; ▩: at 4 °C for 12 h). Different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

separation in the mixed solution systems. The formation of gels was induced by the addition of rennet and it was suggested that the gel structure was influenced by the phase behavior (Ding, Huang, Cai, & Wang, 2019). Moreover, there was no microscopic phase separation in gels after the mixed solution was stored at 25 °C. In comparison, microscopic phase separation was observed in gels after the mixed solution was stored at 4 °C (Fig. 5). At high temperature conditions (>50 °C), κ -carrageenan existed in a disordered convoluted state and transformed into an ordered helix conformation after cooling, followed by aggregation of the helix structure to form gels at further cooling (Ventura, Jammal, & Bianco-Peled, 2013). It can be hypothesized that κ -carrageenan existed in an ordered helix structure at 25 °C. The average distance of sulfate groups changed from 1 nm to 0.4 nm, resulting in an increase in charge density and electrostatic adsorption between sulfate groups and positively charged amino acid residues in casein micelles at tail adsorption (Langendorff et al., 2000; Wang et al., 2014; Xu, Stanley, Goff, Davidson, & Le Maguer, 1992). The casein micelles were negatively charged on the surface and stabilized by the steric effects of casein

glycomacropptide (Holt, 1992). There was no macroscopic or microscopic phase separation in the casein micelle/ κ -carrageenan mixed solution due to the electrostatic repulsion and steric effects. The helix structure of κ -carrageenan was aggregated to form a weak gel while the stored temperature changed from 25 °C to 4 °C. The electrostatic repulsion and weak gel structure prevented macroscopic phase separation, but microscopic phase separation existed (Bourriot et al., 1999).

Due to the rough and porous surface of casein micelles, large polysaccharides could not enter but small molecules (rennet) could enter and acted on the specific sites. (Corredig et al., 2011). The polymer concentration was increased after the addition of κ -carrageenan, leading to an earlier RCT of the gel network structure formation in the mixed gels than that of the casein micelle gel. In addition, κ -carrageenan and casein micelles were both negatively charged in the solution. The electrostatic repulsion increased and the formation of casein micelles was encouraged as the concentration of κ -carrageenan increased (Li, Guo, Li, et al., 2021). There was no microscopic phase separation in the mixed system and the G' increased with the increase of κ -carrageenan concentration. The composite gels network structure was denser and could be tightly bound to more water while the mixed solution was stored at 25 °C than

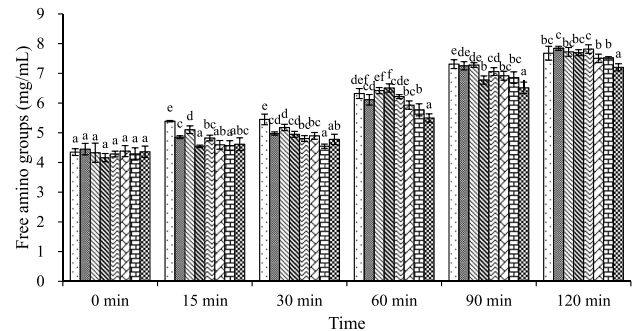


Fig. 7. Effect of concentration of κ -carrageenan on free amino groups content in the simulated gastric digestion solution (□: 0.00 g/100 mL; ▨: 0.01 g/100 mL; ▩: 0.02 g/100 mL; ▪: 0.03 g/100 mL; ▫: 0.04 g/100 mL; ▬: 0.06 g/100 mL; ▮: 0.08 g/100 mL; ▯: 0.10 g/100 mL). Different lowercase letters superscripted indicate significant differences between additions of κ -carrageenan ($p < 0.05$). Results are shown as averages of three replicates \pm one standard deviation.

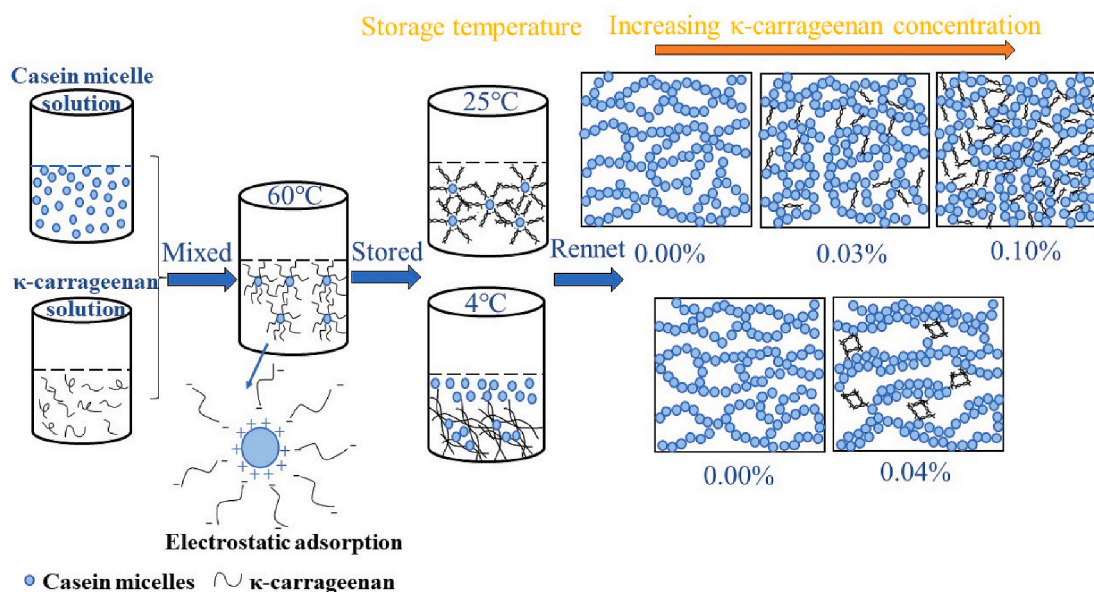


Fig. 8. Schematic diagram of formation mechanism of casein micelle/ κ -carrageenan gel.

4 °C. Before the addition of rennet, the weak gel network formed by the aggregation of the helical structures of κ -carrageenan resulted in microscopic phase separation of the mixed solution during storage at 4 °C. Therefore, the G' decreased and the gel network structure pore sizes were large with the increase of κ -carrageenan concentration, resulting in a decrease in its ability to bind water.

4. Conclusions

In conclusion, this study demonstrated the effects of κ -carrageenan concentration and storage temperature on the properties of composite gels. Casein micelle solution and κ -carrageenan solution were mixed and stored at different temperature conditions. Complexes were formed by electrostatic adsorption without macroscopic phase separation. The mixed system was stabilized by electrostatic interaction at 25 °C. The appearance of macroscopic phase separation was prevented by the electrostatic interaction between proteins and polysaccharides and the weak gel structure formed by κ -carrageenan at 4 °C. The gel was formed by the addition of rennet. Both the κ -carrageenan concentration and storage conditions affected the microstructure of the composite gels. Moreover, there was no microscopic phase separation in composite gels and the network structure was dense with a small pore size when κ -carrageenan concentration increased at 25 °C. Microscopic phase separation was appeared in the mixed system and a sparse with a large pore size structure was formed at 4 °C. The addition of κ -carrageenan reduced the RCT and gel strength. The G' and WHC were increased with the addition of κ -carrageenan at 25 °C while they were decreased at 4 °C. The mixed gels with better WHC were obtained at 25 °C than that at 4 °C. The microstructure of the gels was diverse, which could be used in the dairy product to obtain specific products by changing κ -carrageenan concentrations and storage conditions. Moreover, the composite gels delayed the hydrolysis of proteins during gastric digestion. Therefore, the composite gel could be used as embedding materials to encapsulate bioactive substances.

CRedit authorship contribution statement

Yumeng Zhang: Data curation, Formal analysis, Writing. **Bo Song:** Methodology, Formal analysis. **Xiaodan Wang:** Formal analysis. **Wenyuan Zhang:** Formal analysis. **Huiquan Zhu:** Formal analysis. **Xiaoyang Pang:** Formal analysis. **Yunna Wang:** Investigation,

Validation. **Ning Xie:** Investigation, Validation. **Shuwen Zhang:** Investigation, Validation, Supervision. **Jiaping Lv:** Resources, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

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