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Complex Characterization and Formation Mechanism of Scallop (*Patinopecten yessoensis*) Protein Hydrolysates/κ-Carrageenan/Konjac Gum Composite Gels †

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Abstract: This study aimed to investigate the effects of κ-carrageenan (KC) and konjac gum (KGM) on gelaiton and microstructural properties of scallop male gonads hydrolysates (SMGHs) as well as the governed intermolecular interactions. The *G'* values of SMGHs/KGM/KC significantly increased by 3.6- and 108.5-fold in comparison to that of SMGHs/KC and KGM/KC gels at 0.1 Hz, with corresponding melting temperature increasing from 27.9 (KGM/KC) and 30.0 (SMGHs/KC) to 33.7 °C (SMGHs/KGM/KC), respectively. Moreover, the ternary mixture of SMGHs/KGM/KC formed a condensed and compact network structure with thick walls as well as showed a rougher structure with more aggregation due to the generation of macromolecular biopolymers. Furthermore, electrostatic and hydrophobic interactions were found to be the main interaction forces, while hydrogen bonds were secondary in the gelation of SMGHs/KGM/KC gels.

Keywords: κ-Carrageenan; konjac gum; scallop male gonads hydrolysates; gel properties; electrostatic and hydrophobic interactions

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1. Introduction

Scallop (*Patinopecten yessoensis*) is a kind of marine bivalve mollusk belonging to the family of Pectinidae. In China, its production has increased to 1.7 million tons in 2020. It has been reported that scallop male gonads hydrolysates (SMGHs) exhibit unique gel behaviour with porous three-dimensional gel network in the presence of polysaccharide [1,2]. These observations indeed prompt us to embark on a systematic study to examine the gelling behavior of SMGHs in the presence of polysaccharides, thus further enhancing the shellfish-based protein utility toward improving human health by developing novel organoleptic food products.

Among polysaccharides, KC and konjac gum (KGM) have gained increasing attention due to their inherently hydrophilic nature, which plays vital roles on improving the stabilizing performance of proteins via complexation. Therefore, study on the gel properties and microstructures of ternary gel system formed by SMGHs and KGM/KC is necessary for the development of novel organoleptic functional soft biomaterials in food industry.

The purpose of this study was to investigate the influence of binary polysaccharides KGM/KC on gel properties of SMGHs as well as the intermolecular interactions within them. SMGHs/KGM/KC ternary gel was fabricated and the rheological properties of samples were detected by a rheometer. The group changes in ternary gel were characterized

by fourier transform infrared (FTIR). The gel microstructure and surface morphology were observed by cryo-scanning electron microscopy (cryo-SEM) respectively.

2. Materials and Methods

2.1. Materials and Chemicals

Scallop *P. yessoensis* was purchased from local seafood market (Dalian, China). The male gonads collected from scallop body were cleaned carefully and then boiled for 10 min to inactivate endogenous enzymes. After freeze-drying, the powders of scallop male gonads were stored at –80 °C for further analysis.

KC and KGM with purity both more than 95% were obtained from Aladdin Co., Ltd. (Shanghai, China) and Yuanye Biotech Co., Ltd. (Shanghai, China), respectively. Trypsin was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Guanidine hydrochloride (GuHCl), urea and SDS were provided by Sangon Biotech Co., Ltd. (Shanghai, China). All the other reagents were of analytic grade.

2.2. Preparation of SMGHs

Scallop male gonad powders contained 81.7% of crude protein were dissolved in deionized water at 4% (*w*/*v*) of protein. The reaction suspension was initiated by trypsin (3000 U/g protein) addition with 3 h incubation. The pH of the enzymatic system was kept at 8.0 by the addition of NaOH (0.1 M) with ceaselessly stirring at 37 °C. Finally, the obtained hydrolysates were boiled for 10 min to inactivate trypsin, followed by freeze-dried and stored at −80 °C for further analysis.

2.3. Preparation of Gel Samples

The SMGHs (2.5%, *w*/*v*) powders were mixed with deionized water in the presence or absence of KC and KGM powders (KC: KGM = 5.5: 4.5) at 80 °C for 30 min. The mass concentration of SMGHs, KC and KGM was 2.5%, 0.34% and 0.28%, respectively. The samples were prepared at natural pH without any salt addition. The solutions of SMGHs (2.5%), KC (0.34%) and KGM (0.28%) were also prepared separately as controls. All the samples were cooled to room temperature and the bubbles of samples were removed by centrifuged at 5000× *g* for 10 min. Finally, all the samples were stayed at 4 °C for 16 h for sufficient hydration and gel formation.

For determination of intermolecular forces, chemicals including NaCl (1 M), GuHCl (1 M), urea (4 M) and SDS (2%) were dissolved in deionized water before SMGHs, KC or KGM (0.28%) powders addition, and then the samples were soaked into corresponding solution at 4 °C for 16 h for sufficient hydration and gel formation.

2.4. Characteration

The rheological properties of samples were measured by a rheometer (Discovery HR-1) from TA Instruments Menu Co., Ltd., USA equipped with a parallel plate geometry (d = 40 mm).

Frontier FTIR spectrometer (PerkinElmer, USA) was used to observe the infrared spectra of sample at 25 °C.

The images of gel sample microstructures were obtained by cryo-SEM and viewed by SU8010 SE (Hitachi Co., Ltd., Tokyo, Japan) at 2000 × magnification with the accelerated voltage of 1.0 kV.

2.5. Statistical Analysis

All the experiments were repeated for three times and the data was shown as mean value ± standard deviation. Significant differences of sample means were analyzed by the Student's t-test. A level of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Gel Profiles of SMGHs Affected by KGM and KC

As shown in Figure 1, the single SMGHs (2.5%, *w*/*v*), KC (0.34%, *w*/*v*) and KGM (0.28%, *w*/*v*) as controls could hardly form the gel and perform obvious flow behavior like liquid. Separately, KGM/KC, SMGHs/KC and SMGHs/KGM performed as liquid, weak gel and solid gel, respectively (Figure 1). Moreover, the development of ternary gel of SMGHs/KGM/KC fabricated at the mass concentration of 2.5%, 0.34% and 0.28% maintained the hard gel structure with no flow behavior, conveying the synergistic effect between SMGHs and KGM/KC (Figure 1). Therefore, it is suggested that KGM/KC chains would inter penetrate well within the SMGHs network and augment the network strength in yielding solid and strong gel.

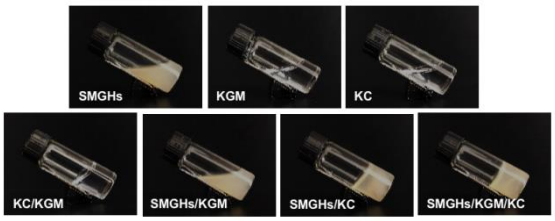

Figure 1. Visual appearance of SMGHs affected by KC and KGM. The mass concentration of SMGHs, KC and KGM was 2.5%, 0.34% and 0.28% (*w*/*v*), respectively.

3.2. Rheological Behavior of SMGHs Affected by KGM and KC

Obviously, the *G*′ and *G*″ values of SMGHs, KC, KGM as well as SMGHs/KGM were extremely low (<10 Pa) within frequency of 0.1-10 Hz (Figure 2A), which were consistent with the liquid appearance of the samples in Figure 1. In terms of gel groups of KGM/KC, SMGHs/KC and SMGHs/KGM/KC, the *G*′ values were significantly higher than *G*″ values, accentuating the superior elasticity of these solid samples and the formation of continuous network structures (Figure 2A). Moreover, SMGHs/KC and SMGHs/KGM/KC gels showed stable *G*′ and *G*″ values independent on frequency, reinforcing an ideal gel with stiff behavior [3]. Furthermore, the *G*′ and *G*″ values at 0.1 Hz of ternary system were significantly higher than that of binary ones, with values of 271.2 and 87.0, 74.6 and 9.9, as well as 2.5 and 0.5 Pa of SMGHs/KGM/KC, SMGHs/KC and KGM/KC, respectively, almost 3.6- and 108.5-fold increment in terms of G′ (Figure 2A). The improvement of the G′ values could be incurred by peptide-peptide and peptide-polysaccharide interactions [4].

Figure 2B presented the thermal profile of the elastic modulus obtained. KGM performed inconspicuous positive impact on improvement of *G*′ values and only promoted the appearance of melting temperature of 22.4 °C of SMGHs/KGM (data not shown). Different with KGM, KC showed prominent optimistic effect on the improvement of SMGHs gel properties, with initially *G*′ values increasing from 0.3 (SMGHs) to 229.8 Pa (SMGHs/KC) at 4 °C, melting temperature of 30.0 °C and gelling temperature of 25.1 °C, respectively (Figure 2B). Moreover, the addition of KGM could further augment the melting and gelling temperature combined *G*′values of SMGHs/KC, with the achieved values of 33.7 and 27.7 °C, 469.0 for SMGHs/KGM/KC gels at 4 °C respectively (Figure 2B). Moreover, the formation of SMGHs/KGM/KC, SMGHs/KC and KGM/KC gels could be distinguished as thermally reversible gels as G′ decreased with the rising temperature and inversely increased during cooling [3]. In the current work, the recovered *G*′ in SMGHs/KC and KGM/KC gels during cooling could not reach the same initial level in heating phase (4 °C) with values of 229.7 and 16.9 Pa, respectively, which might be ascribed to less hydrogen bonds formation than the disrupted ones during heating (data not shown).

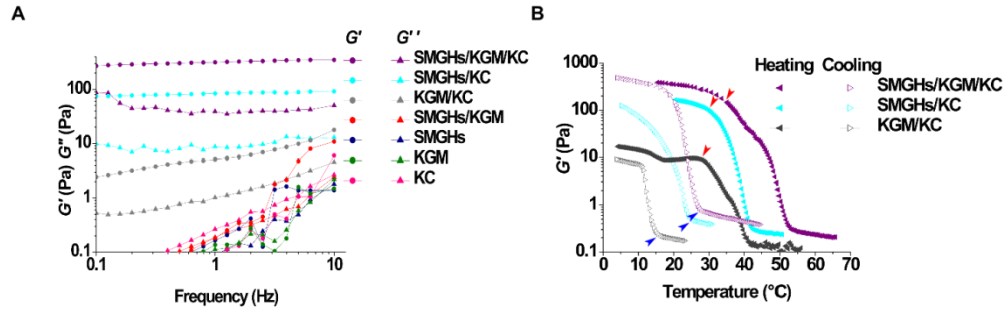

Figure 2. Rheological behavior of SMGHs affected by KC and KGM. (**A**) Storage modulus (*G*′), loss modulus (*G*″) -frequency profiles; (**B**) Storage modulus (*G*′)-heating (filled symbols) and cooling (open symbols) ramp profiles. The red and blue arrows indicate the melting and gelling temperature of the examined samples, respectively. The mass concentration of SMGHs, KC and KGM was 2.5%, 0.34% and 0.28% (*w*/*v*), respectively.

3.3. Changes in Conformation Characterization of SMGHs Affected by KGM and KC

Figure 3 compared the FTIR spectra from SMGHs, KGM and KC as well as corresponding binary and ternary mixtures. In terms of SMGHs/KGM/KC, the hydroxyl group vibration peaks showed obvious blue shift as well as stronger intensity in comparison to SMGHs/KC, from 3351 to 3314 cm^{-1}, reinforcing the generation of intermolecular hydrogen bonds as KGM addition [5]. In addition, a new peak of 1155 cm^{-1} was observed in SMGHs/KGM, SMGHs/KC and SMGHs/KGM/KC systems in comparison to SMGHs (Figure 3), which might be associated with glycoside bond formation due to the development of an ordered and strong gel network caused by the synergy within SMGHs, KGM and KC.

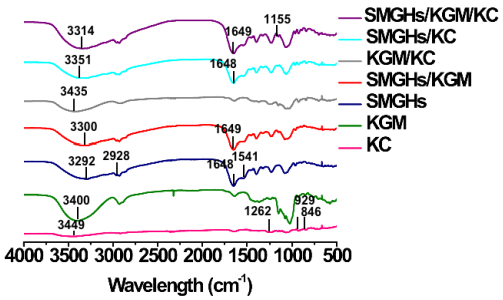

Figure 3. Changes in conformation characterization of SMGHs affected by KC and KGM. The mass concentraton of SMGHs, KC and KGM was 2.5%, 0.34% and 0.28% (*w*/*v*), respectively.

3.4. Microstructure of SMGHs Affected by KGM and KC

As shown in Figure 4A, the loose gel network of KC was strengthened obviously by the addition of KGM, in which KGM/KC became compact and uniform in comparison to single KGM and KC, which might be responsible for the high synergistic effect of the KGM/KC. Moreover, both of SMGHs/KGM and SMGHs/KC could exhibit porous gel network, where SMGHs/KGM showed coarse gel microstructure with larger voids and accidented walls, while SMGHs/KC exhibited homogeneously distributed network with smaller holes and smoother walls, similar to observations by Yan et al. [3]. Furthermore, SMGHs/KGM/KC ternary gels formed a condensed gel of compact structure with denser pore sizes and thicker walls in comparison to other microstructures, accentuating excellent gel properties of the ternary system, which were consistent with the rheological and moisture-distribution data. Generally, magnitude of the force immobilizing the water is inversely related to the pore size [6]. In this case, the addition of KGM/KC to SMGHs promoting a more porous microstructure could explain stronger gel strength and higher water retention.

3.5. Surface Morphology of SMGHs Affected by KGM and KC

As shown in Figure 4B, both of SMGHs and KGM presented a smoother surface in comparison to other samples and SMGHs hold very few fibers. The image of KC showed a uniformly distributed and coarse punctate structure among peaks without aggregation (Figure 4B), Typically, low concentration of KC allows the observation of helix formation and chain-chain aggregation instead of visualization of network structure. The increased aggregation on the surface of SMGHs/KC, SMGHs/KGM and SMGHs/KGM/KC reinforced strong interactions between hydrolysates and polysaccharides, whose structure might be intertwined by polysaccharide strands presenting in the pores formed by the SMGHs [7]. Furthermore, SMGHs/KGM/KC showed a rougher structure with more aggregation (Figure 4B), which might mainly due to the electrostatic interactions between SMGHs and polysaccharides. Thus, the numerous pores among peaks illustrated the obvious network structures of KC, SMGHs/KGM, SMGHs/KC and SMGHs/KGM/KC which were consistent with the observations in Cryo-SEM (Figure 4A).

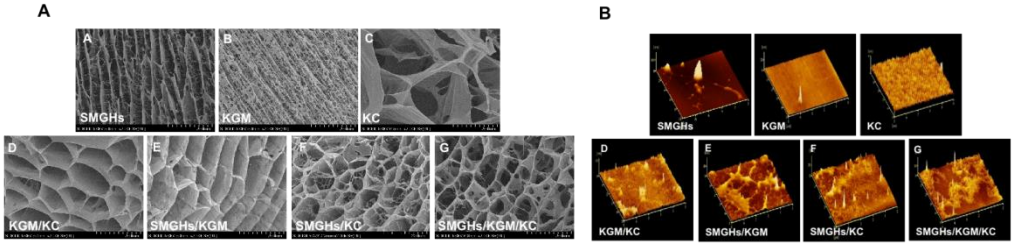

Figure 4. Microstructure images of SMGHs affected by KC and KGM. (**A**) Cryo-SEM images of SMGHs affected by KC and KGM with magnification times of 2000×. (**B**) AFM images of SMGHs affected by KC and KGM. The mass concentration of SMGHs, KC and KGM for cryo-SEM was 2.5%, 0.34% and 0.28% (*w*/*v*), respectively and for AFM was 0.005%, 0.00068% and 0.00056% (*w*/*v*), respectively.

3.6. Intermolecular Forces of SMGHs Affected by KGM and KC

As shown in Figure 5, the addition of four chemical reagents all significantly decreased the *G*′ and *G*″ values of three obtained gel systems. To be specific, NaCl and SDS had stronger effects than urea and GuHCl to deteriorate the KGM/KC gels with extremely flow appearance, which could further reduce the relative shortage modulus *G*′ and loss modulus *G*″ less than 1% (Figure 5A), epitomising the dominant role of hydrophobic and electrostatic interactions in KGM/KC gels. GuHCl and urea treatment could decreased the relative *G*′ and *G*″ less than 15% (Figure 5A). Accordingly, KC is anionic polysaccharide and KGM is neutral polysaccharide, therefore, Na+ as cation would obviously affect the gel properties through interfering the electrostatic interactions between KC and SMGHs. As reported by Wei et al. [8], excess Na^+ (>0.3 M) could neutralize the negative charges of compound gels which further causes the decrease of gel elasticity in KGM/KC. Therefore, it is suggested that electrostatic and hydrophobic interactions are dominated in the KGM/KC gel maintenance.

In terms of SMGHs/KC gels, NaCl principally deteriorated the rheological properties with the transformation from solid to slimy state and relative *G*′ and *G*″ reducing to 14.72% and 18.92%, respectively (Figure 5B). GuHCl slightly destroyed the gel profile of SMGHs/KC and suppressed its relative *G*′ and *G*″ to 38.85% and 36.64%, respectively (Figure 5B). Moreover, urea and SDS hardly changed the SMGHs/KC gel performance with solid state while still weakened the relative *G*′values of SMGHs/KC to 45.72% and 63.52%, respectively (Figure 5B). Typically, high ionic strength could suppress the electrostatic interactions between proteins and polysaccharides via screening the charges of the biopolymers, impeding the proteins/polysaccharides complexes formation [9]. Therefore, it is suggested that the intermolecular forces between SMGHs and KC are mainly electrostatic interactions, and hydrophobic interactions as well as hydrogen bonds are secondary.

As regards to SMGHs/KGM/KC gels, NaCl still showed the strongest destructive effect which could decreased relative *G*′ and *G*″ values to 3.16% and 2.58% (Figure 5c), reinforcing the dominant role of electrostatic interactions. Similar to the visual appearance of SMGHs/KC in Figure 5B, GuHCl slightly changed the shape of the gel, and urea as well as SDS could maintain the gel performance (Figure 5C). Urea treatment decreased the relative G′ values of ternary gels to 62.36%, while 45.72% in SMGHs/KC gels (Figure 5B,C), from which we could assume that less hydrogen bonds generation in SMGHs/KGM/KC gels. GuHCl and SDS weakened the SMGHs/KGM/KC gels analogously with corresponding relative *G*′ and *G*″ values of 15.67%, 7.68% and 17.69%, 10.41%, respectively (Figure 5C), symbolising the contribution of hydrophobic interactions to initial gel structure development. Noteworthily, the effects of GuHCl and SDS in SMGHs/KGM/KC gels were more obvious than those in SMGHs/KC gels, accentuating that hydrophobic interactions were more important in ternary network structure than binary one.

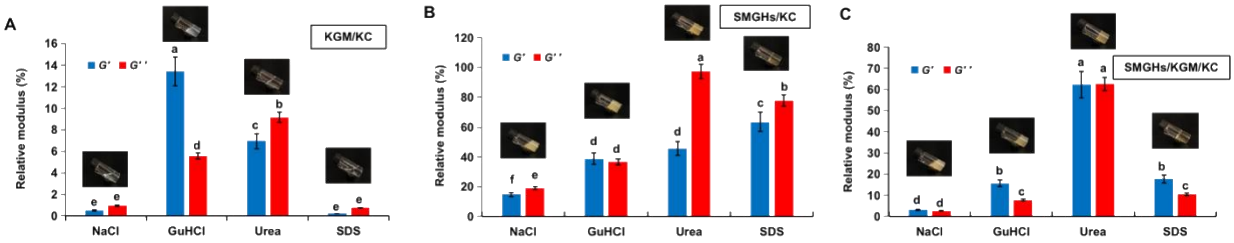

Figure 5. Gel profiles and rheological behavior of KGM/KC, SMGHs/KC and SMGHs/KGM/KC affected by several chemicals. Effect of NaCl (1 M), GuHCl (1 M), urea (4 M) and SDS (2%) on gelation properties of KGM/KC (**A**), SMGHs/KC (**B**) and SMGHs/KGM/KC (**C**) reflected by relative modulus. The final concentration of SMGHs, KC and KGM was 2.5%, 0.34% and 0.28% (*w*/*v*), respectively. The relative storage (loss) modulus *G*′ (*G*″) of samples represented the ratio of storage (loss) modulus *G*′ (*G*″) values of samples in the presence of chemicals to those of controls (assigned to 100%). Different small letters indicate significant differences.

4. Conclusions

Scallop *P. yessoensis* male gonad hydrolysates showed the synergistic interaction with the mixture of KC and KGM. The addition of KGM evidently increased gel properties of SMGHs/KC, which might be attributed to the enhancement of hydrophobic interactions of polymers. Ternary gels of SMGHs/KGM/KC formed a condensed and compact structure of gel network coupled with increased aggregation and rough surface, contributing to excellent gel properties and higher water retention capacity. The present study gives a brief gelation mechanism of SMGHs/KGM/KC gels, in which electrostatic and hydrophobic interactions were dominated and hydrogen bonds were secondary in gel formation. The considerable gel performance of SMGHs/KGM/KC gels might be promised as functional food hydrocolloids with applications of textural modification, fat replacer development and microcapsule formation in food and biological fields.

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Conflicts of Interest:

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