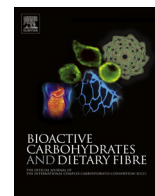




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## Environment induced self-aggregation behavior of $\kappa$ -carrageenan/lysozyme complex

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### ABSTRACT

Self-aggregation behaviors of  $\kappa$ -carrageenan/lysozyme (CRG/Ly) complex inducing by intrinsic and extrinsic factors were investigated from macro and micro aspects, including the ratio, pH, temperature and salt. High Ly content promote aggregation behavior of CRG/Ly complex, while CRG played a suppressive role. Except for CRY5, the complex displayed nano-size distribution. Both CRG addition and the self-aggregation behavior could enhance the thermal stability of Ly. Low  $\zeta$ -potential induced by pH made the CRG/Ly complex easy to self-aggregate into structure with large size due to the gradual association of complex to form larger interpolymeric complexes. The tunable process means to control the dissociation and association behavior between interpolymeric complexes and soluble complex by pH. All systems shared high NaCl tolerance and presented stable transmittance. Heat-induced self-aggregation exhibited a temperature and polymers ratios dependant manner. The mechanisms of environment induced self-aggregation into interpolymeric complexes reflected the re-balanced physical interaction when they suffered from any treatment. Controlling and regulating self-aggregation behavior of protein/polysaccharide system could enrich the soft structures from food respective and also provide practical theory for food process.

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### 1. Introduction

Polyelectrolyte complexes, preparing by mixing solutions of oppositely charged polyelectrolytes, have been found for many years (Laneuville, Turgeon, Sanchez, & Paquin, 2006; Thünemann, Müller, Dautzenberg, Joanny, & Löwen, 2004). Protein and polysaccharide, two common natural polyelectrolyte, are known to have complicated interactions and hard to control (Evans, Ratcliffe, & Williams, 2013; Luo & Wang, 2014). Their complexation is always the active field of research in the past decade because their simultaneous use commonly occurs in food products. And their interactions play a significant role in regulating the structure, texture, and stability of food systems (Doublier, Garnier, Renard, & Sanchez, 2000; Schmitt & Turgeon, 2011; Tran & Rousseau, 2013). The protein/polysaccharide complexes have been widely used as friendly vehicle to control release of proteins and drugs (Liu et al., 2013; Salmaso & Caliceti, 2013), to encapsulate bioactive and other compounds (Yang, Wu, Li, Zhou, & Wang, 2013), and to fabricate

structured organization (Gosal & Ross-Murphy, 2000).

However, the instability of complexes formed by oppositely charged protein and polysaccharide is an inevitable problem. Electrostatic interaction is the prevalent primary interaction controlling the complexation of proteins/polysaccharides, which can also be affected by many extrinsic factors, such as ionic strength, pH, protein/polysaccharide ratio and thermal treatment (Li et al., 2012; Schmitt & Turgeon, 2011). Any external disturbances may induce phase transition. Therefore, there is a need to understand the self-aggregation behavior induced by environmental conditions for better controlling this transition. Although many complex systems have been widely studied that mainly focused on the milk protein, vegetable proteins, cereal protein, egg protein and other protein with anionic or cationic polysaccharides (Hosseini et al., 2013; Lacroix & Li-Chan, 2014; Teng, Luo, & Wang, 2013; Luo, Zhang, Whent, Yu, & Wang, 2011). The intrinsic factors of each proteins/polysaccharides system, such as molecular weight and charge density, determined that each associative behavior was different with other systems (Schmitt & Turgeon, 2011).

Both the two biopolymers of lysozyme and  $\kappa$ -carrageenan are extensively used in food and medicinal application. CRG is a kind of sulfated linear polysaccharide extracted from red seaweed, and

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constitute of galactose and anhydrogalactose units which are linked by glycosidic bonds (Rodrigues, da Costa, & Grenha, 2012). CRG is a kind of bioactive polysaccharides and dietary fiber with strong ionic properties for half-ester sulfate group, and therefore has a high capacity to interact with proteins. Ly, the main protein component of the egg white fraction, has a molar mass of 14.3 kDa and been received attentions for its use as a kind of food preservative (Schuh, Schwarzenbolz, & Henle, 2010). As our group reported before the two biopolymers with opposite charges could easily self-assemble into soluble complex in neutral condition (Xu et al., 2014). But the self-aggregation behavior of the system induced by environment has also not been reported.

Therefore, in the present work, we investigated self-aggregation behavior of CRG/Ly complex induced by intrinsic and extrinsic factors, including initial CRG/Ly ratio, pH, ionic strength and heat treatment. The transmittance, size,  $\zeta$ -potential, micro-structure scanning electron microscope (SEM) and transmission electron microscopy (TEM) were used to monitor the association from optical images and micro structure. The purpose of this study was to evaluate the effects of pH, NaCl concentrations, the CRG/Ly ratios and thermal treatment on the self-aggregation behavior and the environmental tolerance of the complexes.

## 2. Materials and methods

### 2.1. Materials

Lysozyme (Ly, Mw=14.3 kDa) from chicken egg white was obtained from Sinopharm Chemical Reagent co., LTD, and  $\kappa$ -carrageenan (CRG) was purchased from Aladdin Chemistry Co. LTD. Other chemicals were reagent grade and used without purification. All the solutions used in the experiments were prepared using ultrapure water through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

### 2.2. Preparation of complexes

Ly was dissolved in purified water with gentle magnetic stirring for 2 h at room temperature at the concentration of 1.0 mg/mL. The same concentration of CRG solution (1.0 mg/mL) was prepared by stirring the solution at 70 °C for 30 min for complete dissolution. The two biopolymers were mixed with different weigh CRG/Ly ratios of 3:1, 2:1, 1:1, 1:2 and 1:3. The resulted mixtures were defined as CRY1, CRY2, CRY3, CRY4 and CRY5, respectively. Additionally, the effects of pH, salt and heat treatment on self-aggregation behavior of the CRG/Ly complex were further investigated.

### 2.3. Size and $\zeta$ -potential measurements

The nano/micro size and  $\zeta$ -potential measurements were analyzed by dynamic light scattering (Nano-Z, Malvern Instruments, UK) and a Laser Particle Size Analyzer (Malvern 2000, Malvern Instruments, UK). The complexes were directly measured at 25 °C and the  $\zeta$ -potential was determined from the manufacturer's software (version 6.34). Each measurement was performed in triplicates.

### 2.4. Transmittance measurements

Transmittance of the CRG/Ly system was measured at 600 nm as described in previous method at different condition (Lin, Chen, & Liu, 2009), including different pH (4.0–9.0), salt concentration (0–25 mM) and heat treatment (60 °C and 80 °C for 30 min). The purified water was chosen as the blank at the same condition.

### 2.5. Differential scanning calorimetry measurement

Differential scanning calorimetry (DSC) experiments were performed using a 204-F1 (Netzsch, Germany). The freeze-dried CRG/Ly mixtures and Ly (2–5 mg) with 10  $\mu$ L of water were placed in an aluminum pans. Then the aluminum pans were sealed and equilibrated at 25 °C for 24 h (Kumar, Ganesan, Selvaraj, & Rao, 2014). The samples were measured at the scanning rate of 5 °C/min with the temperature ranging from 40 °C to 110 °C. Another aluminum pan without sample was chosen as a reference.

### 2.6. Morphological observation

Scanning electron microscope (JSM-6390LV, Japan) and transmission electron microscopy (H-7650, Hitachi, Japan) were used to further intuitively observe the morphology of the self-aggregation. Before SEM experiment, the vacuum freeze dried CRG/Ly mixtures were coated with about 20 nm gold-palladium under argon atmosphere using a gold sputter module in a high vacuum evaporator. The sputtered time was about 30 s and the accelerating voltage was 15 kV. For TEM test, a drop of CRG/Ly solution was dispensed directly onto a carbon coated copper grid and allowed to dry automatically in a vacuum desiccator. Then the pictures of the prepared samples were obtained with desired magnification.

## 3. Results and discussion

### 3.1. Aggregation behavior

Aggregation behavior was common phenomenon for protein/polysaccharide systems. But the behavioral properties were different and determined by intrinsic and extrinsic factors. The polymer molecules with different molecular weight and charge density intrinsically decided the complex formation and its phase transition. For CRG/Ly system, the complex formed automatically without any treatments. The size variation of the complex with the ratios of CRG/Ly illustrated the aggregation behavior (Fig. 1). The average size increased as the ratios of CRG/Ly increased. The increasing rate was slow first, and then increased dramatically as the Ly contents were further increased. The first increase was beneficial to the higher Ly content and the lower densities of CRG/Ly system that endowed the Ly could efficiently combine with CRG. The high negative charges obtained from CRG could further help to sustain the system stability. At high Ly concentration, more Ly could be available for its physical interaction with CRG thus making a CRG/Ly complex. Additionally, CRG/Ly systems with

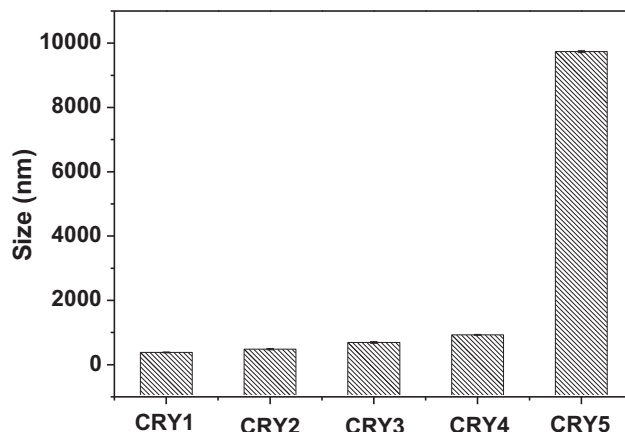


Fig. 1. The size of CRG/Ly system (natural pH about 6.5) with different ratios.

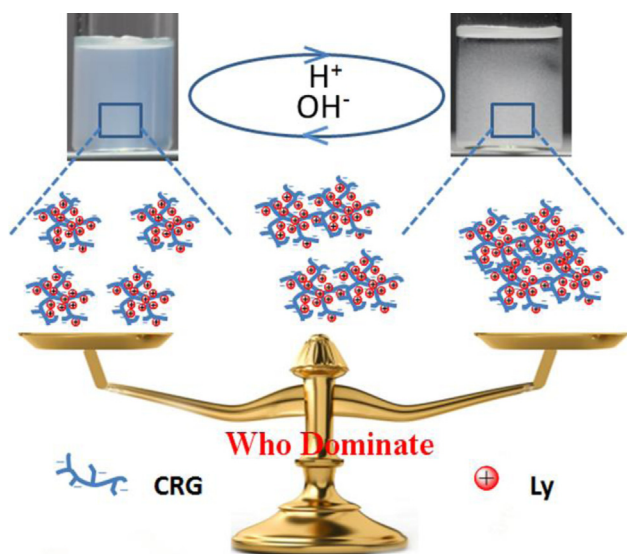


Fig. 2. Self-aggregating model of CRG/Ly complex induced by environmental condition.

lower ratios were sooner electrical charge neutralized resulting in low charged environment for CRG/Ly complex which further induced their easy self-aggregation into interpolymeric complexes with a larger size (Fig. 2). The high protein induced self-aggregation of protein/polysaccharide system has also been found by other researchers. Complexation of bovine serum albumin and pectin showed that moderately high BSA content helped to form the complex, while excessive content could lower the transmittance for self-aggregation of the complex (Li et al., 2012; Ru, Wang, Lee, Ding, & Huang, 2012). Huang et al. (2012) also found that protein to polysaccharide ratio in the mixture had an influence on the charges balance of their complexes. Turbidity of SPI/Chitosan complex decreased as the SPI to Chitosan ratio increased (Huang, Sun, Xiao, & Yang, 2012). The results were well agreed with the size variation of CRG/Ly complex as the function of CRG to Ly ratio.

### 3.2. Morphology observation

For intuitively observing aggregation morphology, the microstructures of CRG/Ly complex via different ratios were investigated by SEM and TEM. The SEM results displayed that the CRG/Ly system was homogeneous with abundant nanoparticles (Fig. 3). No obvious aggregation was found for CRY1, CRY2, CRY3 and CRY4, indicating the CRG/Ly complex was stable in the four systems. However, apparent particle about 1  $\mu\text{m}$  in size could be available for CRY5. The results well coincided with size variation of CRG/Ly complex. The amplified picture showed that the morphology of the self-aggregation was irregular and the size was not uniform. The phenomenon may be caused by the freeze drying processing during the sample preparation.

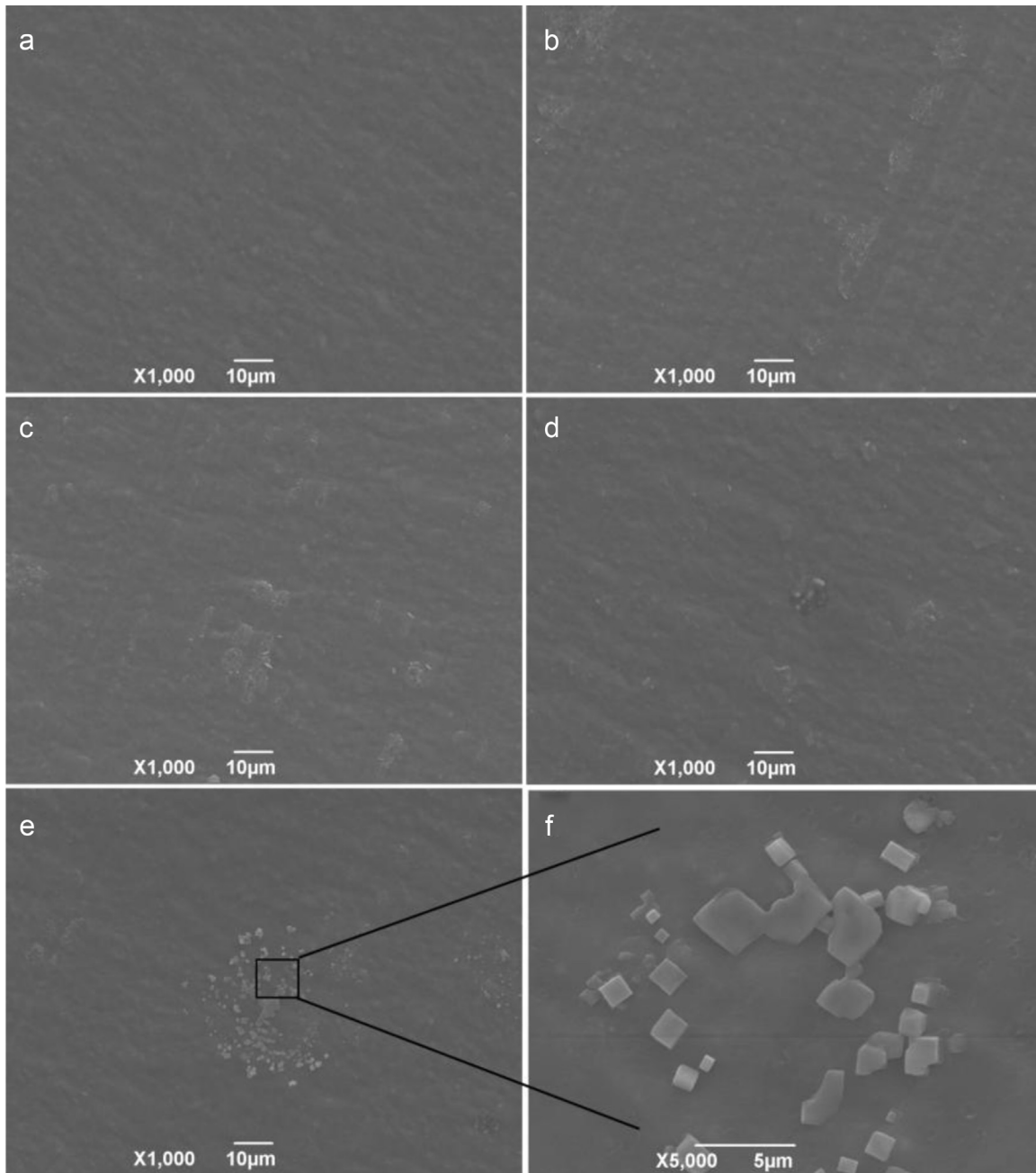
Compared with SEM, TEM (Fig. 4) could factually present the CRG/Ly complex aggregation behavior for naturally drying process. As the TEM shown, CRG/Ly complex more and more aggregated as the ratio of CRG to Ly decreased. For CRY1 and CRY2, the complex scarcely aggregated for high charges provided by CRG. While for CRY3 and CRY4, aggregation became more severe due to lower viscosity and lower repulsive force. When the charges of CRG were neutralized by Ly, CRG/Ly complex aggregated into a larger size to balance the low  $\zeta$ -potential and low the energy of the system (Fig. 2). High Ly content could accelerate the aggregation behavior for CRG/Ly complex. The obvious TEM results excellently displayed the aggregation behavior induced by the changes.

### 3.3. Thermal properties of CRG/Ly complex

Fig. 5 shows the heat flow-temperature pattern of Ly in the presence of CRG. All tested samples exhibited a prominent endothermic peak as shown in Fig. 5a. The major endothermic peaks were attributed to the thermal denaturation of Ly fraction that was reported at about 70 °C (Wong, Lim, Kadir, & Tayyab, 2014). In our test, the  $T_m$  of Ly was 70.2 °C which well agreed with the previous reports. It was strangely found that the effects of CRG on the Ly's thermal stability were different with other studies below. Compared with  $T_m$  of Ly,  $T_m$  of CRG/Ly complex slightly decreased with CRG addition, and then increased when CRG was further increased. Although many studies have been conducted to investigate the protein's thermal stability in presence of polysaccharides, the effects seem to be different in various protein/polysaccharide systems. For example, Ibanoglu found that the thermal stability of Ly decreased in the presence of *t*-carrageenan. But  $T_m$  of BSA and Ly increased in the presence of pectin and guar gum due to the protection of globular proteins against aggregation through blockage of their hydrophobic binding sites by the bulky polysaccharide moiety. While the three polysaccharides could all improve the thermal stability of WPI (Ibanoglu, 2005). Lee and Huang also reported that the thermal stability of  $\alpha$ -lactalbumin and SPI was improved when complexed with chitosan (Huang, Sun, Xiao, & Yang, 2012; Lee & Hong, 2009). The coacervates of hyaluronic acid and Ly displayed a minor decrease in the  $T_m$  as compared with Ly alone (Water, Schack, Velazquez-Campoy, Maltesen, van de Weert, & Jorgensen, 2014). For CRG/Ly complex, the complexation of Ly with CRG may strengthen the dense internal Ly structure resulting into the  $T_m$  enhancement. The high CRG content also played an effect of 'package action' (Yuan, Wan, Yang, & Yin, 2014). The physical protection could further improve the thermal stability of CRY1. As the concentration of CRG was low, self-aggregation of CRG/Ly complex with large size likes a natural protective shelter that could protect the inner Ly from denaturation.

### 3.4. pH induction

The effect of pH on aggregation behavior of polysaccharide/protein complex is always been considered as an important extrinsic factors because pH of the medium strongly affects the charge density of the biopolymers. Herein, we also evaluated the effect of pH which is commonly used in food on the stability of CRG/Ly system. The results showed the CRG/Ly system was kept soluble at a wide pH region (Fig. 6a). To some extent this property was similar with proteins that they are often stable against aggregation in narrow pH range (Xie, Feng, Cao, Xia, & Lu, 2007). But the turbidity of CRY5 was dramatically higher than other system at the same pH condition. The low  $\zeta$ -potential made the CRG/Ly complex easy to self-aggregate into structure with a large size to maintain the system stable. The phenomenon was also substantiated the aggregation of the CRG/Ly complex, as well as the previous results (Figs. 1, 3 and 4). Fig. 6b and 6c showed an increase in transmittance and the reduced  $\zeta$ -potential at the pH values close to pI of Ly for the abundant residual CRG with high negative charges. When pH is kept far away from pI, Ly carry positive charges that neutralized with CRG to reduce the charges of the system. It was noted that the sensitivity of CRG/Ly system to pH variation was related with the initial CRG/Ly ratio. The system with higher Ly content showed the more sensitive to the pH changes, including transmittance and  $\zeta$ -potential. The initial protein/polysaccharide ratio, especially for the protein content, dominating the aggregation behavior of the system has also been reported before (Ru, Wang, Lee, Ding, & Huang, 2012). The aggregation behavior induced by pH was tunable and reversible. This



**Fig. 3.** SEM of XG/Ly mixtures at natural pH (about 6.5) with different ratios, a-CRY1, b-CRY2, c-CRY3, d-CRY4, e-CRY5.

aggregation was responsible for the gradual association of CRG/Ly complex to form larger interpolymeric complexes, but the reversible process was dissociation of interpolymeric complexes into soluble complex (Fig. 2). The tunable property of polyelectrolyte complex induced by pH has also been found in many other biopolymers' complex (Di Costanzo et al., 2001; Sui, Jaber, & Schlenoff, 2006; Wu et al., 2009). We also realized that the soluble CRG/Ly complex with moderate protein content may be used in acidic beverage, making the clear product contains protein and hydrophobic nutrients that could be loaded in internal hydrophobic cavity of the complex, such as curcumin we have studied before.

### 3.5. Salt tolerance

Addition of salt generally suppresses the formation of protein/polysaccharide complex attributing to the ability of screening the

charges of the biopolymers. Excess salt may completely prevent the forming of polymers complex (Wang, Lee, Wang, & Huang, 2007). Appropriate salt tolerance would be important for their potential in different applications as we desired. Fortunately, the CRG/Ly complex displayed high salt tolerant ability as the NaCl concentration ranged below 25 mM. The tolerant behavior made the CRG/Ly system practicable application in the beverage for their low NaCl addition. The NaCl tolerance behavior presented for all CRG/Ly system and exhibited stable transmittance with slight increase in the wide range of NaCl concentrations (Fig. 7b). Even so, the transmittance of the CRY1 and CRY2 exhibited a slight increased trend as the NaCl concentration increased (Fig. 7a). At the same NaCl concentration, the Ly content promoted the aggregation behavior. The phenomenon was responsible for the gradual induction of association between biopolymers complex to form interpolymeric complexes with larger size (Li, Zhao, & Huang, 2014).



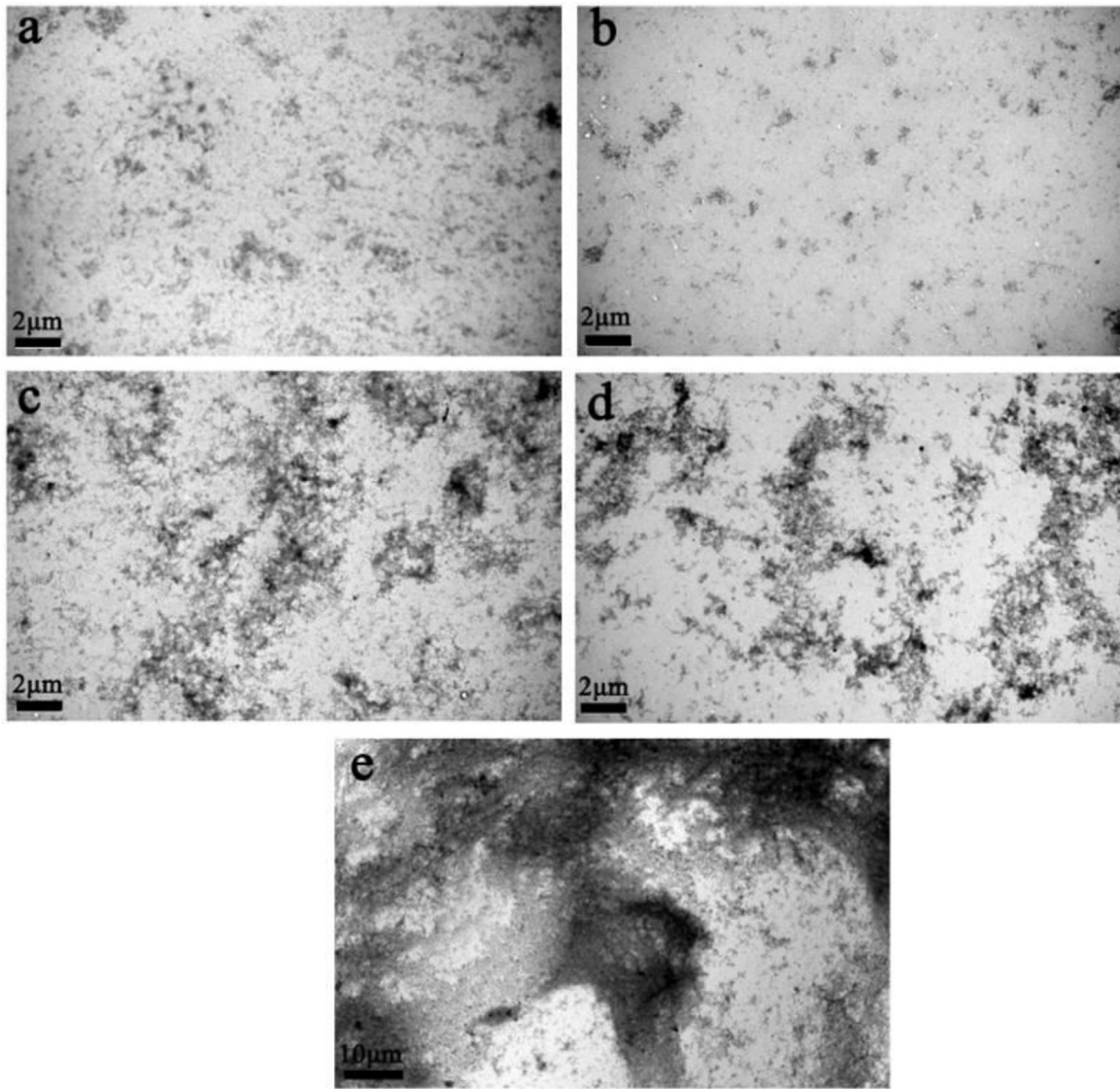


Fig. 4. TEM of XG/Ly mixtures at natural pH (about 6.5) with different ratios, a-CRY1, b-CRY2, c-CRY3, d-CRY4, e, f-CRY5.

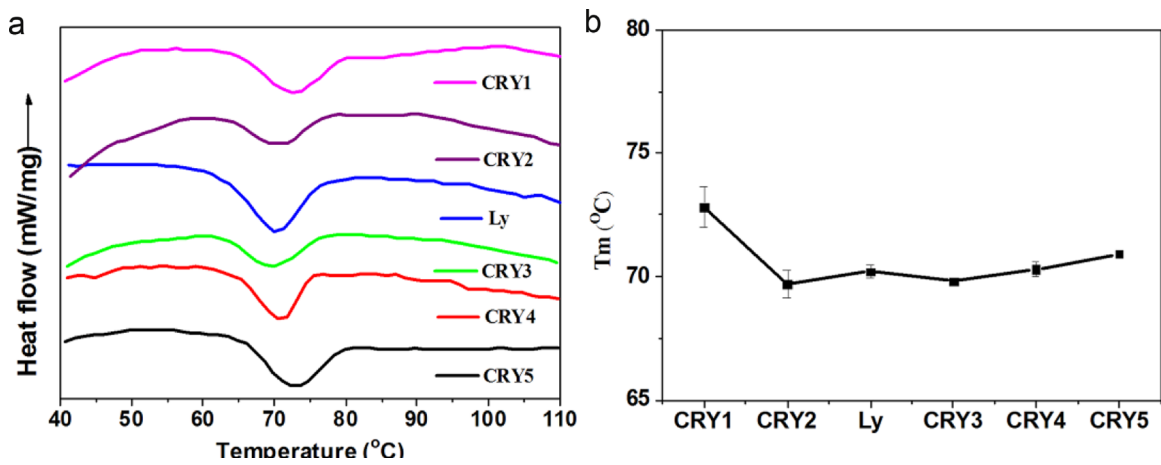


Fig. 5. Thermogram (a) and Tm (b) of Ly and CRG/Ly system with different ratios.

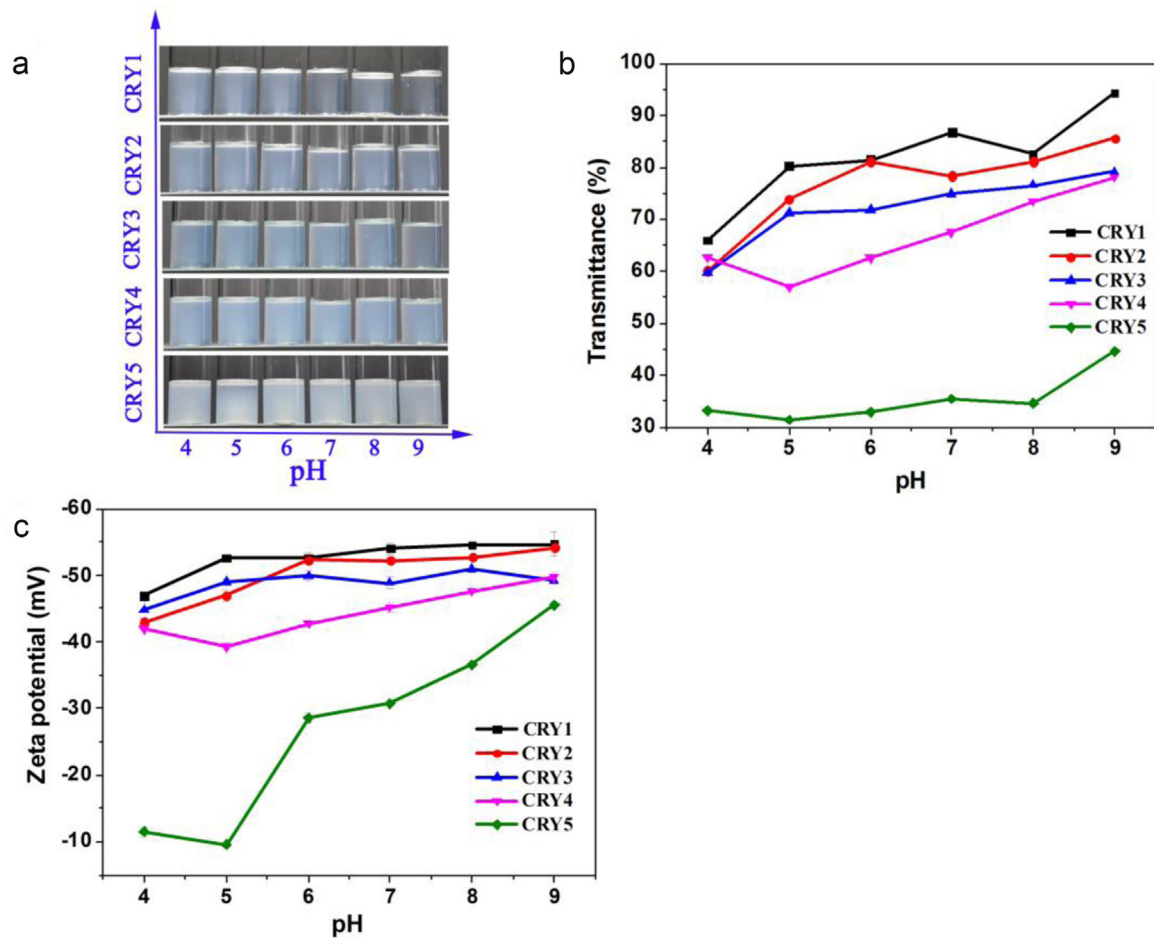


Fig. 6. Optical images (a), transmittance (b) and  $\zeta$ -potential (c) of CRG/Ly system as the function of pH.

### 3.6. Heat-induced aggregation

Heat-induced aggregation commonly occurred in different kinds of complex (Östbring, Rayner, Albertsson, & Erlanson-Albertsson, 2015; Tang, Wen, Lu, Yang, Cheng, & Lu, 2007). As shown in Fig. 7c and 7d, heat treatment increased the turbidity of CRG/Ly system and exhibited a temperature-dependant manner. Temperature elevation promoted the interpolymeric complexes formation. Obvious associative phase separation occurred for CRY4 and CRY5 after 30 min heat treatment (60 °C and 80 °C). For CRY3, the transmittance of 60 °C heat treatment for 30 min was much higher than that disposed at 80 °C. The phenomenon also happened in CRY1 and CRY2. It indicated that the system with higher Ly content more easily associated to self-aggregation and CRG could suppress the phase transition for high viscosity. Although self-aggregation was accelerated by temperature, the CRG/Ly system with high CRG content always kept homogeneous displaying high heat tolerance. The promoted self-aggregation contributing to the additional physical interaction disturbed the balance of initial system, including hydrogen bonding and hydrophobic interactions. Apparently, electrostatic attraction was the dominating driving force for the initial CRG/Ly complex. After thermal treatment, hydrogen bonding and hydrophobic interactions also became competitive driving force to promote the self-aggregation for balancing electrostatic attraction and lowering the energy of the system.

### 4. Conclusions

Environment induced self-aggregation behaviors of CRG/Ly complex were investigated by transmittance, size,  $\zeta$ -potential and micro-structure analysis. The self-aggregation behavior was significantly affected by the CRG/Ly ratios. High Ly content could accelerate the aggregation behavior of CRG/Ly complex, while CRG content played a contrary role. CRG/Ly complex system displayed nano-size distribution except for CRY5. Thermal stability of Ly was improved by CRG addition and the self-aggregation behavior. The dissociation and association behavior between interpolymeric complexes into soluble complex could be regulated by pH. All CRG/Ly system shared high NaCl tolerant behavior and presented stable transmittance with slight increase in wide range of NaCl concentrations. Heat-induced self-aggregation exhibited a temperature and polymers ratios dependant manner. Temperature elevation promoted the formation of interpolymeric complexes. Environment induced self-aggregation into interpolymeric complexes also reflected the re-balanced physical interaction between the polymers and their complex when their system suffered any environmental changes. The self-aggregation behavior was the gradual association of CRG/Ly complex to form interpolymeric complexes with a large size. Regulating self-aggregation behavior of CRG/Ly system could enrich the nano/micro structures from soft material respective and also provide practical theory for food process.

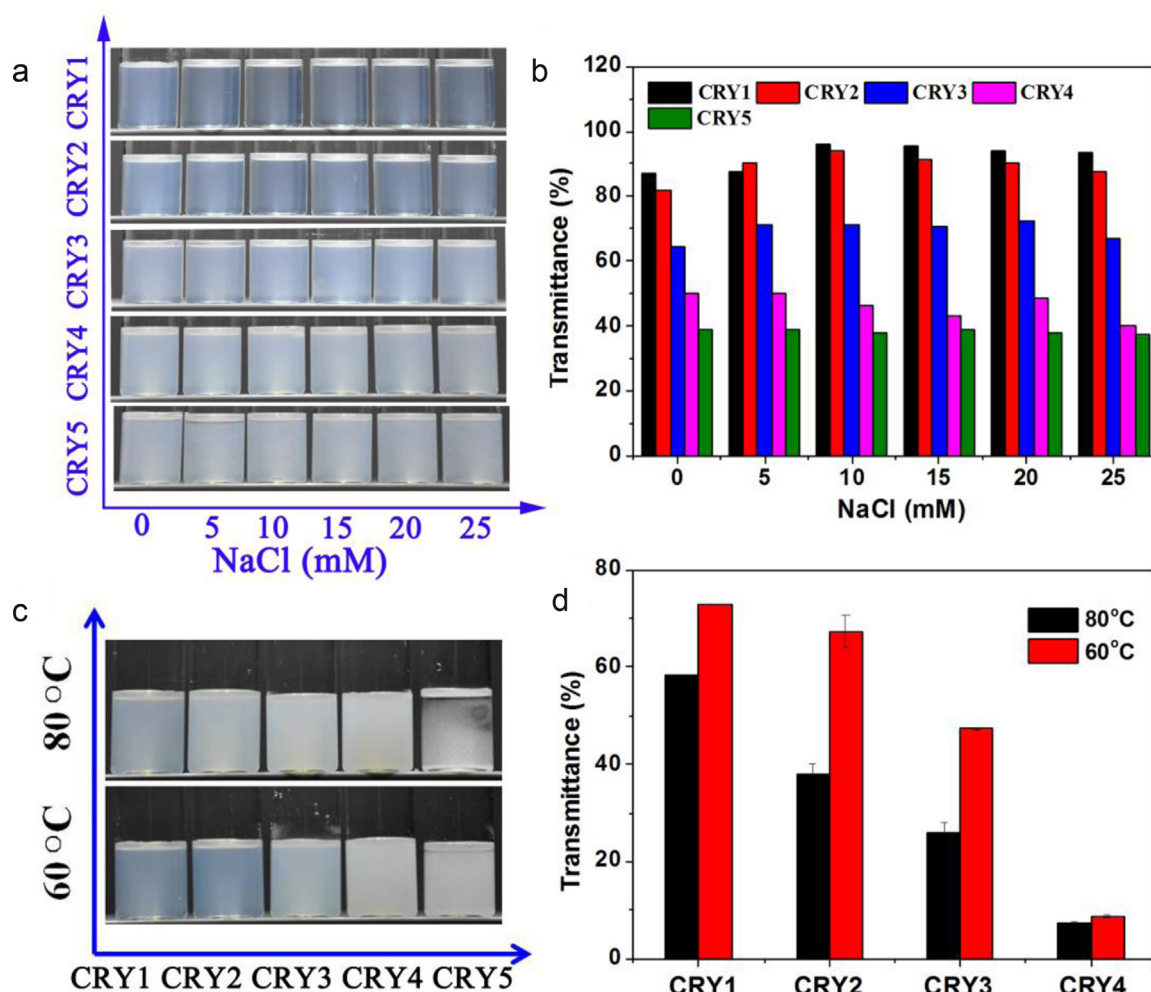


Fig. 7. Effects of NaCl addition (a, and b) and thermal treatment (c, and d) on appearances (a, and c) and transmittance (b, and c) of CRG/Ly system.

## Acknowledgment

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## References

- Di Costanzo, L., Geremia, S., Randaccio, L., Purrello, R., Lauceri, R., Sciotto, D., ... Pavone, V. (2001). Calixarene-porphyrin supramolecular complexes: PH-tuning of the complex stoichiometry. *Angewandte Chemie International Edition*, 40(22), 4245–4247.
- Doublier, J.-L., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, 5(3), 202–214.
- Evans, M., Ratcliffe, I., & Williams, P. (2013). Emulsion stabilisation using polysaccharide-protein complexes. *Current Opinion in Colloid & Interface Science*, 18(4), 272–282.
- Gosal, W. S., & Ross-Murphy, S. B. (2000). Globular protein gelation. *Current Opinion in Colloid & Interface Science*, 5(3), 188–194.
- Hosseini, S. M. H., Emam-Djomeh, Z., Razavi, S. H., Moosavi-Movahedi, A. A., Saboury, A. A., Mohammadifar, M. A., ... Van der Meer, P. (2013). Complex coacervation of  $\beta$ -lactoglobulin-K-carrageenan aqueous mixtures as affected by polysaccharide sonication. *Food Chemistry*, 141(1), 215–222.
- Huang, G.-Q., Sun, Y.-T., Xiao, J.-X., & Yang, J. (2012). Complex coacervation of soybean protein isolate and chitosan. *Food Chemistry*, 135(2), 534–539.
- Ibanoglu, E. (2005). Effect of hydrocolloids on the thermal denaturation of proteins. *Food Chemistry*, 90(4), 621–626.
- Kumar, K. S., Ganesan, K., Selvaraj, K., & Rao, P. S. (2014). Studies on the functional properties of protein concentrate of *Kappaphycus Aalvarezii* (Doty) Doty—an edible seaweed. *Food Chemistry*, 153, 353–360.
- Lacroix, I. M., & Li-Chan, E. C. (2014). Isolation and characterization of peptides with dipeptidyl peptidase-IV inhibitory activity from pepsin-treated bovine whey proteins. *Peptides*, 54, 39–48.
- Laneville, S. I., Turgeon, S. L., Sanchez, C., & Paquin, P. (2006). Gelation of native  $\beta$ -lactoglobulin induced by electrostatic attractive interaction with xanthan gum. *Langmuir*, 22(17), 7351–7357.
- Lee, A.-C., & Hong, Y.-H. (2009). Coacervate formation of  $\alpha$ -lactalbumin-chitosan and  $\beta$ -lactoglobulin-chitosan complexes. *Food Research International*, 42(5), 733–738.
- Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Yao, X., Zhang, Y., ... Jiang, F. (2012). Complexation of Bovine serum albumin and sugar beet pectin: structural transitions and phase diagram. *Langmuir*, 28(27), 10164–10176.
- Li, Y., Zhao, Q., & Huang, Q. (2014). Understanding complex coacervation in serum albumin and pectin mixtures using a combination of the Boltzmann equation and Monte Carlo simulation. *Carbohydrate Polymers*, 101, 544–553.
- Lin, W. J., Chen, T. D., & Liu, C.-W. (2009). Synthesis and characterization of lactobionic acid grafted pegylated chitosan and nanoparticle complex application. *Polymer*, 50(17), 4166–4174.
- Liu, F., Jiang, Y., Du, B., Chai, Z., Jiao, T., Zhang, C., ... Leng, X. (2013). Design and characterization of controlled-release edible packaging films prepared with synergistic whey-protein polysaccharide complexes. *Journal of Agriculture and Food Chemistry*, 61(24), 5824–5833.
- Luo, Y., & Wang, Q. (2014). Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. *International Journal of Biological Macromolecules*, 64, 353–367.
- Luo, Y., Zhang, B., Whent, M., Yu, L. L., & Wang, Q. (2011). Preparation and characterization of zein/chitosan complex for encapsulation of  $\alpha$ -tocopherol, and its in vitro controlled release study. *Colloids and Surfaces B: Biointerfaces*, 85(2), 145–152.
- Östbring, K., Rayner, M., Albertsson, P.-Å., & Erlanson-Albertsson, C. (2015). Heat-induced aggregation of thylakoid membranes affect their interfacial properties. *Food & function*, 6(4), 1310–1318.
- Rodrigues, S., da Costa, A. M. R., & Grenha, A. (2012). Chitosan/carrageenan nanoparticles: effect of cross-linking with triphosphosphate and charge ratios.

- Carbohydrate Polymers*, 89(1), 282–289.
- Ru, Q., Wang, Y., Lee, J., Ding, Y., & Huang, Q. (2012). Turbidity and rheological properties of bovine serum albumin/pectin coacervates: effect of salt concentration and initial protein/polysaccharide ratio. *Carbohydrate Polymers*, 88(3), 838–846.
- Salmaso, S., & Caliceti, P. (2013). Self assembling nanocomposites for protein delivery: supramolecular interactions of soluble polymers with protein drugs. *International Journal of Pharmaceutics*, 440(1), 111–123.
- Schmitt, C., & Turgeon, S. L. (2011). Protein/polysaccharide complexes and coacervates in food systems. *Advances in Colloid and Interface Science*, 167(1), 63–70.
- Schuh, S., Schwarzenbolz, U., & Henle, T. (2010). Cross-linking of hen egg white lysozyme by microbial transglutaminase under high hydrostatic pressure: localization of reactive amino acid side chains. *Journal of Agriculture and Food Chemistry*, 58(24), 12749–12752.
- Sui, Z., Jaber, J. A., & Schlenoff, J. B. (2006). Polyelectrolyte complexes with pH-tunable solubility. *Macromolecules*, 39(23), 8145–8152.
- Tang, Y., Wen, X., Lu, Q., Yang, Z., Cheng, Z., & Lu, C. (2007). Heat stress induces an aggregation of the light-harvesting complex of photosystem II in spinach plants. *Plant physiology*, 143(2), 629–638.
- Teng, Z., Luo, Y., & Wang, Q. (2013). Carboxymethyl chitosan–soy protein complex nanoparticles for the encapsulation and controlled release of vitamin D 3. *Food Chemistry*, 141(1), 524–532.
- Thünemann, A. F., Müller, M., Dautzenberg, H., Joanny, J. -F., & Löwen, H. (2004). Polyelectrolyte complexes. In *Polyelectrolytes with Defined Molecular Architecture II* (113–171). Springer.
- Tran, T., & Rousseau, D. (2013). Stabilization of acidic soy protein-based dispersions and emulsions by soy soluble polysaccharides. *Food Hydrocolloids*, 30(1), 382–392.
- Wang, X., Lee, J., Wang, Y.-W., & Huang, Q. (2007). Composition and rheological properties of  $\beta$ -lactoglobulin/pectin coacervates: effects of salt concentration and initial protein/polysaccharide ratio. *Biomacromolecules*, 8(3), 992–997.
- Water, J. J., Schack, M. M., Velazquez-Campoy, A., Maltesen, M. J., van de Weert, M., & Jorgensen, L. (2014). Complex coacervates of hyaluronic acid and lysozyme: effect on protein structure and physical stability. *European journal of Pharmaceutics and Biopharmaceutics*, 88(2), 325–331.
- Wong, Y. H., Lim, C. H., Kadir, H. A., & Tayyab, S. (2014). Towards increasing chemical and thermal stability of lysozyme with a simulated honey sugar cocktail. *RSC Advances*, 4(96), 53891–53898.
- Wu, C., Ma, R., He, H., Zhao, L., Gao, H., An, Y., & Shi, L. (2009). Fabrication of complex micelles with tunable shell for application in controlled drug release. *Macromolecular Bioscience*, 9(12), 1185–1193.
- Xie, C., Feng, Y., Cao, W., Xia, Y., & Lu, Z. (2007). Novel biodegradable flocculating agents prepared by phosphate modification of Konjac. *Carbohydrate Polymers*, 67(4), 566–571.
- Xu, W., Jin, W., Zhang, C., Li, Z., Lin, L., Huang, Q., ... Li, B. (2014). Curcumin loaded and protective system based on complex of  $\kappa$ -carrageenan and lysozyme. *Food Research International*, 59, 61–66.
- Yang, M., Wu, Y., Li, J., Zhou, H., & Wang, X. (2013). Binding of curcumin with bovine serum albumin in the presence of iota-carrageenan and implications on the stability and antioxidant activity of curcumin. *Journal of Agriculture and Food Chemistry*, 61(29), 7150–7155.
- Yuan, Y., Wan, Z.-L., Yang, X.-Q., & Yin, S.-W. (2014). Associative interactions between chitosan and soy protein fractions: Effects of pH, mixing ratio, heat treatment and ionic strength. *Food Research International*, 55, 207–214.