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Gel Properties and Formation Mechanism of Large Yellow Croaker (*Pseudosciaena crocea*) Roes Protein Isolates Gels †

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Abstract: In this study, the effect of pH on gel properties of large yellow croaker (*Pseudosciaena crocea*) roe protein isolates (pcRPIs) was evaluated. The rheological properties, moisture-distribution and microstructure of pcRPIs gels were also analyzed. The results showed that pcRPIs failed to form gels at pH 4 – 6, while indicated gelation profile at pH 7–9. The optimum pH for pcRPIs to form gels was pH 8 with the gel point at 84 °C, and the *G'* of the pcRPIs gel formed at pH 8 reached 535.4 Pa at the concentration of 100 mg/mL. In addition, as pH increased, pcRPIs gels had smoother surface and more continuous network structure, and the water holding ability of pcRPIs gels increased. Moreover, hydrophobic interactions were the main forces in the formation of pcRPIs gels. These results indicated that pcRPIs had potential to be used as functional materials in the food industry, especially some gel products.

Keywords: large yellow croaker (*Pseudosciaena crocea*); roe; protein isolates; gel properties

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1. Introduction

Large yellow croaker (*Pseudosciaena crocea*) has been extensively cultured in China due to its nutritional value with production higher than 254 thousand tons in 2020. During the processing of large yellow croaker, the roes are mostly discarded as by-products. Roes, accounting for about 15 – 20% of the fish weight, are rich in protein and can be used to develop functional protein products. Protein isolates from large yellow croaker roes are mainly composed of vitellogenin, vitellogenin B and C, having better water absorption and emulsification capacities than soy protein isolates [1]. Therefore, it is necessary to further investigate functional properties of protein isolates from large yellow croaker roes.

Gel property is a very important property of proteins, and protein gelation is a key factor in food that affects the sensory characteristics of food, improves water absorption, has a thickening effect, helps stabilize the system, and affects the appearance of the food [2]. Recently, studies on gelation of protein from aquatic resources are mainly focused on collagen and myofibrillar proteins, such as gelation from fresh croaker fish (*Johnius* sp.) skin [3]. In addition, there are many studies focused on the animals eggs or gonads, such as hen egg [4,5] and male gonad of scallop (*Patinopecten yessoensis*) [6,7]. However, the knowledge about gel properties of protein isolates from *P. crocea* roes is still limited. Thermal gelation is the main method for preparing protein gels. Protein solution at a certain concentration is heated, and the protein molecules are unfolded due to denaturation and aggregate to form a gel.

In this study, the gelation changes of the *P. crocea* roe protein isolates (pcRPIs) during heating were evaluated. The effect of pH on the gel properties, including the rheological

characterization, water migration, the microstructure and chemical interactions of pcRPIs gels were investigated by the rheometer, low field-nuclear magnetic resonance (LF-NMR) relaxometry, fourier transform infrared (FTIR), cryo-scanning electron microscopy (cryo-SEM) and atomic force microscope (AFM).

2. Materials and Methods

2.1. Materials and Chemicals

Large yellow croaker (*P. crocea*) roes were purchased from Qingdao Yujie Group Co. Ltd. (Qingdao, China). All other reagents were of analytical grade.

2.2. Preparation of P. crocea roe *Protein Isolates*

The *P. crocea* roe protein isolates (pcRPIs) were prepared from the freeze-dried powders of *P. crocea* roes (pcRs) according to our previous study [1].

2.3. Preparation of P. crocea roe *Protein Isolate Gels*

The pcRPIs were dispersed in deionized water with the concentration of 100 mg/mL and adjusted to pH 4 – 9 by 0.1 M NaOH or 0.1 M HCl solution. The sample solutions were heated at 85 °C for 20 min to prepare the pcRPIs gel. The gel was kept at 4 °C for 12 h for further equilibration.

2.4. Rheological Measurement

The rheological properties of gels were measured by using rheometer (Discovery HR-1, TA Instruments Menu Co., Ltd., USA) based on the method of Gao et al. [8] and Yan et al. [7]. For determining of gel points, 1 mL of the pcRPIs suspension (100 mg/mL) were loaded on the test platform and were equilibrated for 1 min at 20 °C. The temperature cycle were executed within 20 – 90 °C at a heating/cooling rate of 4 °C/min. At the same time, the storage modulus (G') and loss modulus (G'') were obtained with the frequency of 1 Hz and the gap of 1 mm. Frequency sweeps in the range of 0.1 – 10 Hz were carried out at 25 °C and at a strain of 0.3% which was selected from the linear viscoelastic region. For temperature ramp, the storage modulus G' and loss modulus G'' at 1 Hz and 0.3% strain in the range of 4 – 80 °C at a heating/cooling rate of 4 °C/min with a holding time of 3 min at the final temperature for both the heating and cooling steps were also recorded.

2.5. LF-NMR Relaxometry

The sample was placed in a plastic tube and inserted into the sample bed of NMR scanner. A MesoMR23-060V-1Analyzer equipped with a 0.5 T permanent magnet (Niumag Co., Ltd., Shanghai, China) was used to detect transverse spin-spin relaxation (T_2), and the Carr-Purcell-Meiboom-Gill sequence was used to analyze the T_2.

2.6. Cryo-SEM

The cryo-scanning electron microscopy (Hitachi Co., Ltd., Tokyo, Japan) was uesd to observe the microstructure of pcRPI gels as performed by Yan et al. [6] perfromed.

2.7. AFM

The samples of pcRPIs gels were dispersed in deionized water at the concentration of 0.025 mg/mL. The different samples were dried on mica sheet, and the imaging was carried out by AFM in tapping mode with a Hitachi AFM5500M multimode scanning probe microscope (Hitachi Co., Ltd., Tokyo, Japan). Topographical images scanned at 0.4 Hz and stored in 256 × 512 pixel format were processed using a AFM5000 II software to estimate the height of the molecules.

2.8. Gel Solubility in Various Reagents

In order to determine the intermolecular interaction in the pcRPIs gels, the gel samples were dissolved in four different solutions as described of Tan et al. [9]: 0.6 M NaCl (Sa); 0.6 M NaCl + 1.5 M urea (Sb); 0.6 M NaCl + 8 M urea (Sc) and 0.6 M NaCl + 8 M urea + 0.5 M β-mercaptoethanol (Sd). Protein contents were determined by using Bradford protein assay.

2.9. Statistical Analysis

All the data were expressed as mean ± standard deviation (n = 3). The level of significance ($p < 0.05$) was determined by SPSS 11.5 software.

3. Results

3.1. Gel Point and Visual Appearance of pcRPIs Gels

The changes in the G' and G'' of pcRPIs suspension (100 mg/mL) at pH 7–9 during heating and cooling steps were shown in Figure 1a. The G' of all samples increased with increasing temperature. Moreover, the pcRPIs gelation could be divided into three stages, which was similar to egg yolk gelation 29. Moreover, the crossover of G' and G'' for pcRPIs gels at pH 8 and 9 appeared at 84 °C and 85 °C, indicating gel point, while there was no obvious gel point for pcRPIs suspension at pH 7. Additionally, the pcRPIs under acidic conditions at pH 4–6 did not form gels during heating and cooling (data not shown). Moreover, both of G' and G'' in pcRPIs at pH 8 were higher than those at pH 9 and pH 7 (Figure 1a). These results suggest that pcRPIs are more likely to form gels at pH 8.

As shown in Figure 2, the pcRPIs suspension (100 mg/mL) at pH 7–9 could form gels after being heating at 85 °C for 20 min. Besides, the pcRPIs suspension at pH 4–6 showed fluid state after being heated and there were some insoluble aggregates in the pcRPIs suspension at pH 5–6. These results were consistent with rheological results. However, the pcRPIs at higher pH of 7, 8 and 9 exhibited a further uniform gel likeness performance.

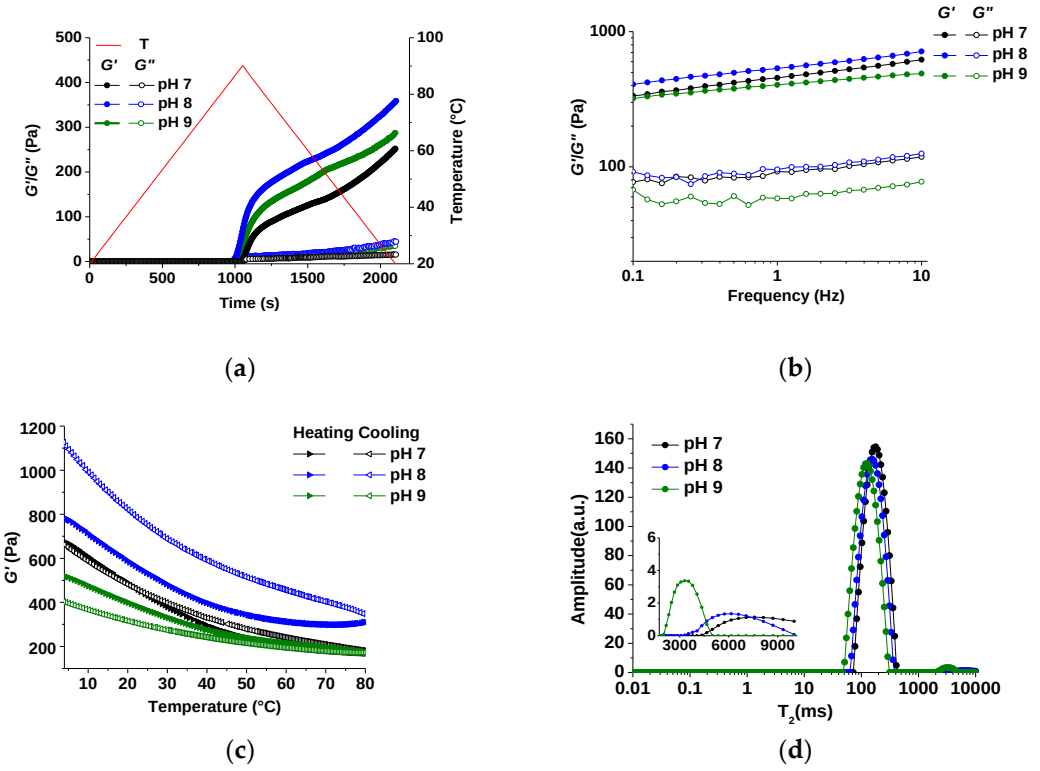

Figure 1. The rheological properties and transverse spin-spin relaxation time curves of pcRPIs gels at different pH (**a**) the changes of storage modulus (G') and loss modulus (G'') of pcRPIs at pH 7–9 during heating and cooling (**b**) the G' and G''

against frequency profiles of pcRPIs gels (**c**) the *G*′ of heating and cooling ramp profiles of pcRPIs gels (**d**) the transverse spin-spin relaxation time curves.

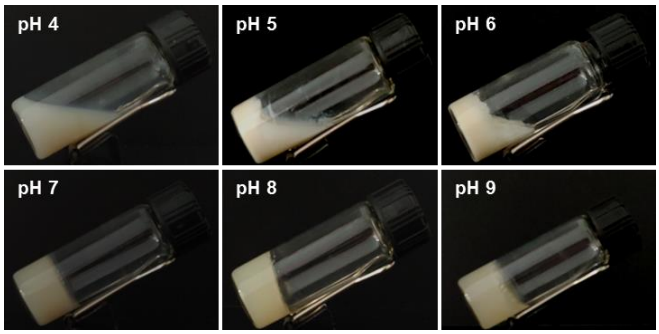

Figure 2. The gelation appearance of pcRPIs gels formed at different pH (4–9).

3.2. Rheological Properties of pcRPIs Gels

Since pcRPIs could not form a gel under acidic conditions, pcRPIs gels at 100 mg/mL formed at pH 7, 8 and 9 were introduced to further analyze rheological properties. As shown in Figure 1b, the rheological properties of pcRPIs gels formed at different pH was evaluated according to *G*′ and *G*″ against frequency, and pcRPIs gels formed at pH 8 had higher *G*′ and *G*″ than those at pH 7 and pH 9, which might due to that increasing the pH at alkaline condition could keep the protein away from its isoelectric point, and improve the protein solubility, thus improve gel properties [2,10].Temperature ramp analysis could further evaluated the change in *G*′ and *G*″ of the pcRPIs gels during heating and cooling phase. As shown in Figure 1c, the *G*′ of pcRPIs gels prepared at pH 7, 8 and 9 firstly decreased upon heating and then increased again during cooling. In addition, the G′ of the pcRPIs gels formed at pH 8 condition was significantly higher than those at other pH conditions, indicating that pH 8 was an optimal condition to prepare pcRPIs gels. The result was consistent with the results from frequency sweep (Figure 1b).

3.3. Water Populations Distribution in pcRPIs Gels

As shown in Figure 1d and Table 1, the T_2 spectrum distribution of of pcRPIs gels had two peaks at 100 – 1000 ms (T_{21}) and >1000 ms (T_{22}), respectively. It has been reported that T_2 obtained at 100-10000 ms is free water [6], so the main form of moisture in the pcRPIs gels was free water. In addition, as the pH increased, the relaxation curve of the pcRPIs gels gradually shifted to the left. The T_{21} and T_{22} of pcRPIs gels formed at pH 9 was significantly lower than those at other pH conditions, and T_{21} of pcRPIs gels formed at pH 8 was significantly lower than those at pH 7 (Table 1).

Table 1. Effect of pH on NMR parameters of large yellow crocea roe protein isolate gels.

Sample	Relaxation Time (ms)	
	T_{21} (ms)	T_{22} (ms)
7	170.37 ± 6.91 [a]	6017.38 ± 483.13 [a]
8	152.62 ± 6.01 [b]	5987.17 ± 347.51 [a]
9	126.04 ± 0.01 [c]	4476.25 ± 259.19 [b]

3.4. Microstructure of pcRPIs gels

The microstructure of pcRPIs gels at pH 4-9 was analyzed by using a cryo-SEM. As shown in Figure 3, the pcRPIs had no obvious network structure at pH 4, and pcRPIs appeared to be aggregated at pH 5 and 6, which is consistent with visual appearance and rheological results (Figures 2 – 4). Indeed, the pcRPIs gels formed at pH 7 had a rougher surface with partial broken network, while the pcRPIs gels formed at pH 8 and pH 9 had

relatively smoother surface and a more continuous network structure, which could be ascribed to that pcRPIs have better solubility at pH 8 and 9 2, thus changed the microstructure of the protein gel.

As shown in Figure 4, pcRPIs gels appeared an aggregate state after heating, in which the particle height of pH 7 was the highest with about 779 nm. The pcRPIs gels prepared at different pH conditions all showed an oval shape. These results suggest that the increase of pH leads to the network structure of the gel more continuous and the particle distribution more uniform.

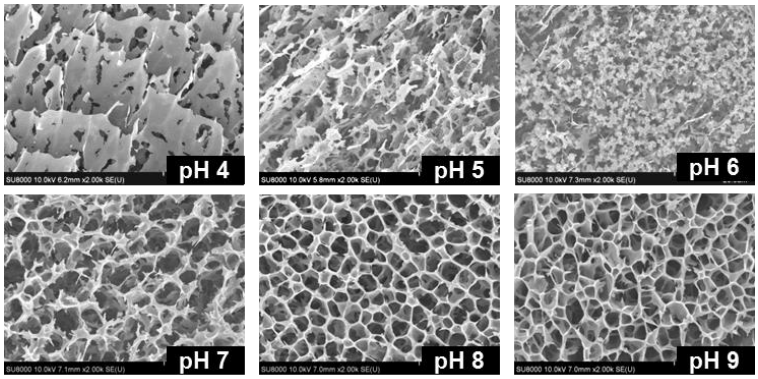

Figure 3. The Cryo-SEM images of pcRPI gels formed at different pH (4 – 9).

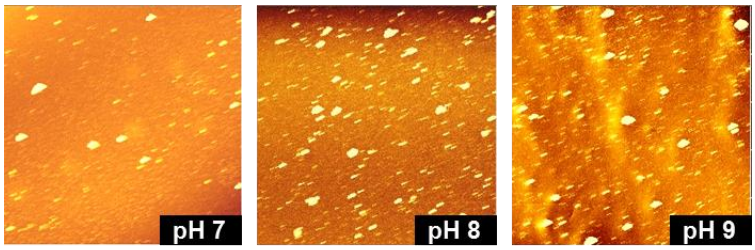

Figure 4. The AFM images of pcRPI gels formed at different pH (7 – 9).

3.5. Chemical Interactions in pcRPIs Gels

As shown in Table 2, both hydrophobic interaction and disulfide bonds ratios of pcRPIs at pH 7 – 9 were significantly higher than those at pH 4 – 6, while the ionic bonds of pcRPIs at pH 7 – 9 was significantly lower than those at pH 4 – 6. Therefore, hydrophobic interaction was seemed to be the main force for forming pcRPIs gels,which was followed by disulfide bonds. Indeed, the ratio of both hydrophobic interaction and disulfide bonds had no significant differences in pcRPI gels formed at pH 7 and 8, while was significantly higher and lower than that at pH 9, respectively.

Table 2. Effect of pH on the chemical interaction ratio of large yellow crocea roe protein isolate gels.

Sample	Chemical Interaction Ratio (%)					
	4	**5**	**6**	**7**	**8**	**9**
Ionic bonds	8.40 ± 0.63 ^{aC}	7.27 ± 0.57 ^{abB}	7.72 ± 0.84 ^{abB}	6.66 ± 0.82 ^{bcD}	6.16 ± 0.30 ^{cD}	7.08 ± 0.43 ^{bD}
Hydrogen bonds	4.71 ± 0.21 ^{aD}	2.87 ± 0.52 ^{cC}	3.11 ± 0.47 ^{cC}	2.92 ± 0.63 ^{cE}	3.20 ± 0.34 ^{cE}	3.98 ± 0.27 ^{bE}
Hydrophobic interaction	17.88 ± 0.61 ^{cB}	8.58 ± 0.68 ^{dB}	6.83 ± 0.95 ^{dB}	19.32 ± 0.51 ^{aB}	21.19 ± 1.84 ^{aB}	18.94 ± 0.32 ^{bB}
Disulfide bonds	4.02 ± 0.45 ^{eD}	6.48 ± 0.36 ^{dB}	8.70 ± 0.49 ^{cB}	9.84 ± 0.41 ^{bC}	11.09 ± 1.30 ^{bC}	14.33 ± 0.81 ^{aC}
Insoluble protein	65.00 ± 1.42 ^{bA}	73.89 ± 4.08 ^{aA}	74.09 ± 2.70 ^{aA}	61.28 ± 2.85 ^{bcA}	59.05 ± 5.81 ^{bcA}	55.68 ± 4.57 ^{cA}

4. Conclusions

In conclusion, the optimum pH for pcRPIs to form a gel was pH 8 with gel point within 84 °C. Moreover, pcRPIs gels formed at pH 8 had better rheological properties. The

main form of moisture in the pcRPIs gels was free water and its water holding ability was enhanced as pH increased with uniform and continuous porous structure, while the gel formed at alkaline conditions had even smoother surface with smaller pore sizes. Therefore, pcRPI gels have potential to be used as functional hydrogels for food, pharmaceutical and biomedical applications.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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Conflicts of Interest: The authors declare no conflict of interest.

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