Effect of ultrasound assisted konjac glucomannan treatment on properties of chicken plasma protein gelation

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Keywords:
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1. Introduction

The global meat chicken industry is developing rapidly due to the relatively low production cost of meat chicken and the advantages of a healthy diet [1]. The United States and Brazil are the major producers of American broilers. Since 1995, China has become the second largest chicken producer after the United States [2]. Therefore, a large number of chicken blood byproducts can be produced. At present, chicken blood accounts for approximately 2.5 to 3.5% of body weight [3]. Whole blood is a kind of red liquid that is composed of water, leukocytes, megakaryocytes, proteins, enzymes and other organic and inorganic substances [4]. It can be divided into two distinct components: cell parts and plasma. Plasma contains approximately 91 to 92% water and 6 to 8% protein, which mainly includes albumin (~60%), globulins, and fibrinogen [5]. The utilization rate of chicken blood is very low, although it is rich in nutrients. Most animal blood, especially chicken blood, is mostly processed into low-value animal primary feed or blood curds employed as a cheap source of protein and iron in Asian diets [6].

Plasma proteins, with good emulsifying, foaming and heat setting properties, can be used as fat substitutes in food products, especially in low-fat meat products, emulsion-type sausage and so on [7]. The role of plasma protein is to form gelation under heating and enhance the water retention, texture characteristics and nutritional value of meat products [8]. Plasma proteins can also be used as transport proteins, which can combine with lipid binding substances to make them water soluble and easy to transport [9]. If chicken plasma proteins are further developed and utilized to prepare food excipients, they can significantly improve the economic benefits of the poultry industry. Konjac glucomannan is odorless and tasteless and aqueous solutions have a strong trailing phenomenon and high consistency [10]. Because of its special

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properties of glucose and mannose β-1,4-glycosidic linkages, it is not affected by the digestive enzymes of the human body, and there is no heat that is generated [11] because it does not contain sugars, fats, starches, proteins and other caloric substances. Due to the special structure and properties of konjac glucomannan, modern medicine has proven that it is a pharmaceutical additive that can effectively reduce cholesterol and blood sugar and weight loss [12]. Therefore, konjac glucomannan will have broad application prospects in the pharmaceutical and functional food industry.

Ultrasound has been broadly applied in the food industry, bioengineering and other manufacturing industries as an auxiliary means because of its cavitation, mechanical effect and thermal effect [13]. Zhang et al. [14] found that ultrasound could promote the interaction of proteoglycan, increase the emulsification, surface hydrophobicity and potential of the system, and make the system more stable under appropriate ultrasound conditions. Kong et al. [15] found that the salt concentration and pH are the key influencing factors on the texture of plasma protein gelation. Furthermore, when the concentration of NaCl was 0.30 mol/L and pH 9.0, the texture and water retention of plasma protein gelation was excellent. To reduce the intake of NaCl and the amount of acid and alkali reagents, konjac glucomannan and ultrasound treatment were added to study the properties of heat-induced gelation of plasma proteins. The objectives of this research were to investigate the effect of ultrasound assisted konjac glucomannan treatment on the gel properties of heat-induced gelation of chicken plasma protein. Gelation strength, water-holding capacity, moisture content in different states, rheological properties and molecular force were determined to evaluate the gelation properties of chicken plasma protein and konjac glucomannan under ultrasound-assisted treatment.

2. Material and methods

2.1. Sample preparation

Konjac glucomannan (purity > 95%, Henan Wanbang Industrial Co., Ltd) was purchased from Sinopharm Chemical Reagent Co., Ltd. A certain amount of konjac glucomannan and plasma protein powder were dissolved in deionized water (DW) and stirred with a magnetic stirrer for 0.5 h to make konjac glucomannan and plasma protein powder dissolved in a polysaccharide protein system.

2.2. Ultrasound treatment

Chicken plasma protein was prepared according to a previous method [16]. The konjac glucomannan and plasma protein powder were prepared at 45 g/L at a ratio of 1:8 and stirred for 120 min at room temperature. The mixture was adjusted to pH 7.0 and then treated at 200 W for 20 min at 20 kHz with a 6 mm ultrasound amplitude probe (SC-II, Chengdu Jiuzhou ultrasonic technology Co., LTD.). The working time was 2 s, and the rest time was 3 s with pulse working mode. To avoid overheating by ultrasound treatment, the beaker-loaded sample was placed in crushed ice for cooling.

2.3. Preparation of heat induced gelation

The ultrasound-treated samples were heated at a heating rate of 5 °C/min and then kept at 85 °C for 20 min. The gelation was cooled with an ice water mixture for 20 min and stored at 4 °C overnight. Untreated plasma protein gelation was used as Control. Plasma protein gelation with konjac glucomannan was recorded as KGG. The plasma protein gelation recorded by ultrasound alone was recorded as UG. Finally, the plasma protein gelation of konjac glucomannan combined with ultrasound treatment was recorded as KGUG.

2.4. Gelation strength

Before determining gelation strength, the gelation samples from different groups in the vessels were equilibrated at 10 °C for 16–18 h. The gelation strength of the samples was determined by a TMS-TOUCH texture analyser (FTC, American). The gelation samples were axially penetrated with a rigger force of 5 g at a speed of 1.0 mm/s. The denaturation rate was 40%. The gelation strength is the maximum pressure of the probe under a pressure of 6.0 mm [17].

2.5. Water-holding capacity of gelation

The water holding capacity (WHC) of the gelation was measured by the method with some modifications [18]. The gelation was cut into a small piece of approximately 5 mm × 5 mm × 5 mm, which was weighed (m1), wrapped in two layers of filter paper and centrifuged at 10,000 r/min for 10 min at 4 °C. The gelation after centrifugation was weighed and recorded as m2. The formula for WHC (%) of gelation is as follows.

\[ \text{WHC}(\%) = \frac{m_1 - m_2}{m_1} \times 100 \]

2.6. Low-field nuclear magnetic resonance (LF-NMR) of gelation

The measurement temperature was 32 °C, and the proton resonance frequency was 22.6 MHz. The gelation samples (5 g) were put into a 15 mm diameter NMR tube, and then assayed by LF-NMR analyser (MesoMR23, Newsmy, Suzhou). The parameters are coil of 25 mm, R value of 200 μs, TW value of 4 000, NECH of 15 000, TE of 0.25, NS of 16, and the number of inversion iterations of 100,000. The transverse relaxation time (T2) of the gelation was obtained, and the Carr-Purcell-Meiboom-Gill (CPMG) sequence was used to measure 3 repetitions in each group. The corresponding relaxation times (T2b, T21, and T22) were calculated by the CONTIN algorithm [19].

2.7. Rheological measurements

The storage modulus (G′) and loss (G″) modulus of the gelation for samples from different groups during the process of heating and cooling were determined by a rotational rheometer with a 1.0 mm gap and P50 parallel plate (MCR302, Anton Paar, Austria) according to the method by Zhou et al. [20]. First, the temperature was linearly raised from 10 °C to 37 °C at a rate of 10 °C/min, then the temperature was kept at 37 °C for 20 min, and then the temperature was linearly raised from 37 °C to 75 °C at a rate of 10 °C/min. To prevent evaporation, the exposed surfaces of the gelation on the parallel plate were covered with liquid paraffin, and all treatments were tested in triplicate. The G′ and G″ of each sample was recorded.

2.8. Particle size measurement

The gelation of the plasma protein and konjac glucomannan was dissolved in phosphate buffer (pH 7.0), and the protein concentration was adjusted to 0.5 mg/mL. The particle size distribution of each sample was determined by a laser particle size analyser [21]. The shading index was set 10–15%. The refractive index of the dispersion medium was 1.361, and the refractive index of protein was 1.472. Distilled water was taken as the background for sampling and analysis. All treatments were tested in triplicate.

2.9. Molecular force in gelation

The chemical force of the gelation of different groups was analysed by the method of Niu et al. [17]. The gelation sample was weighted to 0.5 g and then mixed with 4.5 mL of 0.05 M NaCl (S1), 0.6 M NaCl (S2), 0.6 M NaCl, 1.5 M urea (S3), 0.6 M NaCl, 8 M urea (S4), and 0.6 M NaCl,
8 M urea, 1.5 M β-ME (SS), respectively. After homogenization at 5000 r/min for 2 min and magnetic stirring for 60 min, 4 mL of the supernatant by centrifugation at 12,400 g for 10 min at 4 °C was added with 50% TCA to make the final TCA concentration of supernatant reach 10%. The sample was refrigerated at 4 °C for 18 h and centrifuged at 2500 g for 20 min, and the precipitate was dissolved with 0.5 mol/L NaOH. The supernatant concentration of protein was measured according to the Biuret method. It is worth mentioning that SS was immersed in water at 100 °C for 2 min before mixing. The contents of ionic bonds, hydrogen bonds, hydrophobic interactions and disulfide bonds were expressed according to the protein content dissolved in the above solvent.

2.10. Microstructure of heat-induced gelation

The microstructures of chicken plasma protein and konjac glucomannan after heat-induced gelation in different groups were determined by employing scanning electron microscopy (SEM) (EVO-LS-10, ZEISS, Tokyo, Oberkochen, Germany) as described by Zou et al. [16] with some modifications. The gel samples were cut into small cubes of 5 mm × 2 mm × 2 mm, fixed with 3% glutaraldehyde, eluted by an ethanol gradient and put into a freeze-drying machine. Then, the dried gelation samples were put into an electron microscope sample table after gold powder coating (~10 nm conductive layer). Finally, a photograph was obtained at an accelerating voltage of 10.0 kV.

2.11. Statistical analysis

Statistical analysis was evaluated by ANOVA performed with SPSS 19.0. Significant differences were regarded at $P < 0.05$ and expressed as the mean ± standard deviation (SD). The significance at $P < 0.05$ was evaluated by Tukey’s test.

3. Results and discussion

3.1. Gel strength

Gelation strength is an important indicator to reflect the gelation properties and mouthfeel of gel products [22]. The gelation strength of heat-induced gelation of plasma protein is shown in Table 1. The gelation strength of Control group was 1.32 N, which was significantly lower than that of the other groups ($P < 0.05$). After ultrasound treatment, the gelation strength of UG increased by 108.3% compared with that of Control. The increase in gelation strength of UG may be attributed to the effect of ultrasound, which generated micro beam flow and cavitation on ultrasound promoted the expansion structure and conformational changes of plasma protein and enhanced the capacity of the gel network to entrap and immobilize water [26].

During the heating process, the degree of cross-linking between protein molecules increased [27]. After adding konjac glucomannan, the water holding capacity of the KGG and KGUG groups increased to 9.1% and 28.7% compared with that of the Control, respectively. These data indicated that the hydrophilic groups of konjac glucomannan can form an orderly three-dimensional network structure with proteins through hydrogen bonding, induced dipoles, molecular dipoles and transient dipoles [28]. Interestingly, ultrasound-assisted treatment can accelerate interaction with the surrounding water in the heating process of the plasma protein-konjac glucomannan mixture system [29]. Therefore, the bonding between molecules is significantly improved in KGUG, followed by UG and KGG. Additionally, there was a significant positive correlation between gel strength and water holding capacity.

3.3. LF-NMR analysis

LF-NMR is a rapid, nondestructive and recyclable technology to assess the distribution of different water states in gelation networks, food and other materials [30]. The water distribution in ummobile water (T21) state of the gelation of different samples is shown in Fig. 1. The relaxation time distribution (T2) of LF-NMR of the different gelation groups all has 4 peaks. The corresponding T2 corresponded to four water states: bonding water (T2b), emulsified water (T2b-1), T21 and free water (T22). When the relaxation time is 0.01~8.11 ms (T2b, T2b-1), there are two small peaks, representing strong and weak binding water [31]. The main peak appeared at 49.77~265.61 ms (T21), which represented ummobile water in the heat-induced gelation network. The peak appearing at 811.13~2477.07 ms (T22) represented the free water in the

### Table 1

| Sample     | Gel strength (N)       | Gel water holding capacity (%) |
|------------|------------------------|--------------------------------|
| Control    | 1.32 ± 0.07a           | 70.3 ± 2.35a                   |
| KGG        | 1.83 ± 0.11a           | 76.7 ± 2.61a                   |
| UG         | 2.75 ± 0.16c           | 86.4 ± 2.62c                   |
| KGUG       | 3.19 ± 0.17d           | 90.5 ± 2.65e                   |

Different letters in the same column indicate significant differences ($P < 0.05$), the same below.
gelation. The peak distribution of $T_2$ in these 4 gelation groups was almost identical. The proportion of their binding water was more than 90%, indicating that most of the water was unmobile water in the gelation distribution for different groups. This result can be maintained through the steric hindrance effect for the attraction of bonding water [32]. Interestingly, the $T_2$ of the gelation for the UG, KGG and KGUG groups had a tendency to shift left than that of the Control. This appearance indicated that the corresponding water fluidity was weakened and the associativity was enhanced [33]. Ultrasound treatment and/or konjac glucomannan addition could increase the binding capacity of water molecules in chicken plasma protein gelation, and make their internal moisture exist in a more stable form of water [34,35]. The hydrophilic groups in konjac glucomannan form macromolecules that do not easily move freely with water molecules [36]. In the heating process of the mixed system of plasma protein and konjac glucomannan, they could directly interact with the surrounding water molecules, which improved the intermolecular bonding [37]. Additionally, the effect of micro beam flow and cavitation on ultrasound treatment promoted the expansion of plasma protein structure and promoted the exposure of hydrophobic groups.

### 3.4. Rheological properties

The ability of plasma proteins to form gels is closely related to their aggregation state. Aggregation patterns of plasma proteins produced by different treatment conditions may have a great influence on their gelation ability. The development and rearrangement of plasma protein molecules after ultrasound treatment and/or konjac glucomannan addition followed by heat denaturation are two important processes in gel formation [38]. According to Fig. 2A, that the curves of the storage energy modulus ($G'$) curves of the 4 groups are generally the same. The $G'$ of the Control was the smallest in the whole test process. After ultrasound treatment or konjac glucomannan addition, the $G'$ of the KGG and UG groups was significantly improved ($P < 0.05$). The increased $G'$ indicated that konjac glucomannan can promote the formation of homogeneous and high-intensity protein gel network structures [39]. After ultrasound treatment, the $G'$ of KGUG was further enhanced, indicating that ultrasound had a favourable effect on gelation. This result might be due to the cavitation produced by ultrasound, which could activate more active groups in plasma protein and konjac glucomannan, facilitate intermolecular interactions, and promote gelation of protein and konjac glucomannan [40].

The loss modulus $G''$ can reflect the energy loss due to viscous deformation when the gelation is deformed [41]. The effect of ultrasound combined with konjac glucomannan on the gelation loss modulus $G''$ is shown in Fig. 2B. The shape and trend of the samples were similar to that of the $G'$, which indicated that the change behavior of viscosity was similar to that of elasticity, and the value of $G''$ was always higher than that of $G'$. The above results indicate that the elastic and gelatinous properties of samples play a dominant role in viscoelasticity [42]. After heating, the $G''$ of KGUG was significantly higher than that of the other three groups ($P < 0.05$), indicating that ultrasound combined with konjac glucomannan treatment had a significant effect on the loss modulus.

### 3.5. Particle size

The particle size distribution of the different samples is presented in Fig. 3. The average particle size of the Control was 304.46 nm. After konjac glucomannan addition, the average particle size of KGG increased significantly ($P < 0.05$). In the three-phase system composed of plasma protein, konjac glucomannan and solvent, mainly due to the exposure of hydrophobic groups in the process of plasma protein unfolding, hydrophobic forces were provided for protein aggregation. Induced by a variety of intermolecular interaction forces, hydrophilic konjac glucomannan and plasma protein exhibit multiple noncovalent aggregation phenomena [43], which leads to the formation of large particle sizes of plasma protein and konjac glucomannan aggregates. On the other hand, the carbonyl group in konjac glucomannan contacted the free amino group outside the plasma protein molecule and produced covalent binding in the heating process. This above reaction increased the average particle size of the protein-polysaccharide mixed solution system. Compared with that of the Control and KGG, the average particle size of UG and KGUG decreased significantly after ultrasound treatment. The reason may be that cavitation effect from ultrasound treatment was accompanied by higher energy waves and turbulence, high shear and turbulence [44]. As a result, the unfolding of noncovalent bonds of soluble protein and polysaccharide led to the expansion of the molecular structure after ultrasound treatment, the formation of multiple smaller soluble protein aggregates with small particle sizes and a decrease in the average particle size.

### 3.6. Molecular force in gelation

As shown in Fig. 4, the chemical forces of the samples were determined through the solubility in different solutions. The protein
In general, the increased content of disulfide bonds means a decrease in disulfide bonds in other groups were significantly increased (Compared with the Control, the protein content of hydrophobicity and affected the formation of heat-induced gelation in different groups. Data showed that the hydrophobicity and disulfide bonds mainly maintained the natural structure of the gelation of plasma protein. The formation of heat-induced gelation in chicken plasma protein [45]. They - indicate significant differences (P < 0.05) between different treatments.

Fig. 3. Particle sizes distributions of heat-induced gelation by different treatments for chicken plasma protein and konjac glucomannan.

3.7. Scanning electron microscopy (SEM)

The influence of ultrasound combined with konjac glucomannan on the microstructure of the gel network of chicken plasma protein is shown in Fig. 5. The gel surface of the Control was out of order and a rough honeycomb structure was formed, which presented more holes and a relatively larger diameter. The rough and unstable gel network structure led to its internal water mobility and its binding strength. After konjac glucomannan addition, the gel surface became smoother and more uniform. Konjac glucomannan is a linear molecule that can form a three-dimensional spatial structure. After konjac glucomannan and chicken plasma protein combined with each other, plasma protein molecules are evenly distributed in the linear conformational konjac glucomannan molecular chain network structure [49]. After ultrasound treatment alone, the gel pores became significantly larger, which was mainly due to the ultrasound effect [35]. Moreover, after ultrasound combined with konjac glucomannan treatment, the protein gel surface was smooth, and the chicken plasma protein was well condensed to a fine, uniform and stable gel network structure. This may be due to the enhancement of the homogenization effect of the combined treatment, followed by the decrease of the surface tension, the enhancement of the binding property of the water molecules with orderly spatial sense, dense and more uniform distribution in the protein gel [50]. The microstructure of the gel network closely related to the water holding capacity of these gel samples.

4. Conclusions

Ultrasound treatment and konjac glucomannan addition remarkably improved the gelling properties of heat-induced gelation of chicken plasma protein (KGUG), as indicated by the increased gelation strength and water-holding capacity. KGUG interacted with the surrounding water molecules and improved the intermolecular bonding. The largest rheological properties was showed in KGUG, and the G’ value was always higher than G” in different treated gels. The unfolding of the noncovalent bonds of soluble protein and polysaccharide led to the expansion of the molecular structure after ultrasound treatment, the formation of multiple smaller soluble protein aggregates with small particle sizes and a decrease in the average particle size. Therefore, chicken plasma proteins could be developed and utilized to prepare high-value food excipients by ultrasound treatment and konjac glucomannan addition, leading to significant improvement of the poultry industry economy.

CRediT authorship contribution statement

Ye Zou: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Fangyun Lu: Validation, Formal analysis, Investigation. Biao Yang: Validation, Formal analysis, Investigation. Jingjing Ma: Writing – original draft, Writing – review & editing. Jing Yang: Writing – original draft, Writing – review & editing. Chao Li: Investigation, Writing – review & editing. Xin Wang: Methodology, Investigation. Daoying Wang: Project administration. Weimin Xu: Supervision.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 5. The microstructure images of heat-induced gelation by different treatments for chicken plasma protein and konjac glucomannan (magnification: 1000×).
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