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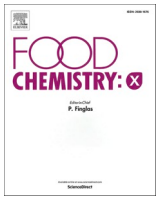

Effects of different polysaccharide contents on the gel properties of transglutaminase-induced ultrasonic-assisted peanut isolate proteins-corn silk polysaccharide products and their influence on riboflavin release behavior

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ABSTRACT

The effects of ultrasonic-assisted glycosylation technology on the gel properties of transglutaminase-induced peanut protein isolate (PPI) and its influence on the release behavior of riboflavin were investigated. The mechanism of the glycosylation action and polysaccharide content on the gel properties of PPI were preliminarily explored. The results showed that glycosylation action made the structural unfolding of PPI, enhanced the molecular interactions between molecules, and formed a more compact gel network structure. Especially when the ratio of PPI to corn silk polysaccharide was 5:1, the G', G", gel strength, and water-holding capacity were significantly improved. The improvement of gel properties was related to the hydrophobic interaction and disulfide bond enhancement between PPI and corn silk polysaccharide. Glycosylation action made the composite gel had a higher loading rate and protective and sustained release of riboflavin. This study provided an idea to solve the problems of poor processing performance of PPI.

1. Introduction

One of the most significant oilseed crops in the world is peanuts. Peanut protein, as the main byproduct after peanut oil extraction, contains a large amount of essential amino acids and has high nutritional value. However, compared to other plant proteins, peanut proteins have inferior functional properties such as solubility and emulsification, which greatly limits their application. In food processing, different properties of proteins correspond to different product properties, so it is necessary to modulate the properties of proteins by means of modification to optimize the organoleptic properties of products and expand the application range of proteins (Wang et al., 2024). Zhang et al. (2024) found that the solubility, emulsification, foaming properties and foam stability of ultrasound and pH shift co-modified peanut protein improved significantly. Furthermore, the allergenicity of peanut protein after ultrasonic-assisted glycosylation was reduced, and its emulsification performance, antioxidant activity, and *in vitro* digestibility were significantly improved (Huang et al., 2024). Protein gelation is a process that creates a continuous, organized network structure by causing the denatured molecules of a protein to aggregate (Hermansson, 1979). The network structure of protein gel can enclose active substances therein, playing a role in protection and delivery, conferring specific functional activities to food and offering a novel development direction for health care and healthy food (Cao et al., 2025; Liu et al., 2023).

Corn silk polysaccharide has physiological activities such as antioxidant, anticancer and hypoglycemic (Zhu et al., 2024). Polysaccharides can enhance the functional characteristics of proteins by engaging in glycosylation processes with proteins (Ma et al., 2023; Zhang et al., 2021). Sun et al. (2024) found that the rheological properties and gel strength of the coupled gel formed by soybean protein isolate and sodium carboxymethylcellulose through glycosylation were improved.

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Hou et al. (2024) discovered that the glycosylation products of pectin and whey protein isolate prepared through the dry method can prominently enhance the emulsifying property of whey protein isolate. Cheng et al. (2022) found that the glycosylation reaction of dextran with soybean isolates significantly improved the solubility of soybean isolates. To enhance the functionality of food gels, polysaccharides are usually added to proteins to form hybrid gel systems (Bora et al., 2023; Wei et al., 2025). In addition, it was suggested that the amount of polysaccharides added also affected the properties of proteins (Pang et al., 2020).

Transglutaminase (TGase) is widely used in protein modification due to its ability to catalyze the binding of the epsilon-amino group of lysine residues in protein molecules to the gamma-amino group of glutamine residues, resulting in the formation of high molecular weight polymers through covalent cross-linking of proteins. Transglutaminase can also react with glucosamine to achieve protein glycosylation, thereby improving the properties of proteins (Zhang et al., 2021). Furthermore, due to the cavitation effect of ultrasound, it is usually employed to enhance the gel characteristics of proteins (Zhao, Han, et al., 2023). Zhang and Wang (2022) found that ultrasonic treatment promoted the formation of disulfide bonds in the soy protein gel catalyzed by TGase, resulting in a more compact, uniform, and stable network structure and higher gel strength.

However, there are few studies on the influence of ultrasound pretreatment combined with TGase in promoting glycosylation reactions on protein gels and the delivery of bioactive compounds. It is hypothesized that the ultrasound-assisted TGase-induced gel of peanut protein isolate can enhance the loading and release ability of riboflavin. Therefore, this study used ultrasonic-assisted TGase glycosylation to form a gel and investigated the effects of varying mass ratios of corn silk polysaccharides on the gel properties of peanut protein isolate. Additionally, glycosylated products were prepared to create gels that could encapsulate riboflavin. This research provides a theoretical basis for expanding the application of peanut protein isolate in food processing.

2. Materials and methods

2.1. Materials

The extraction and identification of corn silk polysaccharides were referred to our previous articles (Han et al., 2022). Peanut isolate protein (PPI, 98 % of protein) was from Bellancom Life Sxiences Dep., USA. Glutamine transferase (200 u/g), pepsin, trypsin, *etc.* were from Shanghai Yuanye Biotechnology Company Limited and the rest of the reagents were all analytically pure.

2.2. Gel preparation of corn silk polysaccharide-peanut isolate protein complexes

Peanut isolate proteins were dissolved in 0.1 M pH = 7 phosphate buffer at a protein concentration of 10 % (*w/v*). Different masses of corn silk polysaccharides were added to achieve protein-to-polysaccharide ratios of 10:1, 5:1, 2:1, and 1:1, respectively, with magnetic stirring for 1 h. The solution was placed at 4 °C overnight, and ultrasonic treatment was carried out by setting the ultrasonic power of 480 W, the ultrasonic time of 60 min, and the pulse mode (pulse duration of 2 s on and 4 s off) according to the evaluation of gelation properties (Fig. S1, Supplementary Material). The sonicated solution was subjected to heat treatment in a water bath at a temperature of 95 °C for a duration of 30 min. Subsequently, it was cooled to the ambient temperature using an ice bath. TGase 20 U/g was added immediately, stirred well, and reacted at 45 °C for 1 h. The gel was left at 4 °C for 20 h to mature. The gel samples were noted as TG-PPI, TG-PC10:1, TG-PC5:1, TG-PC2:1 and TG-PC1:1.

2.3. Gel strength determination

The gel strength of samples was measured using a TA-XT Plus texture meter, with the P-0.5 model probe being chosen for the analysis. The method was slightly improved with reference to Ma et al. (2022). The test speed was 1 mm/s, the probe downward pressure distance was 10 mm, and the touch pressure was 5 g. The curve obtained after the test results was based on the first peak peak point as the gel rupture point, *i.e.*, the gel strength of the samples.

2.4. Determination of gel water-holding capacity

The samples were left at room temperature for 30 min to reach the same temperature as the gel, as explained by Alavi, Emam-Djomeh, et al. (2018). A 5 g gel sample was placed in a 5 mL centrifuge tube and subjected to centrifugation for 10 min at a temperature of 4 °C with a centrifugal force of 10,000 $\times g$ the acceleration due to gravity. The surplus water on the surface of the sample was removed by blotting it with absorbent paper, and the mass before and after centrifugation was recorded. Calculations were performed according to the formula (1):

$$WHC(\%) = \frac{m_a - m_c}{m_b - m_c} \times 100\% \quad (1)$$

Where: m_a is the total mass/g of the gel sample after centrifugation and the centrifuge tube; m_b is the total mass/g of the gel sample before centrifugation and the centrifuge tube; m_c is the mass/g of the centrifuge tube.

2.5. Determination of dynamic rheology

The rotating rheometer was used to characterize the rheological parameters of the samples with slight modifications with reference to Zhang et al. (2020). The sample preparation method was the same as that in 2.2. Immediately after the addition of TGase, a specific quantity of material was extracted and positioned on the test bench, while the distance between the plates was set to 1 mm. A 25 mm parallel probe was used for the probe. The excess sample was scraped off, and the edges were sealed with silicone oil. The temperature was 45 °C, the strain applied was fixed at 0.2 %, the frequency of the experiment was set at 1 Hz, and the scanning period ranged from 0 to 60 min. The variations in the energy storage modulus G' and the loss modulus G" were measured and recorded.

2.6. Determination of moisture distribution

Referring to the method of Li et al. (2018) and the sample preparation method in 2.2, immediately after adding TGase, 1 mL of the sample was transferred into a glass vial and maintained at a temperature of 45 °C for a duration of 1 h. The gel was allowed to mature for 20 h at 4 °C, then the sample was returned to room temperature and the excess water was wiped off the surface. The glass vial was placed into a 15 mm diameter NMR tube and the low-field NMR measurements were performed using the CPMG sequence at 25 °C with 4 repetitive scans. The water relaxation time spectra of the gel samples were acquired, and the cumulative peak integrations were performed to obtain the peak areas labeled as A_{21}, A_{22}, and A_{23}, which corresponded to the relaxation times of water in three different states: T_{21} (bound water), T_{22} (semi-bound water), and T_{23} (free water), respectively.

2.7. Determination of intermolecular forces

The following five reagents were prepared to treat the samples with reference to the method of Matsumoto (1980). Solution a: 0.05 mol/L NaCl; Solution b: 0.6 mol/L NaCl; Solution c: 0.6 mol/L NaCl +1.5 mol/L urea; Solution d: 0.6 mol/L NaCl +8 mol/L urea; Solution e: 0.6 mol/L NaCl +1.5 mol/L urea +0.05 mol/L β- mercaptoethanol. 2 g of gel

samples were added to 10 mL above solutions and mixed, then centrifugation (4 °C, 20,000 g, 15 min) was performed to quantify the concentration of protein in the supernatant. Where: ionic bonding was the difference in protein amount concentration between solution b and solution a; hydrogen bonding was the difference in protein amount concentration between solution c and solution b; hydrophobic interaction was the difference in protein amount concentration between solution d and solution c; disulfide bonding was the difference in protein amount concentration between solution e and solution d.

2.8. Microscopic morphology

The gel samples' microstructure was examined by scanning electron microscopy, following the methodology of Zhao, Han, et al. (2023). The gel samples underwent lyophilization, sectioning, and fixation with conductive glue. Subsequently, the samples were coated with gold for the purpose of structural inspection. The accelerating voltage was set at 5 kV, and the magnification was 1000 × .

2.9. Preparation of riboflavin-loaded gels

Based on the basis of the previous study, a sample with a mass ratio of protein to polysaccharide of 5:1 was selected as the sample ratio for the loading system. The sample solution treatment was the same as the preparation method in 2.1, and the control group was taken as the peanut isolated protein solution without ultrasound and heat treatment (UNTG-PP), the mixture of peanut isolated protein and corn silk polysaccharide with a mass ratio of 5:1 (UNTG-PC5:1), and TG-PPI and TG-PC5:1 in 2.1. Riboflavin was gradually added to the sample solution under stirring, and made the final concentration of riboflavin to be 0.5 % (*w/v*). Immediately after homogeneous mixing, 20 U/g of TGase was stirred well, and the reaction was carried out at 45 °C for 1 h, and then allowed to stand at 4 °C for 20 h. A loaded riboflavin gel was formed, and the containers throughout the process were wrapped with aluminum foil to achieve the effect of light avoidance.

2.10. Measurement of gel yield

After the formation of riboflavin-loaded gel, the gel mass was precisely measured after removing the surplus water from the wet gel surface using filter paper, and the gel yield was determined using eq. (2):

$$\text{Gel yield}\ (\%) = \frac{m_f}{m_d} \times 100\% \quad (2)$$

Where m_f is the weight of the wet gel and m_d is the weight of the dry substance used for gel preparation.

2.11. Determination of riboflavin loading rate

With a slight modification of the method of Geng et al. (2022), the wet gel was lyophilized, and 20 mg of the sample was weighed and added into the simulated intestinal fluid (0.1 M phosphate buffer pH = 7.5) containing 1 % trypsin, and then shaken under the condition of shaking table at 37 °C for 6 h. The agitated solution was subjected to centrifugation at a force of 1000 ×*g* for a duration of 15 min. The spectrophotometer was then used to measure the absorbance of the liquid portion (supernatant) at a wavelength of 445 nm. Different concentrations of riboflavin were dissolved in the simulated intestinal fluid, and the absorbance was measured at 445 nm to prepare riboflavin calibration curves, and the amount of riboflavin in the supernatant was derived from the calibration curves. Loadings (LE) were calculated according to eq. (3):

$$LE(\%) = \frac{\text{amount of loaded riboflavin(mg)}}{\text{amount of total riboflavin(mg)}} \times 100\% \quad (3)$$

2.12. Determination of the swelling rate of riboflavin gels

The lyophilized gel was measured and soaked in undigested simulated gastric fluid (SGF: 0.2 g NaCl dissolved in 100 mL of deionized water, pH adjusted to 1.2 with HCl solution) and simulated intestinal fluid (SIF: 0.1 M phosphate buffer, pH = 7.5) for 360 min, respectively. The surplus water on the surface was meticulously eliminated using a filter paper at 30-min intervals, and the mass of gel samples was weighed (Hu et al., 2020). The dissolution rate was calculated according to eq. (4):

$$\text{Swelling rate}(\%) = \frac{W_t - W_d}{W_d} \times 100\% \quad (4)$$

Where W_t is the weight of the dissolved gel at moment t; W_d is the weight of the gel before dissolution.

2.13. Determination of in vitro release

Riboflavin *in vitro* release assay was executed with minor alterations in accordance with the methodology of Zhang and Wang (2022). The gel samples were broken down into minute fragments and submerged in 20 mL of simulated gastric fluid (SGF containing 0.1 % pepsin (*w/v*), pH 1.2), respectively, with a digestion time of 2 h. The samples were taken every 30 min. At the end of digestion, the gel was applied to synthetic intestinal fluid (SIF containing 1 % trypsin (w/v), pH 7.5), digested for 4 h, and again samples were taken every 30 min. Following the sample process, the gel was transferred into a centrifuge tube and subjected to centrifugation for a duration of 2 min at a temperature of 4 °C and a centrifugal force of 12,000 ×*g* the acceleration due to gravity. The release of riboflavin was detected at 445 nm, and the release rate of riboflavin was computed based on the mathematical formula (5):

$$\text{Riboflavin release rate}(\%) = \frac{\text{Amount of riboflavin released in digestive fluid}}{\text{Total amount of riboflavin loaded in gel}} \times 100\% \quad (5)$$

2.14. Statistical analysis

The experimental data was processed using SPSS 21. Significant differences in all statistical analyses were determined using Duncan's significance test. Graphing was done using the software Origin 2022.

3. Results and analyses

3.1. Gel strength and water holding capacity

Gel strength is one of the most intuitive properties that reflects the quality of the gel. When using a physical property analyzer to characterize gel strength, the force required to force the gel to break for the first time was often chosen to quantify the gel strength (Cheung et al., 2013). According to the gel strength data presented in Fig. 1A, the peanut isolate protein had a low gel strength of just 21.5 g. The addition of corn silk polysaccharides significantly enhanced the gel strength. As the mass of polysaccharides increased, the gel initially showed an increasing

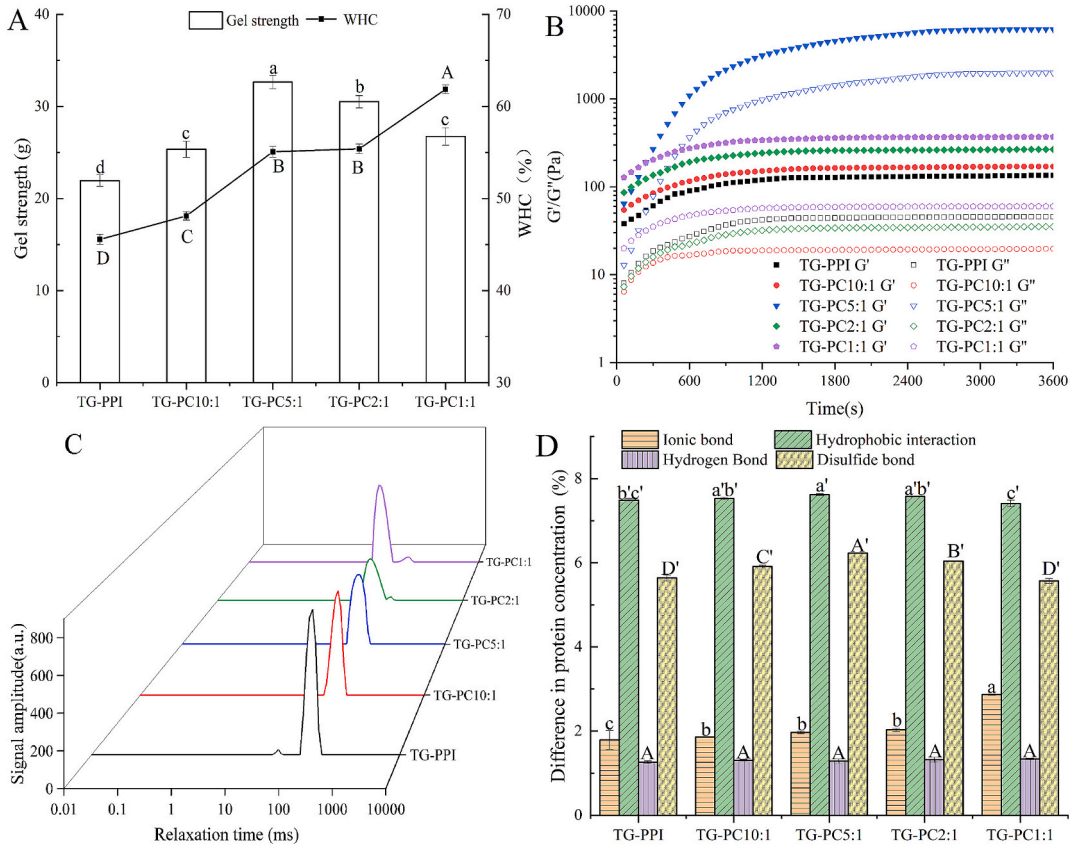

Fig. 1. (A) Gel strength and water holding capacity; (B) Elastic modulus G' and loss modulus G"; (C) Moisture distribution; (D) Intermolecular forces of the gel samples of corn silk polysaccharide-peanut isolate protein complexes with different mass ratios.
(Different letters for the same index represent significant differences)

trend, followed by a slight decrease. The gel reached its maximum strength of 32.7 g at a ratio of 5:1, which is 34 % higher than that of the peanut isolate protein gel. It indicated that the glycosylation reaction was favorable for the TGase to catalyze the gelation of peanut isolate protein. This was due to the fact that corn silk polysaccharides binded to peanut isolate proteins through hydrogen bonding and hydrophobic interactions, which had a specific function in filling the gaps in the network while the gel network was forming, resulting in a well-organized gel network structure (Zhuang et al., 2019). It was also possible that the protein and polysaccharide form a double network structure to enhance the gel strength. However, after adding too much polysaccharide, the polysaccharide itself might be agglomerated, increasing the spatial site resistance, preventing the free lysine from cross-linking with glutamine, destroying the network structure, and leading to a decrease in gel strength (Debusca et al., 2014).

The water-holding characteristic of a gel is its capacity to stabilize water molecules, which is directly linked to the structure of the gel network (Alavi, Momen, et al., 2018). According to the information presented in Fig. 1A, the water-holding property of the samples that underwent glycosylation reaction after the addition of polysaccharides showed a notable increase compared to the samples that did not undergo glycosylation reaction, and the water-holding property progressively rose in conjunction with the augmentation of the mass of corn silk polysaccharides. The maximum value could reach 61 %, which was 26 % higher than that of peanut isolate protein gel, demonstrating that the glycosylation reaction after polysaccharide addition could improve the water-holding capacity of the gel with a more stable network structure. When the mass ratio of peanut isolate protein to corn silk polysaccharide was 5:1, the water holding capacity increased gradually and consistently. This could be attributed to the formation of hydrogen bonds between the hydroxyl group of corn silk polysaccharide, protein, and water molecules. These bonds contributed to the continuous increase in water holding capacity of the gels. However, at this stage, the increase in water holding capacity was primarily due to the presence of polysaccharides and not related to the gelation effect.

3.2. Dynamic rheology analysis

The deformation properties and viscoelasticity of a sample can be measured by rheometer. Proteins undergo structural changes during their transformation from liquid to gel, and their rheological properties are affected. The G' of a protein gel indicates its capacity to store elastic deformation energy, which is associated with the gel's elasticity index. On the other hand, the G" value indicates the amount of energy lost due to viscous deformation when the gel undergoes deformation. A higher G" value indicates a more viscous gel (Zhao, Wang, et al., 2023). Fig. 1B shows the trend of G' and G" changes in different samples during gel formation by TGase cross-linking.

The values of both G' and G" for the gels increased significantly with the addition of polysaccharide compared to the original protein gels. The maximum values were seen when the protein-to-polysaccharide ratio was 5:1, which coincided with the change in gel strength of the samples. This was in agreement with the findings of Zhuang et al. (2021), through which it was found that myofibrillar fibrillar proteins were significantly increased by the addition of 1.0 % of konjac glucosinolates to the mixture G'. The overall G' of the gel samples exceeded the G", suggesting that the system exhibited a higher degree of elasticity compared to viscosity. From Fig. 1B, it is evident that the gel G' values of the samples that underwent glycosylation reactions increased, and the samples showed a rapid increase in G' before 900 s and then leveled off,

probably due to the formation of macromolecular aggregates by cross-linking of protein molecules through TGase, which led to a rapid increase in G'. With the addition of corn silk polysaccharides G' was elevated, probably due to the formation of more covalent binding of peanut isolate proteins to polysaccharides and enhancement of the gel network (Yang et al., 2015). However, the addition of excessive polysaccharides caused a decrease in G' due to the interference of spatial site resistance with the development of the protein gel network. The similar trend of G' and G" changes, with the maximum values of both G' and G' for sample PC5:1, suggested that the glycosylation reaction improves the viscoelasticity of peanut isolate protein gels.

3.3. Moisture distribution analysis

Low-field nuclear magnetic resonance (LF-NMR) is a non-destructive detection technique that analyzes the distribution and migration pattern of water in the gel system through the change of T2 relaxation time, and the shorter the relaxation time, the more tightly the water is combined with the gel structure (Bertram et al., 2004). Fig. 1C displays the moisture distribution spectra of composite gels made from peanut isolate protein and corn silk polysaccharide, with varying mass ratios. From the results in the figure, it could be found that the composite gel system had two main water distribution states, which were bound water (relaxation time of 12.328–14.175 ms) and semi-bound water (relaxation time of 100 ms–200 ms). The water that binded more strongly to the gel structure in a gel system was bound water, which was the least likely to be removed, and the water that binded more weakly was semi-bound water (Zhao, Han, et al., 2023). As the mass of polysaccharides increased, semi-bound water was transformed into bound water, probably due to the addition of polysaccharides connected to protein side chains, which resulted in a more ordered gel network structure, which in turn enhanced the binding ability of water molecules and resulted in a more tightly bound state of water.

Table 1 shows the relative percentages of relaxation times and relaxation peak areas of the two types of water molecules between different samples. From the table, it could be seen that the semi-bound water in the samples PPI, PC10:1, PC5:1 accounted for the main component and reached 100 % at the mass ratio of 5:1, while the samples PC1:1, PC1:2 bound water accounted for the main main peak area ratio, probably due to the addition of excessive polysaccharides, its own agglomeration and the addition of a large number of hydrophilic groups intercepted more bound water to improve the gel water-holding properties. With the addition of corn silk polysaccharides the relaxation times of T_{21} and T_{22} became shorter, demonstrating that the water was more closely associated with the gel network structure (Zhang et al., 2017), which was consistent with the water-holding results.

3.4. Analysis of intermolecular forces

The forces of disulfide bonding, hydrophobic contacts, ionic bonding, and hydrogen bonding played a significant part in preserving the structural integrity of proteins throughout the creation of the gel three-dimensional mesh structure (Yang et al., 2020). The analysis of the gel formation process involved the indirect examination of the primary forces at play. This was achieved by employing various chemical reagents to disrupt the forces within the gel structure and dissolve the proteins. Additionally, the concentration of proteins in the gel samples was measured in different solutions to ascertain any variations. As can be seen in Fig. 1D, the gel molecule exhibited a hierarchy of contact forces, with hydrophobic interactions being the strongest, followed by disulfide bonds, ionic bonds, and hydrogen bonds. This suggests that hydrophobic interactions and disulfide bonds were the primary driving forces for the gel formation in the sample. The introduction of corn silk polysaccharides gradually increased hydrophobic interactions in the gel system. This led to stronger hydrophobic forces due to the improved exposure of hydrophobic groups within the peanut isolate protein molecules after undergoing glycosylation reactions with the polysaccharides. The presence of corn silk polysaccharides caused the disulfide bonds to initially increase and then decrease. This could be attributed to the unfolding of the protein structure as a result of the glycosylation reaction caused by the added polysaccharides (Han et al., 2022). This unfolding exposed more SH groups, which could then be oxidized to form intermolecular disulfide bonds during the glycosylation reaction (Ai et al., 2021). The addition of excessive polysaccharides might inhibit the conversion between free sulfhydryl groups and disulfide bonds due to their own spatial site resistance, which in turn inhibited the formation of the gel structure.

Table 1
Moisture state time and relative content of gel samples with different mass than corn silk polysaccharide-peanut separated protein complex.

	T_{21}(ms)	A_{21}(%)	T_{22}(ms)	A_{22}(%)
TG-PPI	65.793	6.115	231.013	93.885
TG-PC10:1	43.288	8.81	151.991	91.19
TG-PC5:1	–	–	114.976	100
TG-PC2:1	57.224	97.555	174.753	2.445
TG-PC1:1	24.771	96.362	151.991	3.638

3.5. Appearance and microstructure of gel samples

Fig. 2 expresses the microstructures and appearance of the gel samples of corn silk polysaccharide-peanut isolate protein complexes with different mass ratios added. As shown in Fig. 2, as the polysaccharide content increased, the gel color changed from light to dark. When the protein-to-polysaccharide ratio was 1:1, the gel structure was discontinuous and the texture was obviously rough. The peanut isolate protein gel without added corn silk polysaccharide was structurally disordered with a loose network (Fig. 2A), which might be one of the main factors leading to its poor gel properties and water-holding capacity. After the glycosylation reaction occurred, when the ratio of the amount of peanut isolate protein to corn silk polysaccharide was 10:1, the polysaccharide covalently bound to the protein to form a relatively separated gel network structure, which was relatively more homogeneous than that of the peanut isolate protein gel structure without polysaccharide addition (Fig. 2B). With the increase of polysaccharide addition, when the protein-to-polysaccharide ratio was 5:1 the gel network structure was homogeneous and compact, and the surface became smooth, forming a stable three-dimensional mesh structure (Fig. 2 C). Corn silk polysaccharides could promote network formation through covalent binding and acted as fillers in the internal structure (Yang et al., 2021). This could have directly resulted in a rise in both the strength of the gel and its ability to hold water in the samples. Additionally, there might have been an increase in hydrophobic contacts and electrostatic interactions within the gel structure, leading to a stable and densely packed network structure (Wang et al., 2020). Upon continued addition of corn silk polysaccharide, the structure changed significantly, the ordered gel network structure was disrupted, and peanut isolate proteins gradually sexed to form larger agglomerates (Fig. 2D-E). This led to the breakage of the internal structure of the gel junction and the network structure became disorganized. The presence of an excessive amount of polysaccharides led to the phenomenon of aggregation and agglomeration. This increased the resistance between proteins at the spatial level, disrupted the mutual structure between proteins, and consequently affected the formation of the gel network structure. This hindrance in the formation of the gel structure might be one of the factors contributing to the deterioration of the gel properties.

3.6. Yield and loading rate analysis of loaded riboflavin gel samples

According to Fig. 3A, the gel yield of peanut isolate protein increased significantly after being subjected to sonication and heat treatment

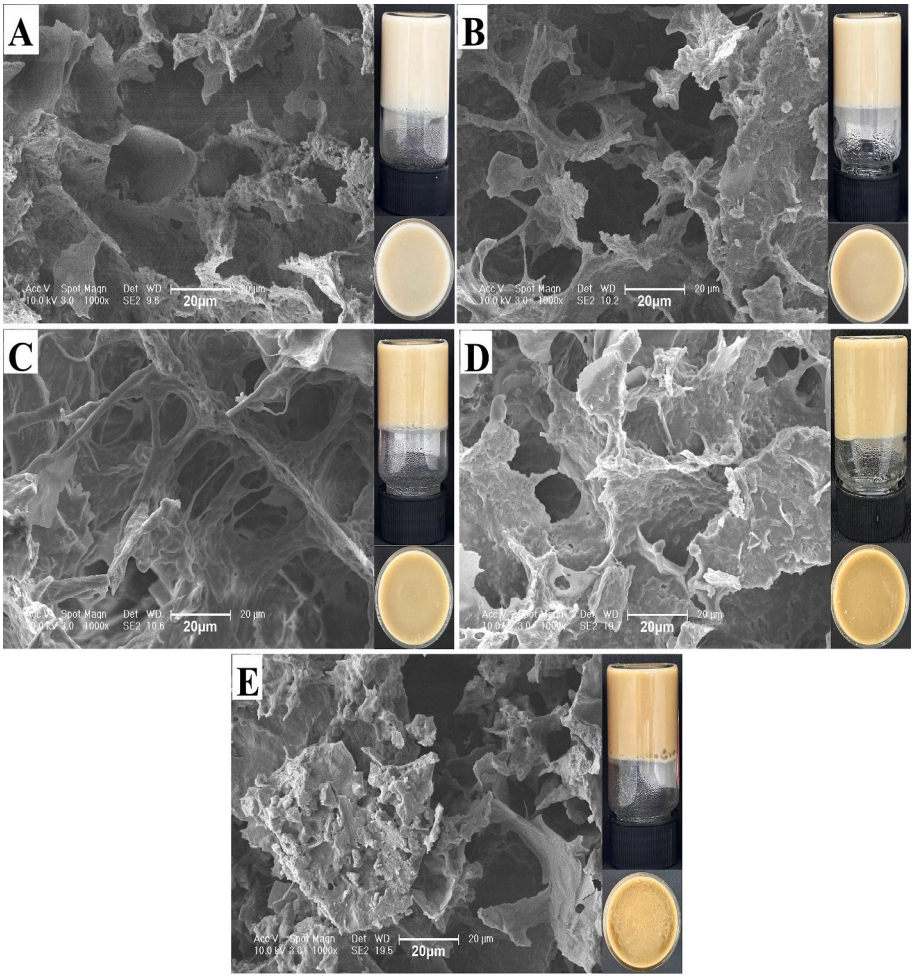

Fig. 2. Appearance and microstructure of gel samples with different mass ratios of corn silk polysaccharide-peanut isolate protein complexes. (A. TG-PPI; B. TG-PC10:1; C. TG-PC5:1; D. TG-PC2:1; E. TG-PC1:1).

compared to untreated peanut isolate protein ($p < 0.5$). The gel yield increased from 4.98 % to 6.63 %. The gel yields after addition of corn silk polysaccharides were all higher than that of simple protein gels, and the gel yield of the gel sample in which the glycosylation reaction occurred reached a maximum. Presumably, the gel network structure more stable and more liquid was trapped in the gel, leading to an increase in solids during gel formation after the glycosylation event took place (Hu et al., 2013). From the figure, it could also be found that the loading rates of the gel samples containing corn silk polysaccharide were all significantly higher than that of peanut isolate protein alone ($p < 0.5$), and the loading rate of the gel samples with ultrasound and heat treatment reached 89.31 %. This might be attributed to the fact that samples undergoing glycosylation reaction rapidly formed a gel network structure catalyzed by the TGase during the process of forming the gel samples and had a compact structure, which encased more riboflavin molecules encapsulated in the gel network structure and the loading rate increased (Zhang & Wang, 2022).

3.7. Dissolution rate analysis of riboflavin-loaded gel samples

Swelling is the result of spontaneous absorption of solvent by the gel matrix, and the swelling capacity of the gel affects the delivery and digestion of the loaded bioactives (Tamanna Begam et al., 2003). When the gel network structure was more compact, the gel had a lower tendency to absorb water and inflate or dehydrate and crumple. Since the structure of the untreated gel samples with added corn filament polysaccharides was prone to collapse after soaking in digestive solution, it was not easy to be measured (UNTG-PC5:1 could only be measured in simulated gastric fluid up to 120 min and in simulated intestinal fluid up to 180 min), and the results were shown in Fig. 3B-C. The gel samples with the addition of polysaccharides had lower dissolution rates than peanut isolate protein gels alone in both simulated gastric and intestinal fluids, probably due to the fact that the polysaccharides functioned as a filler inside the gel network structure, impeding the infiltration of water molecules (Gierszewska et al., 2018). In simulated gastric fluid (SGF), the swelling rate of the gel samples leveled off after 300 min, and the lowest swelling rate (155.6 %) was observed for sample TG-PC5:1, which contained polysaccharides and underwent glycosylation reactions by sonication and heat treatment. In the simulated intestinal fluid (SIF), the gel samples showed a flat rate of swelling after 270 min of immersion and the sample TG-PC5:1 was the lowest (123 %). These results suggested that the glycosylation reaction occurred both reduced the swelling rate of peanut isolate protein gels in SGF and SIF. This might be because the glycosylation reaction exposed more lysine to the unfolding of the peanut isolate protein structure and more covalent cross-linking with the TGase to form a tighter network structure, contributing to the reduction of the swelling rate.

3.8. In vitro release analysis of loaded riboflavin gel samples

Gels have a special network structure that protects biologically active substances, and their protection can be indirectly demonstrated by simulating the digestive behavior of the gel. Riboflavin, also known as vitamin B_2, is released from the food matrix and digested to reach the

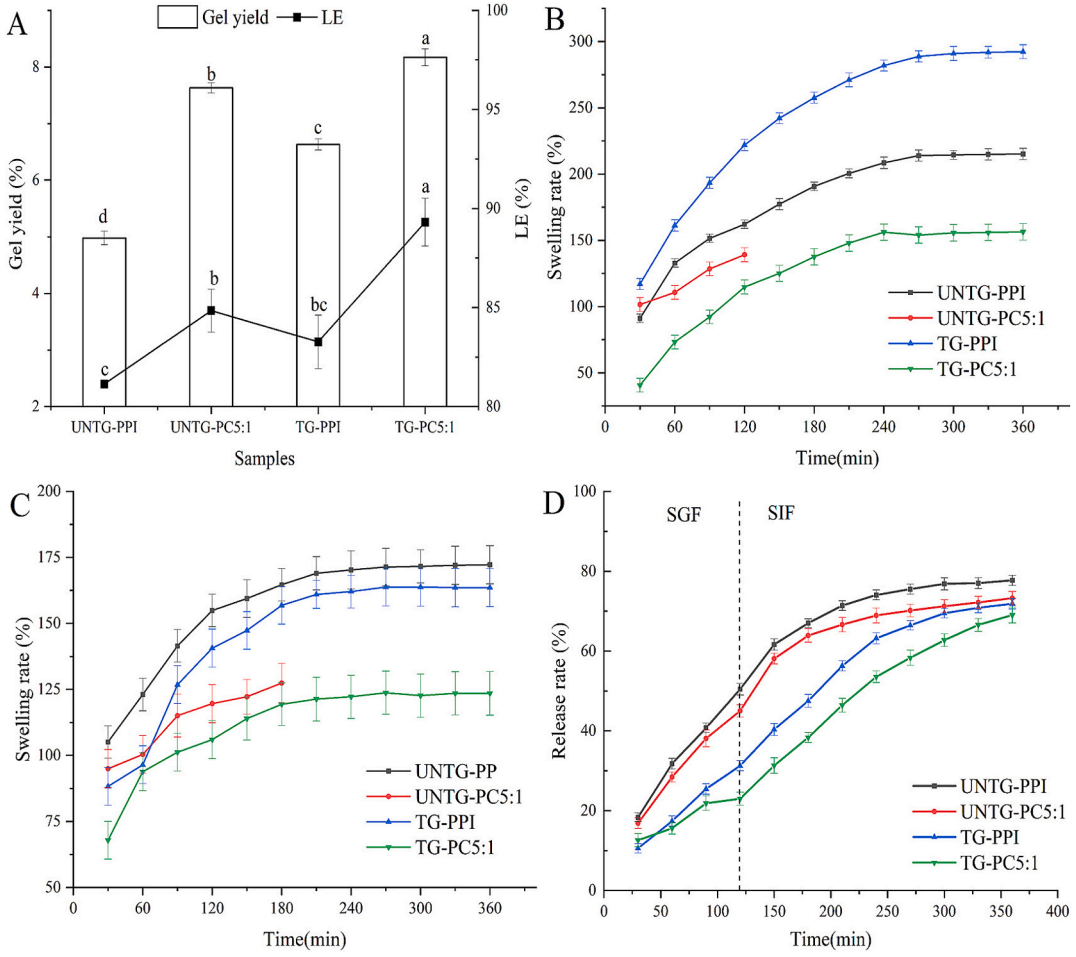

Fig. 3. (A) Gel yield and curcumin loading rate; (B) Dissolution rate in simulated gastric fluid (pH 1.2, without digestive enzymes); (C) Simulated intestinal fluid (pH 7.5, without digestive enzymes);(D) Release profiles in simulated digestive process of curcumin-loaded gel samples.

intestinal tract for absorption before it can exert its functional activity and participate in some biochemical reactions. Therefore, it is crucial to study the *in vitro* release behavior of samples loaded with bioactive substances in gels.

Fig. 3D shows the *in vitro* release rate profile of riboflavin-loaded gel samples after gastrointestinal digestion, from which it can be seen that the gel samples without ultrasound and heat treatment showed 77.7 % UNTG-PPI release after gastrointestinal digestion, the polysaccharide-added samples showed 73.2 % UNTG-PC5:1 release, and the treated gel samples showed a final release of TG-PPI of 71.8 %, whereas the TG-PC5:1 release rate was 69 %. It indicated that the polysaccharide component in the polysaccharide-protein gel system could reduce the hydrolysis of proteins by pepsin and gastric acid (Alavi, Emam-Djomeh, et al., 2018), making the gel structure more complete and the safeguard of the active substances stronger. Hence, the addition of corn silk polysaccharide reduced the release rate of the gel samples to some extent. The gel sample containing both polysaccharides and treated had the lowest release rate, which was reduced by 10.4 % compared to that of the UNTG-PPT sample, demonstrating that the occurrence of the glycosylation reaction formed a tighter three-dimensional mesh structure, which reduced the dissolution rate and protected the release of riboflavin to some extent.

4. Conclusions

The results showed that The addition of corn silk polysaccharide had a significant effect on the gel properties of the composite. The glycosylation action of the polysaccharide combined with the peanut isolate protein changed the protein structure, enhanced the hydrophobic interactions, disulfide bonds, and hydrogen bonds between molecules, thereby improving the rheological properties, gel strength, and water-holding capacity, leading to the transformation of free water to bound water. Microscopic structure showed that the polysaccharide enhanced the density of the gel network structure by combining with the protein. But excessive polysaccharide would increase the spatial hindrance between protein molecules, thereby affecting the gelation process. When the ratio of peanut isolate protein to corn silk polysaccharide was 5:1, the gel had good water-holding capacity (61 ± 0.48 %) and gel strength (32.7 ± 0.71 g), and the loading rate of riboflavin reached 89.31 %. Ultrasonic combined with glycosylation enhanced the gel yield and encapsulation efficiency of the peanut isolate protein gel loaded with riboflavin, reduced the swelling characteristics of the peanut isolate protein gel, and slowed the release of riboflavin from the peanut isolate protein gel during simulated gastrointestinal digestion.

CRediT authorship contribution statement

Nannan Hu: Writing – original draft, Methodology. **Lixin You:** Writing – review & editing, Data curation, Conceptualization. **Xinxin Han:** Software, Resources. **Shuo Wang:** Investigation, Formal analysis. **Weihua Qi:** Visualization. **Lin Xiu:** Writing – review & editing, Supervision, Project administration. **Dan Cai:** Validation, Funding acquisition.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102380.

Data availability

The authors do not have permission to share data.

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