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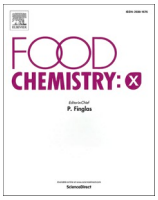

Effects of inulin with different polymerization degrees on the structural and gelation properties of potato protein

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ABSTRACT

This study investigated the effect of inulin with different polymerization degrees (DP), including L-inulin (DP 2–6), M-inulin (DP 10–23) and H-inulin (DP 23–46), on the structural and gelation properties of potato protein isolate (PPI). Results revealed that textural properties (hardness, cohesiveness, springiness and chewiness) and water-holding capacity (WHC) of PPI-inulin composite gels were positively correlated with the inulin DP and addition content at 0–1.5% (*w/v*), but deteriorated at 2% due to phase separation. The addition of 1.5% H-inulin showed the most significant increment effects on the WHC (18.65%) and hardness (2.84 N) of PPI gel. Furthermore, M-/H-inulin were more effective in increasing the whiteness and surface hydrophobicity, as well as in strengthening hydrogen bonds and hydrophobic interactions than L-inulin. Fourier transform infrared spectroscopy analysis and microstructural observation indicated that inulin with higher DP promoted more generation of β-sheet structures, and leading to the formation of stronger and finer network structures.

1. Introduction

Recently, plant proteins have gained burgeoning interest on a global scale for their advantages in environmental sustainability, abundant production, high bioavailability, and superior techno-functional properties. Potato protein is increasingly acknowledged as a particularly valuable plant protein resource due to the balanced amino acid profile with a high proportion of lysine, free allergenicity and diverse bioactivity (Herreman et al., 2024). Researchers and enterprises have explored the utilization of potato protein in various foods, including as a natural emulsifier for ice cream, fining agent for wine, effective delivery system for hydrophobic bioactives, substitute for dairy proteins in allergen-free infant formula, and flavor enhancer and ripening accelerator for cheese (Hu, He, Zhang, & He, 2024; Lomolino, Zannoni, Zabara, Da Lio, & De Iseppi, 2020). Nevertheless, compared with other plant proteins (soy, pea and peanut proteins), the industrial application of potato protein in food production remains limited due to its high hydrophobicity, low water-holding capacity (WHC) and poor gelation properties (Schmidt et al., 2019; Zhu et al., 2022).

The addition of exogenous polysaccharides has been a common strategy to improve the gel-forming ability and textural properties of proteins. Polysaccharides could interact with protein side groups through the Maillard reaction, alert the water distribution through hydrogen binding, and change the protein aggregation behavior and rheological properties by phase separation, which synergistically contributed to the formation and enhancement of the protein network. Inulin, as a typical neutral polysaccharide, has been widely used as an effective gelling enhancer and texture modifier in protein-based foods with distinctive water-solubility, excellent WHC, probiotic activity and no off-flavor or aftertaste (Han et al., 2022; Stanojevic, Barać, Pešić, & Vucelic-Radovic, 2020). Previous studies (Nieto-Nieto, Wang, Ozimek, & Chen, 2015; Xu et al., 2021) have reported that inulin could greatly improve the gel strength of soybean, pea, and oat proteins by forming hydrogen bonds with the carboxyl and amino groups on proteins, as well as enhancing the hydrophobic forces and disulfide bonds between proteins. Therefore, it is hypothesized that the addition of inulin could similarly improve the gelation properties of potato protein through intermolecular interactions. However, at present, limited reports are available on the characteristics and formation mechanism of potato protein-inulin composite gels.

Inulin is a natural fructan joined by β (2 → 1) glycosidic bonds with a terminal β-glucose unit. The degree of polymerization (DP) of inulin varies from 2 to 60, which is mainly dependent on the plant source and isolation or production methods (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015). Based on the differences in DP, inulin is generally classified into short-chain (DP ≤10), native (DP 2–60) and

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long-chain (DP ≥ 23) types (Luo et al., 2017). The DP determines the physicochemical and techno-functional properties of inulin (Wang, Wan, Liu, Xia, & Ding, 2019). In general, inulin with low DP has a superior water solubility and water-retention capacity, whereas those with high DP exhibit higher viscosity and stronger gel-forming properties at lower concentrations (Luo et al., 2017). Thus, it is possible that the inulin with different DP will exert different effects on the molecular interactions and gelation properties of proteins. Tseng, Xiong, and Boatright (2008) reported that inulin with high DP was more capable of improving the thermal stability and gel-forming properties of soy protein isolate than those with low DP by promoting protein-protein interactions and altering the protein aggregation pattern. On the contrary, Guo et al. (2021) and Liu et al. (2021) found that inulin with higher DP strongly hindered the aggregation of gluten by weakening hydrophobic interactions and disulfide bonds, finally resulting in more fragile and more open network structures as well as a softer gel. Thus, the results of these previous studies on the mechanism by which the DP of inulin affected the formation of protein gel were inconsistent or contradictory, which could not clearly indicate the possible impact of inulin DP on the potato protein gel and the related underlying mechanism.

Therefore, this work aims to systematically investigate the influence of inulin with different DP on the physicochemical properties of potato protein gels, including color attributes, WHC, textural properties and microstructures. In order to further elucidate the mechanism by which the DP of inulin affects the gel formation at the molecular level, the changes in surface hydrophobicity, the content of free amino acid, protein interaction forces, and secondary structures were studied. This study will provide a theoretical support and important guidance for using inulin as a gel enhancer in protein-based products and broadening the potential application of potato protein in food industry.

2. Materials and methods

2.1. Materials

Commercial potato protein isolate (PPI) with a protein content of 91.30% ± 0.25% (dry basis) was purchased from Shaanxi Aiboni Biotechnology Co., Ltd. (Xi'an, China). Three kinds of inulin with low DP (L-inulin, DP: 2–6), medium DP (M-inulin, DP: 10–23) and high DP (H-inulin, DP: 23–46) were supplied by Jiaowang Natural Products Co., Ltd. (Chongqing, China).

2.2. Gel preparation

The 25% (*w/v*) PPI dispersion was prepared by adding 5 g of PPI into 20 mL of distilled water and stirring for 40 min at room temperature. Then, inulin with different DP was suspended into the PPI dispersion, keeping the concentration at the level of 0.5%, 1%, 1.5% and 2% (w/v). The PPI-inulin mixed dispersion was heated in a 90 °C water bath for 30 min, followed by cooling down to room temperature and storing at 4 °C overnight (Ryu et al., 2023). The pure PPI dispersion with a concentration of 25% (w/v) was designated as the control.

2.3. Gel color measurement

The color attributes of the gel samples, including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*), were determined by an instrumental colorimeter (YS-3060, Shenzhen ThreeNH Technology CO., Ltd., China). The whiteness value was calculated using Eq. (1):

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

2.4. WHC

The WHC was measured according to the method described by Huang et al. (2024). The composite gels were wrapped in two layers of filter paper and placed into centrifuge tubes (50 mL). Then, the gel samples were centrifuged at 4500 rpm for 15 min, followed by discarding the water in the centrifuge tube and removing the filter paper. The weights of samples before and after centrifugation were recorded as W_1 and W_2, respectively. The WHC of the gels could be calculated using Eq. (2):

$$WHC = \frac{W_2}{W_1} \times 100\% \quad (2)$$

2.5. Texture profile analysis (TPA)

The textural characteristics of PPI-inulin composite gels, including hardness, cohesiveness, springiness, and chewiness, were determined by a texture analyzer (TMS-Pro, Food Technology Corporation, USA) equipped with a 100 N load cell. Uniaxial double compression tests were conducted under the TPA mode with a 36 mm-diameter cylindrical probe. TPA measurements were conducted at pre-test, test and post-test speeds of 1 mm/s, trigger force of 0.05 N and compression distance of 5 mm.

2.6. Visual appearance and microstructural observation

The visual appearance of the composite gels was recorded using a digital camera. The microstructure of the composite gels was observed using a field-emission scanning electron microscope (SEM, JSM-7500F, JEOL Ltd., Tokyo, Japan) according to the method of Min, Ma, Kuang, Huang, and Xiong (2022). Briefly, PPI-inulin composite gels were cut into slices and lyophilized. Then, the samples were manually broken to expose the fracture surface and fixed on a metal stub with conductive adhesive. After sputtering with platinum (JFC-1600, JEOL Ltd., Tokyo, Japan), SEM images were obtained at an accelerating voltage of 5 kV.

2.7. Protein interaction forces

Molecular interaction forces involved in gel formation were assessed by detecting the protein solubility in different dissociation reagents according to the method of Guo et al. (2023). Five selective chemical solutions were used to cleave certain bond types as follows: (1) 0.05 mol/L NaCl, (2) 0.6 mol/L NaCl, (3) 0.6 mol/L NaCl +1.5 mol/L urea, (4) 0.6 mol/L NaCl +8 mol/L urea and (5) 0.6 mol/L NaCl +8 mol/L urea +0.5 mol/L 2-β-mercaptoethanol. The gel samples were freeze-dried, ground, and filtrated through 80 mesh in advance. The samples (0.25 g) were dispersed in each dissociation reagent (10 mL) and stirred for 60 min, followed by centrifugation at 4500 rpm for 15 min. The protein contents in supernatants were determined by Coomassie brilliant blue method. The relative contents of electrostatic interactions, hydrogen bonds, hydrophobic interactions, and disulfide bonds were calculated by the differences in protein contents in solutions (2) and (1), solutions (3) and (2), solutions (4) and (3), and solutions (5) and (4), respectively.

2.8. Surface hydrophobicity

Surface hydrophobicity (H_0) was measured with 8-anilino-1-naphthalenesulphonic acid (ANS) as the extrinsic fluorescence probe. The sample solutions were prepared at concentrations of 0.03–0.5 mg/mL in a phosphate buffer solution (0.01 mol/L, pH 7.0). Then, 20 μL of 8 mM ANS solution was mixed with 4 mL of the sample solution and kept in the dark for 15 min. The fluorescence intensity was determined at an excitation wavelength of 390 nm and an emission wavelength of 490 nm by an RF-5301 fluorescence photometer (Shimadzu, Tokyo, Japan). H_0 was calculated as the initial slope of fluorescence intensity versus the protein concentration plot by linear regression analysis (Chen, Zhang, Liu, Li, & Wang, 2023).

2.9. Quantification of free amino groups

The content of free amino groups was quantified by the o-phthalaldehyde (OPA) spectrophotometric assay with leucine as standard. The OPA reagent was prepared by dissolving 40 mg of OPA in 1 mL of methanol, followed by mixing with 25 mL of 0.1 M sodium tetraborate, 2.5 mL of 20% (*w/v*) sodium dodecyl sulfate (SDS) solution, and 100 μL of β-mercaptoethanol, and then diluting to 50 mL with deionized water. A total of 200 μL of the sample (2 mg/mL) was vortexed thoroughly with 4 mL of OPA reagent and reacted at 35 °C for 2 min. Subsequently, the absorbance was detected at 340 nm using an ultraviolet-visible spectrophotometer (UV-1201, Shimadzu, Japan) (Chen et al., 2023). Deionized water was used as a blank.

2.10. FTIR determination

FTIR determination was conducted using a TENSOR 27 spectrometer (Bruker, Germany). A total of 1 mg of freeze-dried and powdered sample was ground finely and pressed with 100 mg of potassium bromide for measurement. The spectra were acquired at a wavelength range of 4000–400 cm^{-1} with 64 scans at a resolution of 4 cm^{-1} (Jiang et al., 2022). PeakFit 4.12 software (Seasolve, Framingham, MA, USA) was applied to assess the relative percentage of protein secondary structures.

2.11. Statistical analysis

All experiments were repeated in triplicate. The results were reported as means ± standard deviation. Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, USA). The significant difference amongst different samples was evaluated by one-way analysis of variance and Duncan's test at a 5% significance level.

3. Results

3.1. Color attributes of PPI-inulin composite gels

Color attributes are quantifiable indicators of the gel's visual appearance, which directly determine the acceptability of consumers. As shown in Table 1, the L* value decreased dramatically from 75.49 ± 0.32 to 65.46 ± 0.28 with the addition content of L-inulin increasing from 0 to 2%, accompanied with pronounced increases of a* and b* values. However, the addition of M- and H-inulin resulted in completely reverse trends. This result could be attributed to the fact that L-inulin had a higher percentage of carbohydrate units containing reducing groups than that of M- and H-inulin, thus the Maillard reaction between the free amino groups in potato protein and the reducing groups in L-inulin during heating deepened the gel color (Mensink et al., 2015). A similar phenomenon was reported by Poinot et al. (2010) who observed that the addition of inulin accelerated the Maillard reaction and increased a* and b* values of white bread. In contrast, the compact and uniform network structures in PPI-M/H-inulin composite gels (as indicated in SEM images) increased the effect of diffuse reflection, thereby increasing lightness values. Additionally, inulin with a high molecular weight could self-aggregate to form particulate gel which filled in the protein gel networks, thus leading to a higher refractive index and stronger light scattering. Likewise, Zhou, Hu, Xiang, and McClements (2023) reported an evident increase in the L* value of the potato protein gel with increasing pectin concentration, which was associated with variations in the density and dimensions of the porosity in gel structures.

The whiteness of the gel with M- and H-inulin was significantly higher ($P < 0.05$) than that of the control and gel with L-inulin. This result could be confirmed from the macrostructure observation. It implied that inulin with high DP was prone to induce the formation of ordered structures with linear aggregation, whereas the inulin with a short chain promoted the formation of the opaque gel containing larger and more random particle aggregates (Man et al., 2023).

Table 1
Effects of inulin content and polymerization degree on the color attributes and textural properties of heat-induced potato protein gel.

Sample	Inulin content (*w/v*)	L*	a*	b*	Whiteness	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Control (PPI)	0	75.49 ± 0.32Aa	0.22 ± 0.01 Da	8.30 ± 0.02 Da	74.12 ± 0.31Aa	0.52 ± 0.01 Da	0.32 ± 0.01 Da	2.62 ± 0.04 Da	0.43 ± 0.02Ea
PPI-L-inulin	0.5%	73.04 ± 0.37Ba	0.28 ± 0.01Ca	8.58 ± 0.15Ca	71.71 ± 0.31Bc	0.62 ± 0.01Cc	0.34 ± 0.01Cc	2.74 ± 0.01Cc	0.59 ± 0.01Dc
	1%	72.63 ± 1.72Bc	0.32 ± 0.02Ba	8.88 ± 0.09Ba	71.22 ± 1.64Bc	0.74 ± 0.02Bc	0.37 ± 0.01Bc	2.84 ± 0.03Bc	0.78 ± 0.02Cc
	1.5%	68.80 ± 0.83Cc	0.38 ± 0.02Aa	9.20 ± 0.06Aa	67.47 ± 0.81Cc	0.78 ± 0.01Ac	0.41 ± 0.01Ac	2.99 ± 0.03Ab	0.96 ± 0.02Ac
	2%	65.46 ± 0.28Dc	0.39 ± 0.02Aa	9.27 ± 0.10Aa	64.23 ± 0.29Dc	0.79 ± 0.01 Ac	0.36 ± 0.02Bc	2.96 ± 0.01Ab	0.84 ± 0.03Bc
PPI-M-inulin	0.5%	76.9 ± 10.72Cb	0.20 ± 0.02Ab	8.26 ± 0.04Ab	75.47 ± 0.69Cb	0.96 ± 0.01Db	0.37 ± 0.01Db	2.88 ± 0.01Cb	1.01 ± 0.03Cb
	1%	78.04 ± 0.63BCb	0.16 ± 0.03Bb	8.09 ± 0.03Bb	76.59 ± 0.60Cb	1.23 ± 0.02BB	0.39 ± 0.01Cb	2.93 ± 0.02Bb	1.42 ± 0.03Bb
	1.5%	79.29 ± 0.43Bb	0.12 ± 0.01Cb	7.76 ± 0.09Cb	77.88 ± 0.39Bb	1.54 ± 0.02Ab	0.46 ± 0.01Ab	3.02 ± 0.01Ab	2.14 ± 0.06Ab
	2%	81.00 ± 1.30Ab	0.10 ± 0.02Cb	7.53 ± 0.11Db	79.56 ± 0.19Ab	1.17 ± 0.01Cb	0.42 ± 0.01Bb	2.97 ± 0.02Bb	1.46 ± 0.02Bb
PPI-H-inulin	0.5%	79.74 ± 0.31Dc	0.20 ± 0.02Ab	8.17 ± 0.04Ab	78.16 ± 0.28 Da	2.03 ± 0.01 Da	0.42 ± 0.01Ca	2.97 ± 0.01 Da	2.54 ± 0.06 Da
	1%	82.33 ± 1.13Ca	0.16 ± 0.01Bb	7.83 ± 0.19Bc	80.66 ± 0.95Ca	2.54 ± 0.02Ca	0.43 ± 0.01Ca	3.12 ± 0.02Ba	3.41 ± 0.07Ca
	1.5%	84.64 ± 1.05Ba	0.11 ± 0.04Cb	7.28 ± 0.22Cc	82.99 ± 0.90Ba	3.36 ± 0.02Aa	0.52 ± 0.02Aa	3.17 ± 0.01Aa	5.57 ± 0.18Aa
	2%	87.19 ± 1.04Aa	0.07 ± 0.02Db	7.24 ± 0.21Cb	85.28 ± 0.98Aa	3.02 ± 0.01Ba	0.46 ± 0.01Ba	3.02 ± 0.01Ca	4.20 ± 0.09Ba

Note: Different uppercase letters in the same column mean significant differences ($P < 0.05$) in varying amounts of inulin with the same DP. Different lowercase letters in the same column mean significant differences ($P < 0.05$) between inulin with different DP and the same inulin amount.

3.2. WHC of PPI-inulin composite gels

WHC is an essential quality indicator of gels, which is closely related to inner microstructures and the number of hydrogen bonds (Teng et al., 2024). As shown in Fig. 1A, the WHC of heat-induced PPI gel was 67.96% ± 1.00%, which was comparable to the result (65.58%–69.46%) of the previous study (Joeres et al., 2023). The WHC of potato protein gels positively correlated with the added inulin content in the range of 0.5%–1.5%, whereas it decreased sharply when the inulin content was beyond 1.5%. These results are consistent with the report of Zhang et al. (2020), which showed that the WHC of myosin gels initially increased as the addition of inulin increased from 0% to 2% and then decreased with the increase of inulin content. These changes can be explained by the fact that inulin could bind water through hydrogen bonds at low concentrations. Moreover, as shown in SEM images, the incorporation of inulin promoted the formation of a porous protein network with a high capacity to trap water and retain it. However, the incompatibility of macromolecular biopolymers at high concentrations disrupted the continuity of gel network structures, thus reducing the WHC of the composite gels by capillary forces (Xu et al., 2021).

With the increase of the DP of inulin, the WHC of PPI gel increased gradually. The WHC value increased from 67.96% ± 1.00% to 73.07% ± 0.37% as L-inulin concentration varied from 0% to 1.5%, and reached 80.20% ± 0.61% and 86.61% ± 0.41%, respectively, with the addition of 1.5% M-inulin and H-inulin. These results could be interpreted that L-inulin, as a natural dietary fiber with rich hydroxyl groups and high solubility, could effectively dissolve in the protein gel network and restrict water translocation by hydrogen binding, thereby diminishing the quantity of free water and increasing the WHC. On the contrary, the entanglement between the long molecular chains of H-inulin with potato proteins promoted the formation of dense and fine gel structures with evenly distributed small pores, thereby entrapping water molecules effectively. Similarly, Piao et al. (2023) observed a higher increase in WHC of surimi gel with the addition of 12% (*w*/w) H-inulin than that with equivalent L-inulin. However, in contrast to the present results, Liu et al. (2021) reported that the addition of 10%–40% (w/w) L-, M-, and H-inulin all significantly reduced ($P < 0.05$) the WHC of gluten gel, and inulin with a higher molecular weight led to a lower WHC. The researchers assumed that inulin prevented the protein from binding water by forming a barrier around the gluten protein molecules. These discrepancies may be due to the differences in treatment conditions, protein gelation properties, and molecular interaction ways amongst the different plant proteins, inulin, and water. Therefore, the incorporation of inulin could not only increase the dietary fiber content and improve the nutrient profile of the gel-type food, but also was conducive to the improvement of the juiciness and final yield of PPI gels.

3.3. Textural properties of PPI-inulin composite gels

Texture is the dominant structural and organoleptic characteristic that influences the tenderness, juiciness and swallowing properties of food. Hardness is defined as the resistance of a material to deformation and scratching, and it is expressed as the peak stress (Wang, Xiao, Guo, Tian, & Ai, 2024). Cohesiveness refers to the internal bonding strength

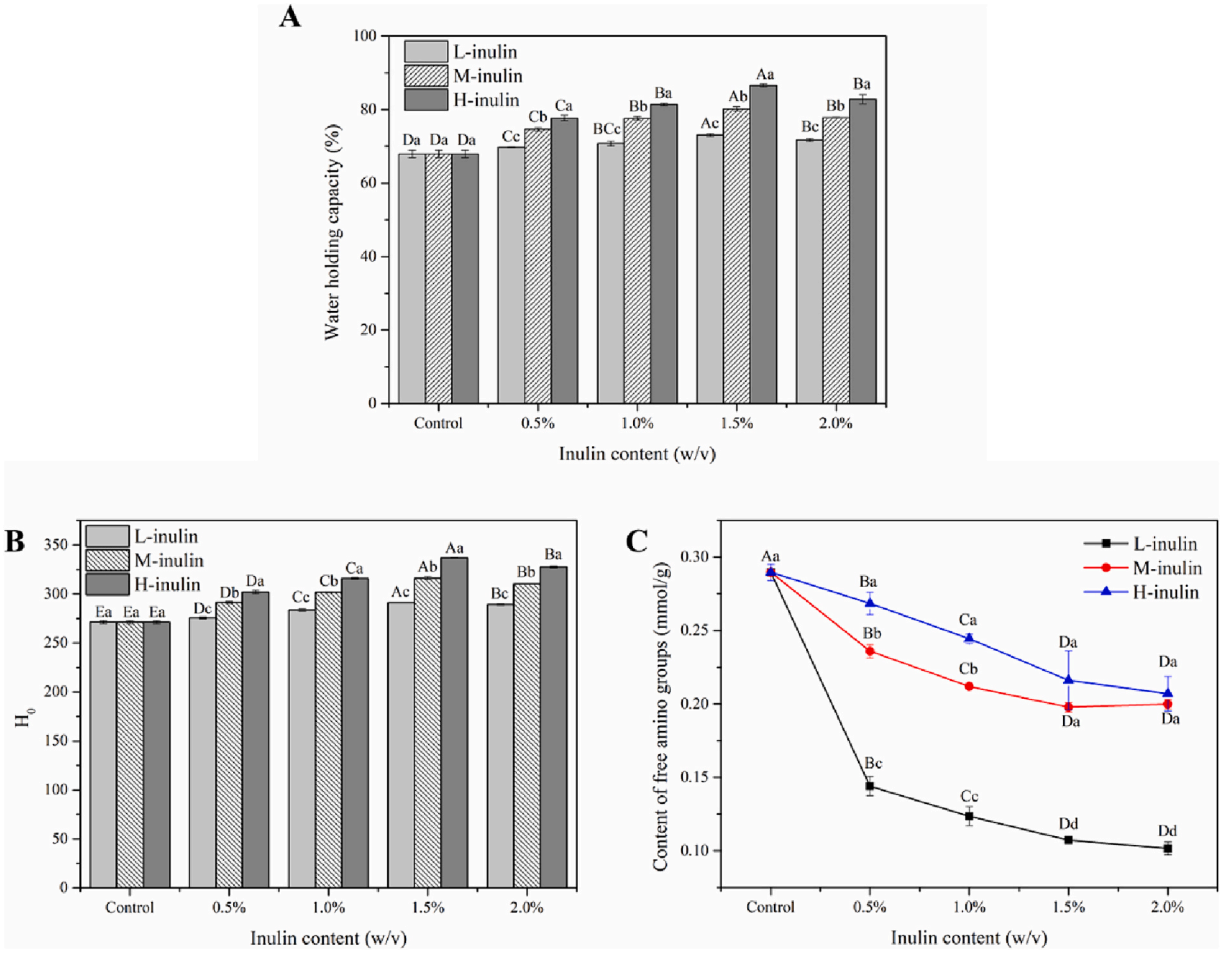

Fig. 1. Effects of inulin content and polymerization degree on the water holding capacity (A), surface hydrophobicity (B) and content of free amino groups (C) of heat-induced potato protein gel. Different uppercase letters mean significant differences ($P < 0.05$) in varying amounts of inulin with the same DP. Different lowercase letters mean significant differences ($P < 0.05$) between inulin with different DP and the same inulin amount.

that binds a material into clumps, and it is calculated as the work area ratio of two compressions (Zhu et al., 2022). Springiness is the extent that a deformed material recovers to its undeformed state after withdrawing deformation force, whereas chewiness refers to the energy required to fully chew a substance into a swallowable state (Piao et al., 2023).

As shown in Table 1, the incorporation of L-inulin had a mild impact on the improvement of the hardness, cohesiveness, springiness and chewiness of the PPI gel, whereas the addition of M/H-inulin significantly increased ($P < 0.05$) all of these TPA mechanical parameters. Moreover, the enhancement effects of inulin on the gel textural properties positively correlated with the inulin content in the appropriate range (0–1.5%). The hardness and chewiness of PPI-H-inulin composite gel increased significantly ($P < 0.05$) from 0.52 ± 0.01 N and 0.43 ± 0.02 mJ to 3.36 ± 0.02 N and 5.57 ± 0.18 mJ, respectively, as the addition content of H-inulin increased from 0 to 1.5%. Similarly, Huang et al. (2024) reported that the presence of inulin remarkably increased the hardness, springiness and cohesiveness of myofibrillar protein gels, and this effect was concentration-dependent. This result indicated that the presence of inulin contributed to the formation of a stronger biopolymer network compared with the control group. Furthermore, L-inulin was distributed uniformly in the protein network as nanoparticles, and it filled the cavities of the protein gel network, thus improving the gel texture. Inulin with long molecular chains would occupy a large hydrodynamic volume, which allowed it to easily entangle and interact with potato proteins, thereby resulting in a firmer and more elastic gel (Cortez-Trejo, Gaytán-Martínez, Reyes-Vega, & Mendoza, 2021). However, when the amount of L-inulin increased from 1.5% to 2.0%, the hardness and springiness remained almost constant, whereas the cohesiveness and chewiness decreased significantly ($P < 0.05$) from 0.41 ± 0.01 and 0.96 ± 0.02 mJ to 0.36 ± 0.02 and 0.84 ± 0.03 mJ, respectively. Furthermore, the addition of M-inulin and H-inulin at the level of 2.0% led to significant reductions ($P < 0.05$) in all tested textural characteristics compared with that with 1.5% addition content. These results indicated that the presence of excessive inulin would disrupt or weaken the internal connections in composite gels, thereby reducing the ability of these gels to resist stress.

3.4. Macro- and micro-structures of PPI-inulin composite gels

Visual appearances and inner microstructures of the heat-induced PPI-inulin composite gels are shown in Fig. 2. The pure PPI gel

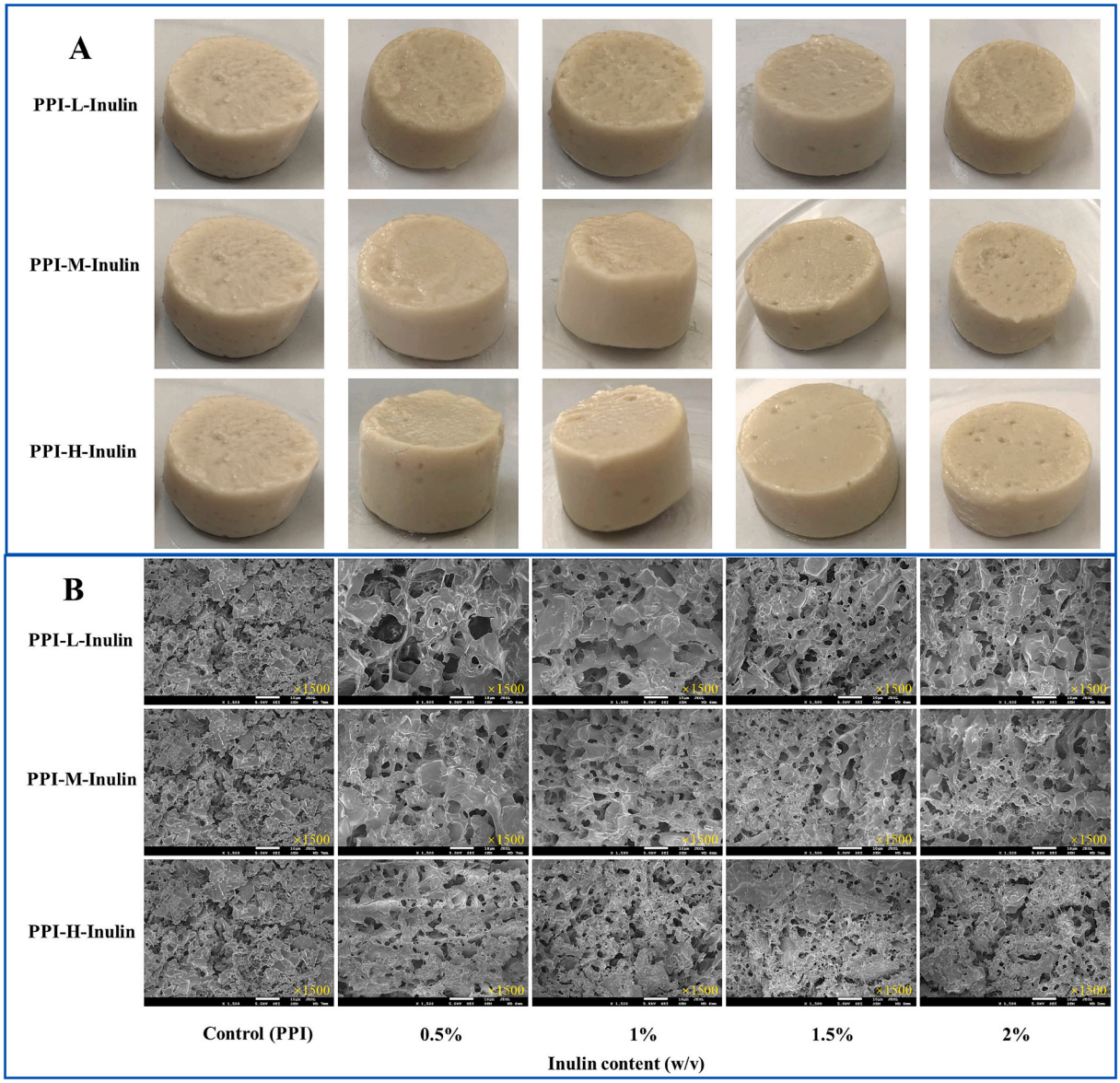

Fig. 2. Camer (A) and SEM (B) images of heat-induced PPI-inulin composite gels.

presented a weak and coarse appearance without an evident interconnected microstructure. The addition of inulin led to smoother and firmer appearances and crosslinked three-dimensional network structures with evenly localized pores. This result may be due to the fact that inulin, as a hydrophilic polysaccharide, competed for water with PPI, which provided a more hydrophobic environment for proteins and promoted the hydrophobic interaction amongst protein molecules (Han et al., 2022). Moreover, the binding of inulin to water generated local pressure and increased the relative concentration of potato protein (Han et al., 2022). This effect accelerated the contact and aggregation of protein molecules, thus promoting the formation of gel network structures. Increasing the concentration and DP of the added inulin, the pore size in the gel networks became smaller and more uniform. These changes might be due to the fact that inulin with a higher concentration and longer molecular chain was more prone to macromolecular entanglement and led to greater solution viscosity, contributing to the formation of more finely structured and highly interconnected network structures. However, when the amount of inulin exceeded 1.5%, the gels exhibited more and larger pores, and some fraction of the protein matrix formed block-like clustering and larger agglomeration. It was probably due to the fact that the self-aggregation and local gelation of inulin caused phase separation and hindered the cross-linking of protein molecules. These large pores might be responsible for the reduction of WHC at 2% inulin. Therefore, the result indicated that the addition of inulin, especially that with high DP and 1.5% concentration, was advantageous for the formation of a continuous and strong protein gel network with a uniform pore distribution.

3.5. Effect of inulin on the surface hydrophobicity of PPI gel

To obtain further information about the effects of inulin on the surrounding microenvironment of protein molecules and the exposure degree of the hydrophobic groups within the interior of potato proteins, surface hydrophobicity was determined. As shown in Fig. 1B, the presence of inulin generally increased the surface hydrophobicity of PPI gels, which suggested that the combination of water by inulin enhanced the hydrophobic environment of proteins, and induced the exposure of hydrophobic amino acid side chain groups on the potato protein chains. With the concentration of L-, M- and H-inulin increasing from 0 to 1.5%, the surface hydrophobicity increased remarkably from 271.22 ± 1.61 to 291.04 ± 0.35, 316.09 ± 1.66, and 336.86 ± 0.50, respectively. These increments might be ascribed to the formation of α-helix structures containing hydrophobic centers when inulin was in a polar environment (Han et al., 2022). These α-helix structures tended to spontaneously associate with the hydrophobic groups on proteins to attenuate the interface free energy of the mixed system, thus inducing more exposure of hydrophobic groups. When inulin was added at the level of 2%, the surface hydrophobicity exhibited a decreased trend, which was consistent with the changes in WHC. It suggested that excess inulin might interact with the carboxyl and amino groups on proteins through hydrogen bonds and generate a hydrophilic layer around the protein molecules, thereby leading to the reduction of accessible binding sites for ANS. Additionally, the excluded volume effect amongst the macromolecular biopolymers at high concentrations would result in the partial refolding of hydrophobic groups into the interior of proteins.

The surface hydrophobicity of PPI gels showed an evident dependence on the DP of the added inulin. At the same addition level of inulin, the higher DP of inulin led to a higher surface hydrophobicity. This result implied that inulin with higher DP was more prone to entanglement with protein molecules and inhibited the refolding of heat-induced stretching protein molecular chains, thereby maintaining high exposure levels of hydrophobic groups. This effect would further enhance the hydrophobic interactions amongst the protein molecules and consequently promote the aggregation and crosslinking of protein molecules. Likewise, Piao et al. (2023) also reported higher surface hydrophobicity of surimi gels containing inulin with long molecular chains compared with those with short molecular chains.

3.6. Effect of inulin on the content of free amino groups of PPI gel

The changes in the content of free amino groups indirectly characterize the molecular interaction intensity between PPI and inulin, as well as the degree of protein self-aggregation and cross-linking. As shown in Fig. 1C, the content of free amino groups decreased significantly ($P < 0.05$) with the increase of inulin concentration in the range of 0–1.5%. This result was consistent with the previous report of Wang et al. (2020), who found that the carbonyl groups of the reducing end of inulin interacted covalently with the free amino groups of whey protein isolate after wet-heating treatment at 70 °C, thereby decreasing the free amino group content. Jiang et al. (2022) also observed that the grafting degree of PPI-inulin conjugates positively correlated with inulin concentration. Nevertheless, the number of free amino acid groups remained almost constant when the content of inulin increased from 1.5% to 2%, indicating that the molecular interaction between inulin and potato protein was saturated at 1.5%.

The incorporation of inulin with different DP led to considerable differences in the content of free amino groups. With the addition of 0.5% L-inulin, the content of free amino groups decreased drastically from 0.29 ± 0.01 mmol/g to 0.14 ± 0.01 mmol/g, whereas the value decreased slightly to 0.27 ± 0.01 mmol/g with the addition of H-inulin. Inulin with low DP had high solubility and flexibility, which enabled it to contact with the amino acid residues and participate in the Millard reaction easily. This finding was consistent with the data of color attributes. In addition, inulin with high DP entangled with heat-induced stretching protein molecular chains, thereby inhibiting the refolding of denatured proteins and decreasing the reduction amplitude of free amino groups.

3.7. Effect of inulin on the intermolecular forces of PPI gel

The microstructures and textural properties of PPI-inulin composite gels were essentially determined by intermolecular forces, including electrostatic interactions, hydrogen bonds, hydrophobic interactions and disulfide bonds. As shown in Table 2, the PPI gel and PPI-inulin composite gels were all constructed and maintained mainly by hydrophobic interactions and hydrogen bonds, followed by electrostatic interactions, and then weak disulfide bonds. This result was consistent with the previous reports of Ryu and McClements (2024) and Tanger, Andlinger, Brümmer-Rolf, Engel, and Kulozik (2021). Potato protein contained a high proportion of hydrophobic amino acids (Hu et al., 2024). Upon heating, the hydrophobic residues that were initially buried inside would be exposed and would react with one another via hydrophobic interactions. However, patatin, the main component of potato protein, contains only one free thiol group, leading to the lack of internal disulfide bonds in the potato protein gel networks (Tanger et al., 2021).

As the DP and addition content (0–1.5%) of inulin increased, the hydrogen bonding and hydrophobic interactions were enhanced gradually, which was consistent with previous studies on the effects of inulin on pea protein isolate, chicken myofibrillar protein, and surimi gel (Han et al., 2022; Piao et al., 2023; Xu et al., 2021). This effect is probably due to abundant hydroxyl groups of inulin which could interact with carboxyl and amino groups on proteins through hydrogen bonds. Furthermore, the result of surface hydrophobicity suggested that inulin, especially those with longer molecular chains and higher addition content, was more prone to induce the exposure of hydrophobic groups on proteins, thus significantly enhancing the hydrophobic interactions. The electrostatic interactions and disulfide bonds were slightly weakened with increasing the DP and content of inulin. These results may be associated with the fact that inulin is a neutral polysaccharide, and it cannot generate electrostatic interactions and form covalent bonds easily with protein molecules (Liu et al., 2021). Inulin with long

Table 2
Effects of inulin content and polymerization degree on the intermolecular forces of heat-induced potato protein gel.

Sample	Inulin content (w/v)	Ionic bond (g/100g)	Hydrogen bond (g/100g)	Hydrophobic interaction (g/100g)	Disulfide bond (g/100g)
Control (PPI)	0	5.87 ± 0.28ABa	17.07 ± 0.45Ca	37.21 ± 0.21 Da	1.94 ± 0.42Aa
PPI-L-inulin	0.5%	5.92 ± 0.21Aa	17.85 ± 0.53BCb	38.69 ± 0.58Cb	2.13 ± 0.29Aa
	1%	5.94 ± 0.25Aa	18.01 ± 0.14Bc	39.20 ± 0.42Cc	2.17 ± 0.45Aa
	1.5%	5.45 ± 0.29BCa	20.47 ± 0.21Ab	42.48 ± 0.35Ab	0.92 ± 0.16Ba
	2%	5.25 ± 0.14Ca	20.10 ± 0.81Ab	41.37 ± 0.58Bb	1.06 ± 0.08Ba
PPI-M-inulin	0.5%	5.64 ± 0.12Aa	18.50 ± 0.28Cb	40.77 ± 0.42Ba	1.99 ± 0.16Aa
	1%	5.29 ± 0.11Bb	19.24 ± 0.28Bb	41.10 ± 0.21Bb	1.34 ± 0.14Bb
	1.5%	4.16 ± 0.18Cb	20.90 ± 0.35Aab	42.11 ± 0.42Ab	0.69 ± 0.14Ca
	2%	4.04 ± 0.08Cb	20.93 ± 0.21 Aab	42.16 ± 0.45Ab	0.74 ± 0.21Cb
PPI-H-inulin	0.5%	4.78 ± 0.14Bb	21.27 ± 0.21Ba	41.33 ± 0.29Ca	1.39 ± 0.21Bb
	1%	3.65 ± 0.12Cc	21.71 ± 0.21Aba	43.08 ± 0.45Ba	0.97 ± 0.21BCb
	1.5%	3.26 ± 0.54Cc	22.87 ± 1.68Aa	43.87 ± 0.14Aa	0.83 ± 0.14Ca
	2%	3.31 ± 0.46Cc	21.71 ± 0.08ABa	43.82 ± 0.21Aa	0.79 ± 0.14Cab

Note: Different uppercase letters in the same column mean significant differences ($P < 0.05$) in varying amounts of inulin with the same DP. Different lowercase letters in the same column mean significant differences ($P < 0.05$) between inulin with different DP and the same inulin amount.

molecular chains and high concentration may impart steric hindrance and interfere with the electrostatic interactions and SH-SS exchange interactions amongst protein molecules. Likewise, Liu et al. (2021) reported that the presence of inulin with high molecular weight occupied a large space in the gluten matrix and hindered the mobility of protein molecular chains, thereby reducing the interconversion between S-S and SH groups and the contribution of ionic bonds. The increase in inulin content from 1.5% to 2.0% resulted in unnoticeable changes in the fraction of each interaction force in gels, except for a slight decrease in hydrophobic interactions for PPI-L-inulin composite gel. It suggested that when continuous composite gel network structures were once formed, the addition of excess inulin in protein has little impact on the intermolecular forces that maintained the structures of the gel network. Therefore, the enhanced hydrophobic interactions and hydrogen bonds by inulin play critical roles in improving the textural characteristics of the composite gels, such as hardness and chewiness.

3.8. *Effect of inulin on the secondary structures of PPI gel*

FTIR spectroscopy is a powerful technique to provide information on the molecular conformation changes of proteins and interactions of protein-polysaccharide systems. The changes in secondary structures were explored by analyzing the amide I band (1700 cm^{-1}-1600 cm^{-1}) through Fourier self-deconvolution and Gaussian curve-fitting (Fig. 3) (Yang et al., 2022). The relative contents of secondary structures in composite gels are presented in Table 3. Potato protein contained a relatively high content of β-sheet and a low content of α-helix, which was considered a typical plant protein structure (Carbonaro, Maselli, & Nucara, 2015). The DP of inulin showed more significant effects on the secondary structures than the addition content. The addition of L-inulin promoted the conversion of α-helix to β-sheet, whereas M-inulin led to the conversion of β-turn and random coil to β-sheet. As 1.5% H-inulin

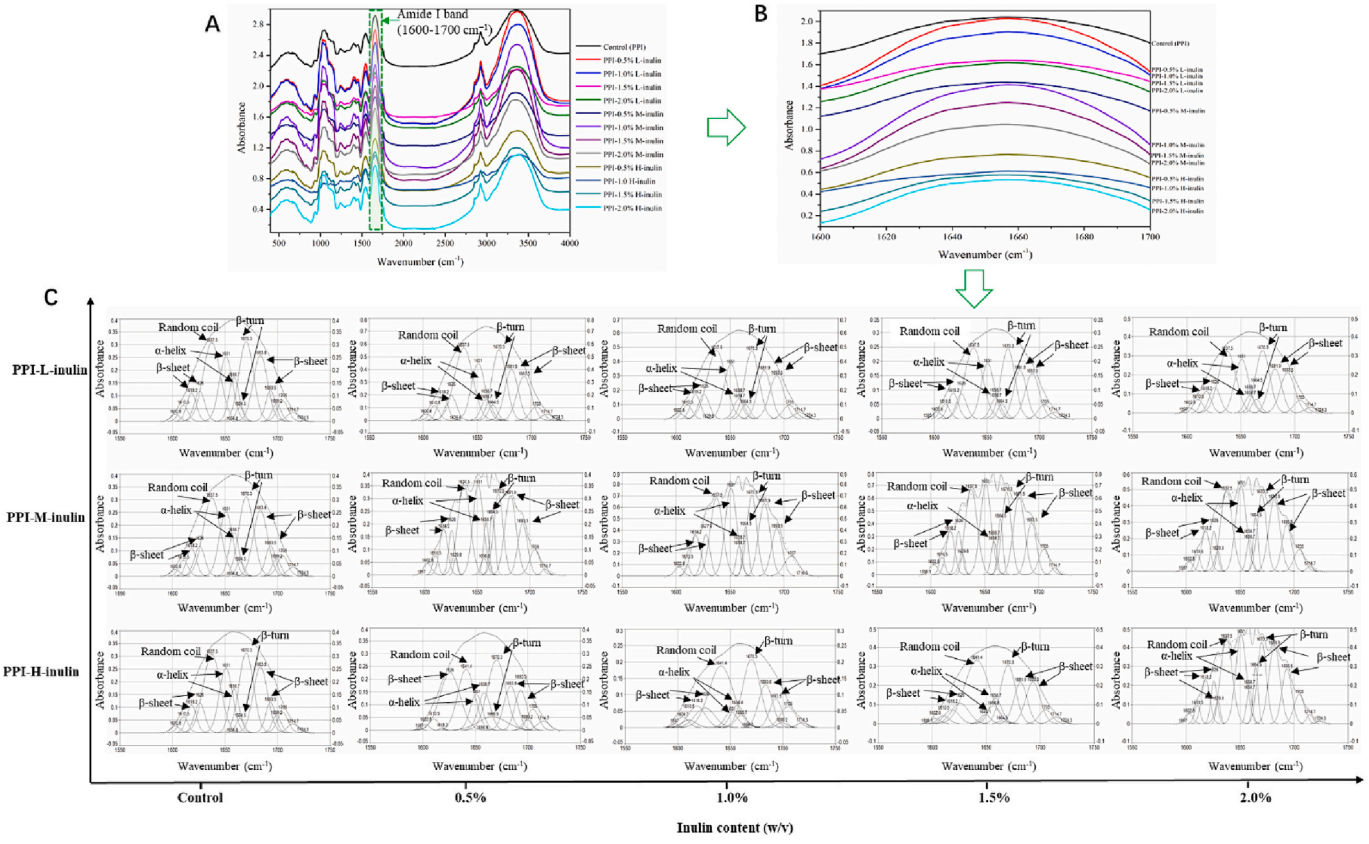

Fig. 3. FTIR spectra of potato protein gels with different inulin contents and polymerization degrees in the range of 400-4000 cm^{-1} (A) and 1600–1700 cm^{-1} (B), and FTIR curve-fitted sub-peaks spectra (C).

Table 3
Effects of inulin content and polymerization degree on the secondary structures of heat-induced potato protein gel.

Sample	Inulin content (w/v)	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)
Control (PPI)	0	22.98 ± 0.43Aa	31.90 ± 0.22Ba	21.86 ± 0.44Aa	23.26 ± 0.20BCa
PPI-L-inulin	0.5%	18.50 ± 0.68Cb	34.15 ± 0.42Abb	21.41 ± 0.22Aa	25.94 ± 1.32Aa
	1%	18.93 ± 0.00BCb	34.95 ± 0.06ABc	21.62 ± 0.09 Aa	24.51 ± 0.03ABa
	1.5%	18.76 ± 0.45BCb	35.14 ± 0.16Ac	21.72 ± 0.16 Aa	24.37 ± 0.77ABa
	2%	21.12 ± 1.86Aba	33.88 ± 2.65Abb	22.53 ± 1.20 Aa	22.47 ± 0.42Ca
PPI-M-inulin	0.5%	24.09 ± 0.53Aa	37.86 ± 0.91Ba	18.93 ± 0.51Bb	19.12 ± 0.92Bb
	1%	22.08 ± 0.57BCa	39.51 ± 0.35Ab	19.69 ± 0.43Bb	18.72 ± 0.64Bb
	1.5%	21.82 ± 0.01Ca	39.44 ± 0.01Ab	19.46 ± 0.01Bb	19.28 ± 0.02Bb
	2%	21.99 ± 0.07BCa	39.13 ± 0.05Aab	19.50 ± 0.00Bb	19.37 ± 0.03Bb
PPI-H-inulin	0.5%	18.59 ± 1.16Bb	38.99 ± 0.65Ba	21.08 ± 0.12Aa	21.34 ± 0.39Bb
	1%	18.44 ± 0.72Bb	41.39 ± 0.49ABa	19.83 ± 0.55Bb	20.33 ± 0.78Bb
	1.5%	18.94 ± 0.20Bb	42.97 ± 0.49Aa	19.71 ± 0.02Bb	18.38 ± 0.31Cb
	2%	20.33 ± 1.63Ba	42.11 ± 2.49ABa	19.23 ± 0.04Bb	18.33 ± 0.90Cb

Note: Different uppercase letters in the same column mean significant differences ($P < 0.05$) in varying amounts of inulin with the same DP. Different lowercase letters in the same column mean significant differences ($P < 0.05$) between inulin with different DP and the same inulin amount.

was introduced into the potato protein system, the ratios of β-sheet increased significantly ($P < 0.05$) from 31.90% ± 0.22% to 42.97% ± 0.49%, whereas the ratios of α-helix, β-turn and random coil decreased from 22.98% ± 0.43%, 21.86% ± 0.44% and 23.26% ± 0.20% to 18.94% ± 0.20%, 19.71% ± 0.02%, and 18.38% ± 0.31%, respectively. Similar results have been obtained by Piao et al. (2023) and Huang et al. (2024) who reported that inulin facilitated the partial transformation of protein from α-helix to β-sheet for surimi and myofibrillar protein. β-sheets were ordered conformations that were stabilized by strong intermolecular hydrogen bonding (Wang, Xiao, et al., 2024). A high proportion of β-sheet structure was responsible for the high thermal stability and stable gel network (Wang et al., 2024). Thus, the substantial increase in β-sheet structures by the addition of inulin indicated that inulin promoted the protein polymerization and favored the formation of more organized and stable structures in potato protein gels, which was verified by the enhanced textural properties. The β-turn structure was an open reverse turn referred to "loop" regions, and it required less hydrogen bonds than β-sheet, whereas random coil was referred to the flexible and loose structures (Mozafarpour, Koocheki, Milani, & Varidi, 2019; Wang et al., 2017). Thus, the reduction of β-turn and random coil structures by M-/H-inulin implied that the entanglement between protein and inulin with high DP enhanced the intermolecular association and rearrangement amongst protein molecules, consequently leading to more stable and ordered structures in heat-induced protein networks.

3.9. Mechanistic discussion

Based on the results above, the possible interactions between potato protein and inulin with different DP during heat-induced gelation are summarized in Fig. 4. During heating, potato protein molecules underwent dissociation and partially or completely lost the spatial conformation of the initial structures. Unfolding protein molecules exposed the buried reactive sites and turned into a flexible state that favored protein-polysaccharide interactions. The addition of inulin provided several hydrogen bond donors in the mix system. Moreover, inulin tended to form a helical structure with a hydrophobic center in water. Thus, the incorporation of inulin, irrespective of the DP and concentration, strengthened the hydrogen bonding and hydrophobic interactions in the composite gels, thereby increasing the gel strength and WHC.

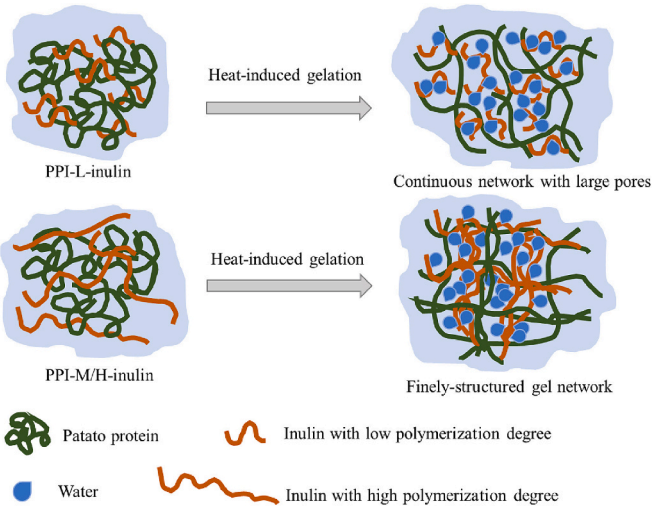

Fig. 4. The schematic illustration of the intermolecular interactions between potato protein and inulin with different polymerization degrees during heat-induced gelation.

The physicochemical properties of the PPI-inulin composite gels showed an evident dependency on the DP of inulin. Specifically, L-inulin likely interacted with potato protein through the Maillard reaction, thereby reducing the content of free amino acids and deepening the gel color. Moreover, L-inulin was inserted into the protein matrix as a filler and competed for water with proteins, thereby accelerating the contact of protein molecules and promoting protein aggregation and cross-linking. By contrast, inulin with high DP tended to entangle with protein molecules through non-covalent bonding and inhibited the refolding of heat-induced stretching protein molecular chains, consequently leading to a compact and fine gel network with more β-sheet structures.

Furthermore, the gel strength was positively correlated with the inulin concentration in the range of 0–1.5%. The increasing inulin concentration introduced more numbers of hydrogen bonds per unit area in the PPI-inulin mixed system and reduced the average distance amongst protein molecules, which created greater probability for protein molecules to aggregate and crosslink. In addition, the appropriate additional amount of inulin shielded charges amongst proteins and reduced the electrostatic repulsion forces, thereby accelerating protein aggregation. However, excessive inulin (2.0%) may lead to a macromolecular crowding environment in the PPI solution. The volume exclusion and phase separation in the crowded solution interfered the kinetics of protein hydration and association reactions involved in protein folding, assembly and aggregation. Moreover, the self-assembly of excessive inulin formed separate hydrogel and large aggregates after heating and cooling treatments, which would hinder the crosslinking of unfolding protein molecular chains. Consequently, excessive inulin (2.0%) led to a decreasing trend in the WHC and textural properties of PPI-inulin composite gels, as well as an increase in the porosity of gel microstructures.

4. Conclusion

In this study, the addition of inulin generally had a positive impact on the gel characteristics of potato protein, including the WHC, texture and microstructures. This gel enhancement performance of inulin was

strongly dependent on the DP and addition concentration. The composite gel of PPI with 1.5% H-inulin showed the highest values in hardness, cohesiveness, springiness, chewiness, and WHC, with a finely structured and highly interconnected network structures. However, the addition of 2.0% inulin caused reductions in texture profiles and increases in pore size of gel networks due to phase separation. Inulin with high DP and concentration (0–1.5%) was more effective in increasing the surface hydrophobicity and strengthening the hydrogen bonds and hydrophobic interactions. FTIR analysis indicated that the DP of inulin played a more critical role in improving the ratio of β-sheet structures than the addition content. Inulin with high DP promoted more conversion of other secondary structures to β-sheet, thus leading to the formation of firmer appearances and more uniform network structures in potato protein gels. This work provides a theoretical basis and technical support for the application of inulin as a gel enhancer, texture modifier, and nutrition improver in plant protein-based foods.

Credit author statement

Qiongling Chen: Conceptualization, Investigation, Data interpretation, Writing- Original draft preparation; Xiaowen Wang: Methodology, Data interpretation, Writing - Review & Editing; Yu Wang: Data interpretation, Validation; Tianqi Guo: Software, Data acquisition; Peihan Guan Software, Data acquisition; Jinyu Hou: Data acquisition, Zhenjia Chen: Investigation, Writing - Review & Editing, 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.

Data availability

Data will be made available on request.

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