Gel properties of *Nicandra physalodes* (Linn.) gaertn. seeds polysaccharides with tea polyphenols and its application

Qiu-Yue Ma [a], Qian-Da Xu [a,b], Nan Chen [a], Wei-Cai Zeng [a,b,*]

[a] *Antioxidant Polyphenols Team, Department of Food Engineering, Sichuan University, Chengdu 610065, PR China*
[b] *The Key Laboratory of Food Science and Technology of Sichuan Province of Education, Sichuan University, Chengdu 610065, PR China*

ARTICLE INFO

ABSTRACT

The novel gelling polysaccharides (NPGP) were extracted and characterized from *Nicandra physalodes* (Linn.) Gaertn. seeds, while properties and potential application of NPGP gels with tea polyphenols were further explored. NPGP was composed of GalA, Glc, Rha, Gal, Xyl, Ara, and Man at a molar ratio of 71.87:17.13:3.10:2.55:2.19:1.64:1.52, with molecular weight of 6.32×10^4 Da and low methoxylation degree of 45.21%. The gelling properties of NPGP gel induced by tea polyphenols showed that tea polyphenols significantly improved the structural and rheological properties of NPGP gel, due to the formation of dense network by hydrogen bonds and the increase of crystalline degree of NPGP. NPGP gels with tea polyphenols could significantly ameliorated the texture, water-holding capacity, aggregation, leading force, and moisture distribution of surimi during freeze-thaw cycles. All results suggest that NPGP gels with tea polyphenols has fine properties and show potential to be applied as natural additives in food industry.

1. Introduction

Nicandra physalodes (Linn.) Gaertn., is an erect annual herb belonging to the family of *Solanaceae*. It first appeared in South America and is now widely distributed in several Asian nations, most notably China (Zhang et al., 2024). The seeds in its berries are the main product with a variety of physiological functions such as annealing, diuresis, wind dispelling and anti-inflammation and so on (Yuan et al., 2023). Moreover, its seeds have traditionally been used to make a jelly-like food, which is commonly called "Bing Fen" and popular in China. In addition, it has been reported that *N. physalodes* seeds are rich in polysaccharides, which can form a thermally reversible cold-induced gel with good water retention properties (Guo et al., 2021). However, the gel strength of this polysaccharides gel only formed from these polysaccharides is not strong, which affects its further applications in food processing.

In some previous studies, it was found that several suitable concentrations of plant polyphenols were employed as additive to form network structure for polysaccharides, polyphenol-polysaccharide interactions may affect the physicochemical and functional properties of plant foods, such as emulsification and gelling properties (Guo et al., 2022). Among plant polyphenols, tea polyphenols (TP) are so popular to

be used as additives in food industry. Tea polyphenols are the main active components of tea and widely used in food industries due to their well-documented biological activities their potential benefits for food quality and human health (Gao, Xu, & Zeng, 2020). Consequently, tea polyphenols have the potential to improve the strength and properties of *N. physalodes* seeds polysaccharides gel (Lv et al., 2023).

Polysaccharides gels are often used as water-retaining agents for frozen aquatic products. Compared with some synthetic agents with potential toxicity and harmful risks, some alternative polysaccharides gels derived from natural resources have received more attention as significant approaches in recent years. Surimi is the most common frozen product in processed fish products and also a kind of raw material for aquatic products. Freezing is a common method to inhibit the deterioration of surimi during processing and storage (Zhu, Zhou, & Sun, 2019). However, the quality of frozen surimi, especially after long-term freezing or repeated freeze-thaw process, will be reduced (Tan et al., 2022), and the juices of surimi will be seriously lost and its nutritional value will also be reduced (Chen et al., 2022). Thus, in the preset study, the efforts are made to characterize the structure of polysaccharides from *N. physalodes* seeds and form the polysaccharide gels using tea polyphenols. In addition, the effects of tea polyphenols on the gel properties of this polysaccharides gel were determined. Furthermore, by

---

* Corresponding author at: Antioxidant Polyphenols Team, Department of Food Engineering, Sichuan University, Chengdu, 610065, PR China.
E-mail address: weicaizeng@qq.com (W.-C. Zeng).

using surimi as food model, the potential application of this polysaccharides gels with tea polyphenols to protect the quality of surimi during freeze-thaw cycles was evaluated.

2. Materials and methods

2.1. Materials and reagents

Nicandra physalodes (Linn.) Gaertn. seeds were purchased from a local market in Chengdu, China and stored at 4 °C (ID: 2022-06-16). Tea polyphenols (TP, purity ≥98%, and the main active components were identified to be catechins, including epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate were purchased from Tongze Biotechnology Co. (Xi 'an, China). Common carps (*Cyprinus carpio* Linnaeus) were obtained from a local wholesale aquatic market in Chengdu, China, in July 2023, and were immediately transported to the laboratory alive in water.

Standard monosaccharides, dimethyl sulfoxide (DMSO), trifluoracetic acid (TFA), dichloromethane (DCM), and potassium bromide (KBr) were provided by Aladdin Biochemical Technology Co. (Shanghai, China). Urea, sodium dodecyl sulfate (SDS), and bovine serum albumin (BSA) was purchased from Yunde Technology Co. (Chengdu, China). The remaining substances and reagents were all of analytical grade. The experimental water was produced by the UPT water purification system (Ulupure, Chengdu, China).

2.2. Extraction and purification of polysaccharides from N. physalodes seeds

The *N. physalodes* seeds (100 g) were pocketed into gauze and then immersed in deionized water (1 L) at 50 °C for 50 min. Afterwards, the seeds were extruded and filtered out to collect the crude polysaccharide solution. The proteins in crude polysaccharide solution were removed with Sevage reagent. Next, the filtrate was precipitated and purified with 10 times volume of 40%, 60%, 80% ethanol solution, respectively. The precipitate was washed by absolute ethanol and then freeze-dried to obtain *N. physalodes* seeds polysaccharide (NPGP). The yield of NPGP was 5.21% (5.21 g NPGP/ 100 g sample). The total saccharide content, uronic acid content and degree of methoxylation (DM) of NPGP were determined by the phenol-sulfuric acid method, *M*-hydroxybiphenyl assay and acid-base titration (Guo et al., 2021), respectively.

2.3. Structural characterization of NPGP

2.3.1. Homogeneity and molecular weight determination
The homogeneity and molecular weight of NPGP were determined by high-performance gel-permeation chromatography (HPGPC) with TSK GEl-G5000PW$_{XL}$-CP columns (Tosoh Biosep, Yamaguchi, Japan). The specific experimental methods and operational details were referred to previous studies (Zeng, Zhang, & Jia, 2014). The analysis was carried out using the sample (0.5 g/L, 20 μL) and dextran standards (DXT91K, DXT62K, DXT60K, DXT55K, and DXT41K, 0.1 g/L, 20 μL).

2.3.2. Determination of fourier transform infrared (FTIR) spectra
The FTIR spectra was recorded using an FTIR spectrometer (Nicolet Is10, Thermo Fisher, USA). The spectra range was 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} and the scan number of 16 times. NPGP (1 mg) was mixed with dry potassium bromide (100 mg), ground and crushed for determination.

2.3.3. Measurement of monosaccharide composition
The monosaccharide composition of NPGP was determined by gas chromatography–mass spectrometry (GC–MS, Shimadzu GCMS-QP 2010, Japan), and the detailed method was referred to the previous study (Zeng et al., 2014).

2.4. Preparation of gels

Tea polyphenols (TP) were added into the NPGP solution (10 μg/mL) to reach the final concentration of 0, 1, 5, 10 and 15 μg/mL, respectively. Afterwards, the mixture was incubated at 4 °C for 30 min to form the gels.

2.5. Determination of gels texture

The texture of gels (5 g, 22 mm in diameter) was measured according to previous method (Tan et al., 2022). Texture profile analysis (TPA) testing was performed with a texture analyzer (TA.XT Plus Stable Micro Systems Ltd., Godalming, UK) equipped with a P/36R probe. The probe speeds were 2, 1, and 2 mm/s for pre-, mid-, and post-tests, respectively. Each samples were tested at least 6 times at 25 °C.

2.6. Determination of gels rheological properties

The rheological properties of the gels were determined by a modular ompact rheometer (MCR32 Anton Paar Co., Ltd., Austria). Each group of gels was prepared into slices (5 mm in height) and placed on parallel plates (60 mm in diameter) for the rheological test. The frequency scanning mode was performed at 1.0% strain (within the linear viscoelastic region), and the frequency sweep range was 0.1 to 100 rad/s. The storage modulus (G′) and viscous modulus (G″) were recorded.

2.7. Determination of X–ray diffraction (XRD) of freeze-dried gels

The crystal structure of the freeze-dried gels was studied by a wide-angle X-ray diffractometer (D8 Advance, Bruker, Ltd., German). The gels were lyophilized and ground to powder before analysis, and the gels without tea polyphenols were used as control. The instrument was equipped with a CuKα lamp and a nickel filter (40 kV, 40 mA), with a scanning diffraction angle of 5° to 55° (2θ) at a rate of 5°/min and a step size of 0.02°.

2.8. Determination of gelling forces

The urea and SDS were added to the TP-NPGP stocking solution to reach the final concentration of 2 mol/L and 0.4 g/L, respectively.The mixture was stirred for 5 min at 25 °C and stored overnight at 4 °C. Then, hardness values of gel samples were determined as described in Section 2.4.2. Gel without urea and SDS was used as control.

2.9. Effects of gels on the quality of surimi during freeze-thaw cycles

2.9.1. Preparation of frozen surimi balls
The fresh carps (weight 750 ± 50.0 g) were slaughtered and washed, and the viscus, bones, and skin of carps were removed. After that, carp meat was homogenized at 6000 g for 5 min at 4 °C to obtain the surimi. Subsequently, surimi was mixed with tea polyphenols (5 mg/kg, based on the weight of surimi) and NPGP (0, 2, 4, 6, 8, 10 mg/kg, based on the weight of surimi), and the mixture was further stirred at 6000 g for 3 min at 4 °C. Then, the mixture was made into meatballs (5.00 ± 0.05 g), and the surimi meatballs were subjected to triple freeze-thaw cycles, with each cycle being frozen at −20 °C for 20 h and thawed at 25 °C for 4 h (Cen et al., 2023).

2.9.2. Determination of whiteness
Whiteness was determined by a color difference colorimeter (CM-5, Konica Minolta, Japan). The results were expressed by whiteness values, and the formula was expressed as follows: whiteness $= 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$, where L^* is lightness, a^* is redness and greenness, and b^* is yellowness and blueness, respectively.
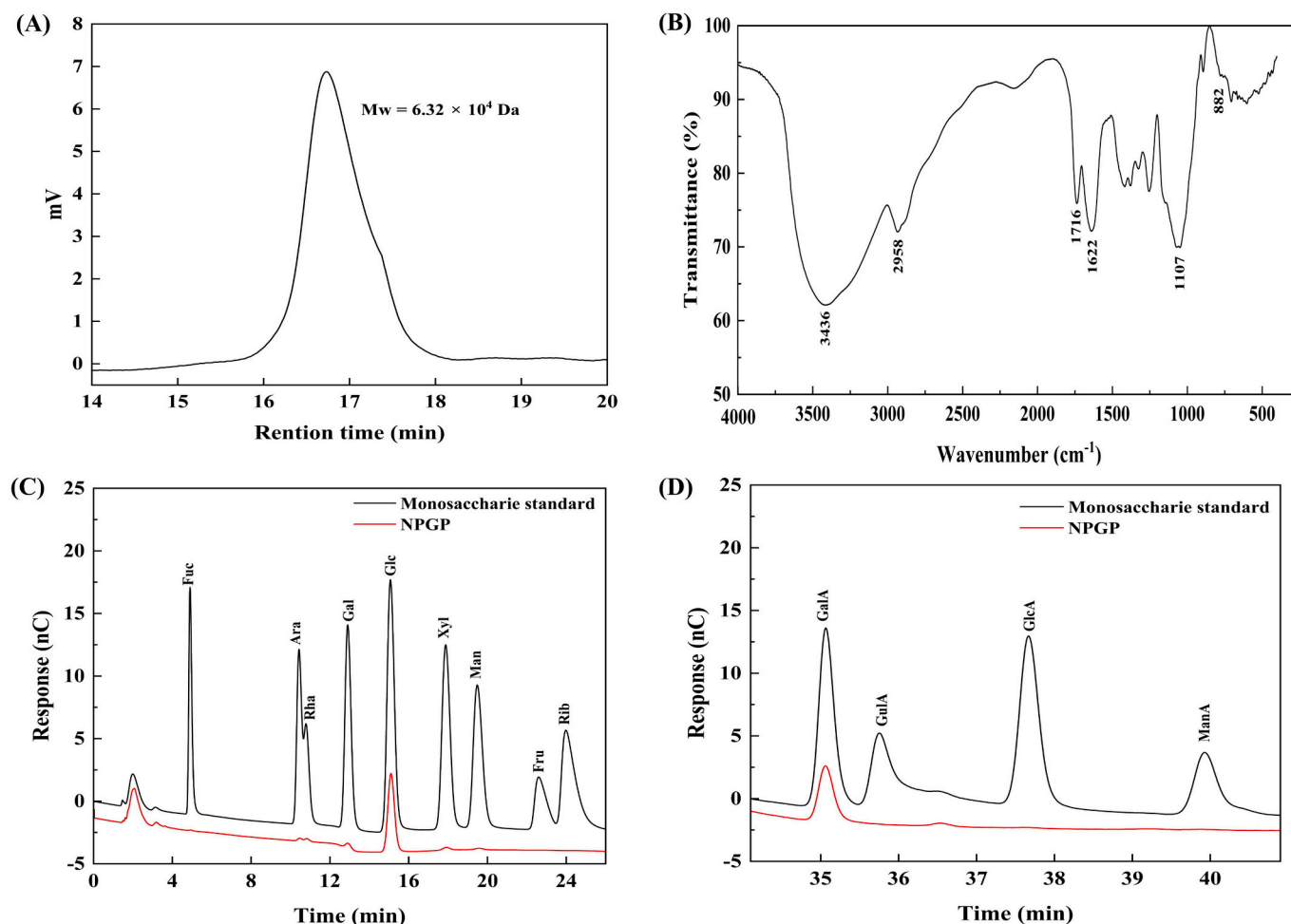
Fig. 1. Structural characterization of polysaccharides (NPGP) from *N. physalodes* seeds. (A) High-performance gel-permeation chromatography profile, (B) Fourier transform infrared spectra, (C) Monosaccharide composition, (D) Uronic acids composition.

2.9.3. Determination of texture

The texture was measured in the same way as in *Section 2.4.2* using a texture analyzer equipped with a P/10 probe. The probe speed was 2 mm/s, the trigger force was 10 g, and the compression distance was 5 mm.

2.9.4. Determination of water-holding capacity (WHC)

All surimi samples were weighed at 5.0 g (W_1) and centrifuged (5000 g, 5 min) in three layers of filter paper at 4 °C. The supernatant was removed, and the precipitate was weighed again (W_2). The water-holding capacity was calculated as follows: WHC (%) = $W_2/W_1 \times 100$.

2.9.5. Determination of zeta potential (ζ-potential)

Myofibrillar protein (MP) was prepared from carp meat according to the previous study (Tan et al., 2022), and then stored at 4 °C for further use. The concentration of MP was determined by the Biuret method. Subsequently, MP solution (1 g/L) was mixed with tea polyphenols (5 mg/kg, based on the weight of MP) and NPGP (0, 2, 4, 6, 8, 10 mg/kg, based on the weight of MP). The ζ-potential of mixture was measured using a potential analyzer (Nano-ZS90, Malvern Instruments, UK), and the detailed method was according to the previous study (Li et al., 2017).

2.9.6. Determination of molecular forces

The content of ionic bonds, hydrogen bonds, and hydrophobic interactions of surimi were determined according to the methods in the previous study (Fu et al., 2023). The surimi samples (0.5 g) were mixed with 5 mL of solution A (0.05 mol/L of sodium chloride), B (0.6 mol/L of sodium chloride), C (0.6 mol/L of sodium chloride and 1.5 mol/L urea), and D (0.6 mol/L of sodium chloride and 8 mol/L urea), respectively. Subsequently, the mixture was centrifuged at 4 °C (8000 g, 10 min) and the protein content in supernatant was determined by the Biuret method. The differences in protein content between solutions B and A, C and B, and D and C represent the presence of ionic, hydrogen, and hydrophobic interactions, respectively.

2.9.7. Determination of moisture distribution

The water distribution of surimi was detected by a low-field nuclear magnetic resonance (LF NMR) analyzer (NM120, Niumag Co., ltd., Shanghai, China) with a frequency of 22 MHz at 32 °C. The surimi (1 g) containing NPGP (10 mg/kg) was transferred into an NMR tube for testing, and the surimi without NPGP was used as the control. The sequence of Carr Purcell-Meiboom-Gill (CPMG) was used to record the time of transversal relaxations (T_2).

2.10. Statistics analysis

Statistical analysis was using SPSS (version 26.0 for Windows, SPSS Inc., Co, USA). The data were reported as mean ± standard deviation (SD) from three replicates. The analysis of variance (ANOVA) and the Tukey test were used to determine the significant differences ($P < 0.05$).

Table 1
The effects of tea polyphenols (TP) on texture of *N. physalodes* seeds polysaccharides (NPGP) gels.

| TP concentrations (µg/mL)	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Resilience
0	88.12 ± 0.42[e]	0.87 ± 0.12[e]	0.61 ± 0.24[ab]	52.76 ± 0.23[d]	0.43 ± 0.23[a]
1	92.11 ± 0.59[d]	0.89 ± 0.09[d]	0.62 ± 0.27[a]	54.83 ± 0.02[cd]	0.42 ± 0.42[ab]
5	98.74 ± 0.49[c]	0.92 ± 0.18[c]	0.59 ± 0.17[c]	55.80 ± 0.14[c]	0.40 ± 0.35[b]
10	107.21 ± 0.55[b]	0.94 ± 0.26[b]	0.60 ± 0.33[b]	57.80 ± 0.07[ab]	0.38 ± 0.41[c]
15	110.11 ± 0.60[a]	0.96 ± 0.13[a]	0.60 ± 0.23[b]	58.88 ± 0.06[a]	0.36 ± 0.58[d]

Note: Different small letters in the same column indicate significant difference ($P < 0.05$).

3. Results and discussions

3.1. Composition and structural characterization of N. physalodes seeds polysaccharide

Chemical composition analysis showed that the total saccharide content, uronic acid content, and degree of methoxylation (DM) of *N. physalodes* seeds polysaccharide (NPGP) were 93.53 ± 0.82%, 72.53 ± 0.64%, and 45.21 ± 0.31%, respectively. The ultraviolet spectra showed no absorption at 280 or 260 nm, which indicated that NPGP was free of nucleic acids and protein. As shown in Fig. 1A, NPGP exhibited a single and symmetrical sharp peak. Thus, NPGP was a homogenous polysaccharide and its weight-average molecular weight (Mw), number-average molecular weight (Mn), and Mw/Mn were calculated to be 6.32 × 10^4 Da, 5.16 × 10^4 Da, and 1.22, respectively. As shown in Fig. 1B, NPGP exhibited strong broadband absorption peaks unique to poly-saccharides at 3436 and 2958 cm^{-1}, representing the O—H and C—H stretching vibration (Yuan & Macquarrie, 2015). The absorption peaks at 1622 and 1716 cm^{-1} were mainly due to the stretching vibration of carboxylation and C=O, which indicated the presence of uronic acid in the NPGP structure (Wang, Wang, Huang, Liu, & Zhang, 2015). More-over, the characteristic absorption bonds at 1107 and 882 cm^{-1} implied that the glycosidic bond of NPGP was mainly composed of pyranose ring and α-configurations linkages (Yuan & Macquarrie, 2015), further revealing that NPGP had typical structural characteristics of pectin. As shown in Fig. 1C and D, NPGP was mainly composed of galacturonic
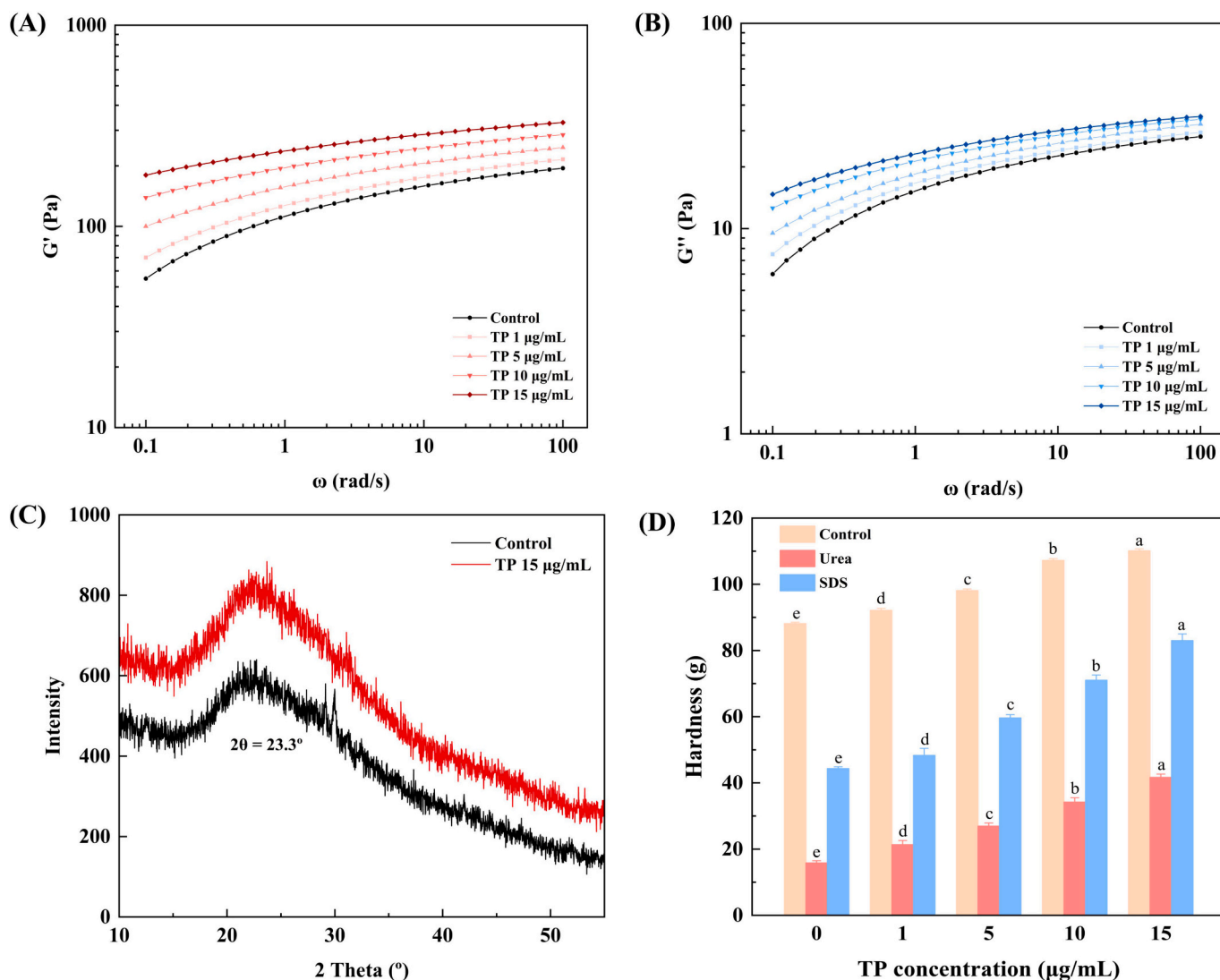
Fig. 2. Effects of tea polyphenols (TP) on the properties of gels. (A) Storage modulus (G′), (B) Viscous modulus (G″), (C) X-ray diffraction spectra, (D) Gelling forces.
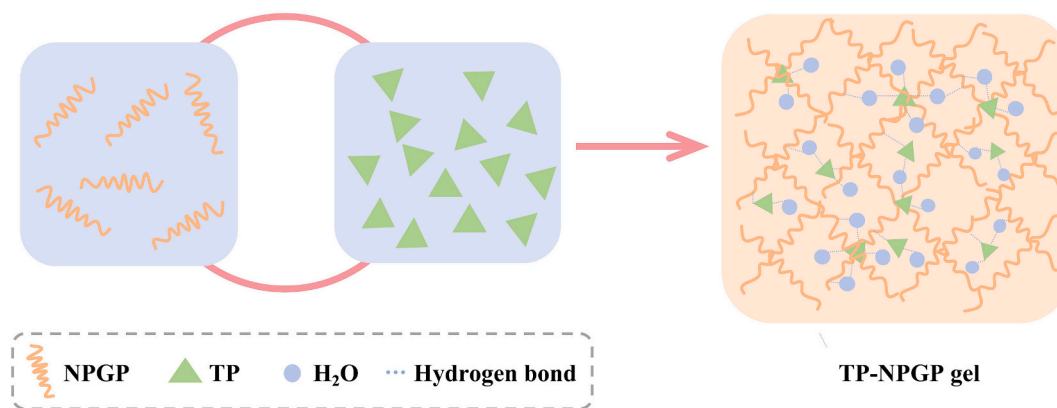
Fig. 3. Schematic mechanism for the interaction between tea polyphenols (TP) and *N. physalodes* seeds polysaccharides (NPGP).

acid (GalA), glucose (Glc), rhamnose (Rha), galactose (Gal), Xyluse (Xyl), arabinose (Ara), and mannose (Man) at a molar ratio of 71.87:17.13:3.10:2.55:2.19:1.64:1.52. Previous research structural analysis suggested that NPGP polysaccharide was consisting of dominant GalA unit (87.8%) and its DM is approximately 28% (Guo et al., 2021). Liu et al., also analyzed the structure of NPGP that it possessed a DM of 46.93%, GalA content of 65.80%, and average molar weight of 6.31×10^5 Da (Liu et al., 2022). These structural parameters again indicate that NPGP belongs to low methoxyl pectin (LMP), but the degree of methoxylation (DM) value and GalA content are noticeably different, which probably due to the different extraction methods of NPGP. Additionally, the previous study showed that the gel properties of a polysaccharide are mainly determined by its molecular aggregation, and higher molecular weight might favorably contribute to the formation of network structures (Yuan et al., 2022).

3.2. Textural properties of gels

As shown in Table 1, all gels showed relatively high hardness and gumminess, but low springiness, cohesiveness and resilience, indicating that gels were easily deformed and hard to recover to the original shape. In addition, with increase of TP concentration, the texture properties of gels except for the restitution were slightly improved, especially the hardness (from 88.12 to 110.11 g). The results showed that TP could promote the NPGP gelation and enhance the gel structure, which might be due to the increased number and closeness of the gel network (Li et al., 2022). It has been reported that the significant hydrophilicity of tea polyphenols can reduce the solvation degree of polysaccharide molecules (Cheng, Xiang, Tang, Zhu, & Liu, 2021). They further provide further providing hydrogen bonds to stabilize the junctional region structures while immobilize the free water in polysaccharide gels (Guo et al., 2021). Therefore, TP was able to improve the textural properties of polysaccharide gels.

3.3. Rheological properties of gels

In the range of angular frequency tested, the G′ of sample (Fig. 2A) was bigger than the corresponding G″ (Fig. 2B), illustrating that the composite system had a colloidal behaviour. Meanwhile, G′ and G″ of NPGP gel increased with the angular frequency, which indicated that NPGP gel was a kind of weak gel. However, G′ and G″ of NPGP gel increased with the increase of TP addition amount, showing that TP could enhance the entanglement between the molecular chain segments in gel and then improve the network structure of gel system. According to these results and previous reports (Yang et al., 2023), TP and NPGP might be combined to form intermolecular aggregates through hydrogen bond interactions and hydrophobic interactions. Afterwards, the water molecules were immobilized within the network structure, which

effectively prevented the water flow and made the whole gel aggregating in a relatively limited space (Li et al., 2022). Therefore, a denser network structure was formed and the elastic strength of gel was improved, which was consistent with the texture results of gels in Section 3.2.

3.4. XRD analysis of gels

Natural pectin polysaccharides generally exist in the form of semi-crystalline and/or amorphous structures without obvious crystal structure, so the pectin polysaccharides crystals will show a relatively wide characteristic peak range in XRD spectra (Guo et al., 2021). As shown in Fig. 2C, a major diffraction peak was observed at $2\theta = 23.3°$, indicating that NPGP formed a semi-crystalline and amorphous structures. With the addition of TP, the diffraction peaks located at $2\theta = 23.3°$ was more significant and sharper, perhaps because TP increased the crystalline degree of the polysaccharides. It is reported that phenolic compounds can enhance the interaction forces between the polysaccharides molecules, and the molecular arrangement in the polysaccharides are more orderly, which ultimately leads to an increase in crystalline degree of polysaccharides (Lee & Chang, 2020).

3.5. Gelling forces of gels

Commonly, urea can be used to break the hydrogen bond interaction between polymer molecules, and sodium dodecyl sulfate (SDS) is employed as an anionic surfactant that can break the hydrophobic interaction between compounds (Guo et al., 2021). As shown in Fig. 2D, urea significantly reduced the gel strength and completely destroyed the gel structure, while SDS also reduced the gel strength but was weaker than urea. Consequently, the hydrogen bond interaction played a dominant role in the formation of the gels. Additionally, compared with the groups without tea polyphenols, tea polyphenols attenuated the negative effects of urea and SDS on the hardness values of gel in a dose-dependent manner. This might be related to the stimulative effect of TP on gelling. Some previous studies report that polysaccharides carry a large number of hydrophilic groups (Lee & Chang, 2020). Thus, water molecules are easy to form a water molecular layer outside the polysaccharides through hydrogen bonds, further preventing the proximity of other polysaccharides molecules (Yuan et al., 2023). In the present study, tea polyphenols might provide a large number of phenolic hydroxyl groups that interact with NPGP through hydrogen bonds (Zhang et al., 2024), as shown in Fig. 3. Meanwhile, tea polyphenols could fix more free water in the gel network, further promoting the formation of gel network binding regions and enhancing the interaction in the gel system.

Table 2
Texture and whiteness of surimi treated with *N. physalodes* seeds polysaccharides (NPGP)and tea polyphenols.

NPGP concentrations (mg/kg)	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Resilience	Whiteness
0	88.12 ± 0.42[de]	0.87 ± 0.12[e]	0.52 ± 0.24[a]	52.76 ± 0.23[d]	0.43 ± 0.23[a]	56.43 ± 0.26[a]
2	92.11 ± 0.59[d]	0.89 ± 0.09[d]	0.55 ± 0.27[b]	54.83 ± 0.02[c]	0.42 ± 0.42[ab]	55.97 ± 0.52[ab]
4	98.74 ± 0.49[c]	0.92 ± 0.18[c]	0.57 ± 0.17[c]	55.80 ± 0.14[c]	0.40 ± 0.35[b]	55.53 ± 0.57[bc]
6	105.21 ± 0.51[b]	0.94 ± 0.26[b]	0.61 ± 0.33[d]	57.80 ± 0.07[b]	0.38 ± 0.41[c]	54.15 ± 0.20[c]
8	110.11 ± 0.60[ab]	0.96 ± 0.13[a]	0.64 ± 0.23[e]	58.88 ± 0.06[b]	0.36 ± 0.58[d]	54.08 ± 0.38[cd]
10	114.11 ± 0.60[a]	0.96 ± 0.42[a]	0.68 ± 0.19[f]	61.64 ± 0.24[a]	0.34 ± 0.46[e]	54.03 ± 0.48[d]

Note: Different small letters in the same column indicate significant difference ($P < 0.05$).
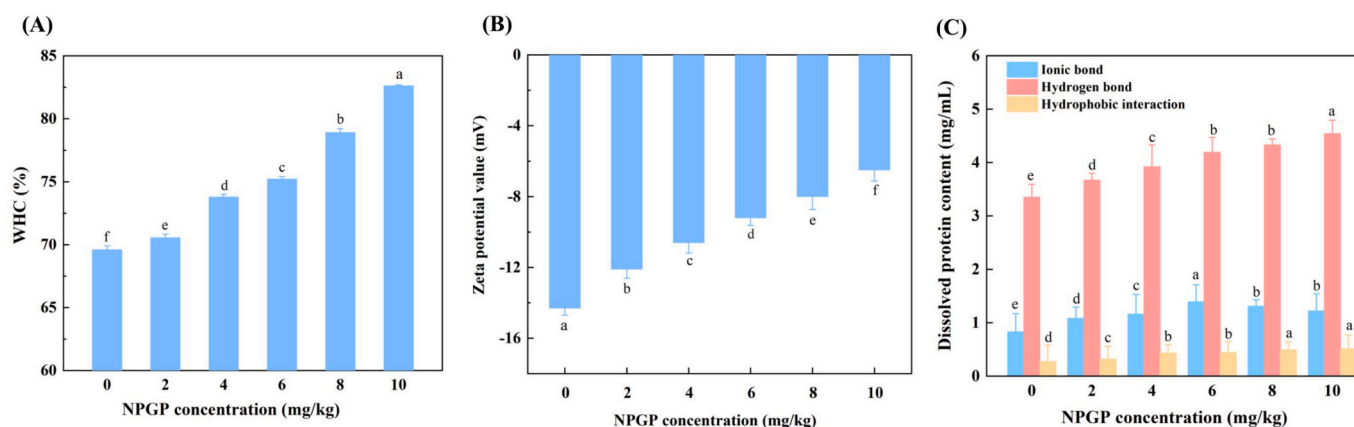

Fig. 4. Effects of *N. physalodes* seeds polysaccharides (NPGP) gel with tea polyphenols on the (A) water-holding capacity, (B) zeta potential, and (C) molecular force of surimi during freeze-thaw cycles.

3.6. Effects of NPGP on the quality of surimi

3.6.1. Texture and whiteness of surimi

As shown in Table 2, NPGP showed the significant ($P < 0.05$) effect on the texture of surimi. With the addition of NPGP, the hardness and chewiness of surimi were significantly higher than those in the control group, whereas no significant change in springiness was observed. On the one hand, NPGP might contribute to the electrostatic interaction in surimi by interacting with myofibrillar protein and water to improve the stability of surimi (Chen et al., 2022). On the other hand, the hydrated NPGP gel was filled into the pores of the protein network structure, forming a more uniform and stable network structure, thereby improving the gel texture of surimi. Moreover, the whiteness value of the surimi is slightly reduced, which was due to the light gray color of NPGP itself. Compared with the control group, the whiteness of surimi decreased with the increase of NPGP.

3.6.2. Water-holding capacity (WHC) of surimi

WHC of surimi is an important parameter to measure the quality of surimi, which reflects the protein-water interaction and structural changes in surimi. As shown in Fig. 4A, the WHC of surimi gradually increased in a dose-dependent manner with the increase of NPGP addition amount. These results indicated that NPGP had favourable water retention protection on surimi, which might be related to the fine gelation capability of NPGP. Combined with the structure characterization (Fig. 1), NPGP had a large number of hydrogen bonds and hydrophilic groups, which could bind with excess free water in surimi and promoted the interactions among NPGP, myofibrillar protein and water (Fu et al., 2023). And thus, a denser and more uniform network structure with a certain strength was formed, which better locked water inside (Lv et al., 2023). The results were consistent with the texture results in Section 3.6.1.

3.6.3. ζ-Potential of surimi

ζ-potential can be used to characterize the degree of macromolecule aggregation and the stability of the dispersion system. As shown in Fig. 4B, the ζ-potential values of all groups were negative, indicating that the electrostatic interaction was present and the repulsive force was greater than the aggregation force. Additionally, the absolute values of ζ-potential decreased with the increase of NPGP concentration, which indicated that the repulsive force became weaker and the mixture tended to condense (Zhang, Pan, Jiang, & Shi, 2023). Therefore, the results suggested that NPGP might facilitate interactions between myofibrillar proteins, thereby improving the aggregation of myofibrillar proteins.

3.6.4. Molecular forces of surimi

Generally, non-covalent bonds (ionic bonds, hydrogen bonds, and hydrophobic interactions) are the important molecular forces in protein products, which affect the properties of protein. Fig. 4C showed the changes of ionic bonds, hydrogen bonds, and hydrophobic interactions in surimi with different amount of NPGP. Hydrogen bonds were the dominant non-covalent bonds in surimi, and their amount increased with the increasing NPGP. It is reported that polysaccharides can interact with proteins, so as to unfold the spatial structure of proteins, thereby exposing more binding sites to form hydrogen bonds (Mohammed et al., 2021). The amount of ionic bonds showed a trend of increasing initially and then decreasing with increase of NPGP. According to the structure characterization (Fig. 1), NPGP contained a large amount of COO⁻, which is negatively charged. Thus, with the increase of NPGP, the negative charge increased, so as to increase the ionic bonds. However, with the continuous increase of NPGP, other non-covalent bonds (such as hydrogen bonds) might be formed, and the ionic bonds formed might also be broke, resulting in a decrease of ionic bonds. The hydrophobic interaction was less, and there was a weak increasing trend with the increase of NPGP. It is probably due to the reason that NPGP could promote the aggregation of myofibrillar proteins (Fig. 4B), so as to expose the hydrophobic groups of myofibrillar proteins (Yekta,
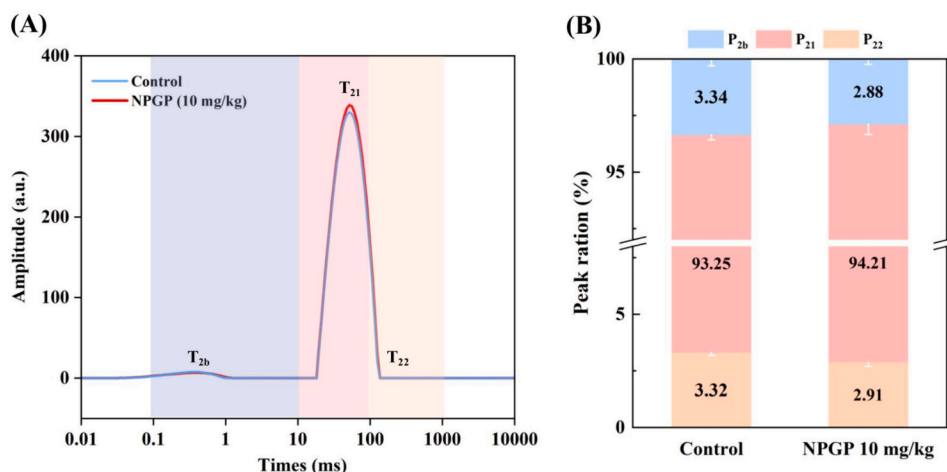
Fig. 5. (A) Curves of relaxation time, and (B) moisture distribution of surimi treated by *N. physalodes* seeds polysaccharides (NPGP) gel with tea polyphenols during freeze-thaw cycles.
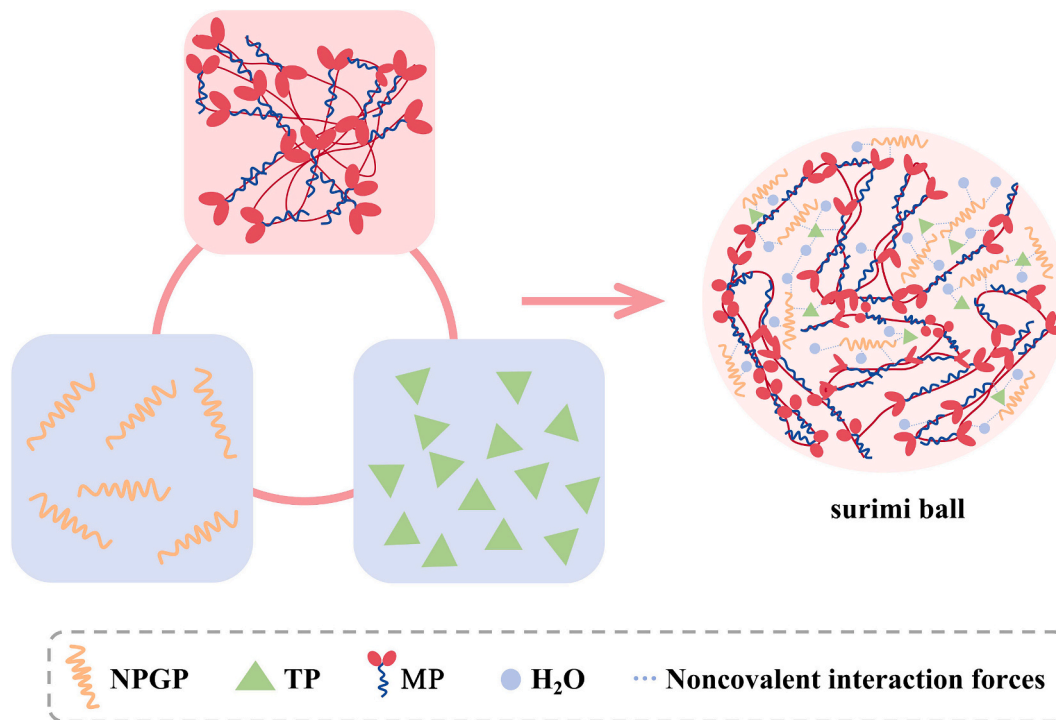

Fig. 6. Schematic mechanism for the interaction of myofibrillar protein (MP) and *N. physalodes* seeds polysaccharides (NPGP) gel with tea polyphenols (TP) in surimi.

Assadpour, Hosseini, & Jafari, 2023), thereby increasing the hydrophobic interaction between myofibrillar proteins.

3.6.5. Moisture distribution of surimi

The water distribution of surimi after freeze-thaw cycles was shown in Fig. 5A. The relaxation time is usually used to reflect the water distribution of the sample. T_{2b} (0.1 to 10 ms) reflects weak binding water, T_{21} (10 to 100 ms) reflects non-flowing water, and T_{22} (100 to 1000 ms) reflects free water. Meanwhile, their peak area ratio was shown in Fig. 5B as P_{2b}, P_{21}, and P_{22}, respectively. Compared with control group, P_{21} of NPGP group significantly increased ($P < 0.05$), while P_{2b} and P_{22} decreased. This result indicated that the free water (P_{22}) in surimi gradually transformed to non-flowing water (P_{21}). It probably because the NPGP promoted the formation of denser gel networks that reduced the water flow between cells during freeze-thaw cycles (Lv et al., 2023).

Meanwhile, the weak binding water (P_{2b}) content decreased, possibly owing to the reason that NPGP might prevent the transformation of free water (P_{22}) and non-flowing water (P_{21}) to weak binding water (P_{2b}) during freeze-thaw cycles. These results indicated that NPGP was more likely to retain the easily flowing water in surimi, which might be due to the reason that NPGP itself had a certain capability to absorb moisture and moisturize. It reports that polysaccharides can interact with myofibrillar proteins through numerous hydrogen bonds, resulting in a denser gel network that binds to free water in myofibrillar proteins products (Cao et al., 2022). Furthermore, the relevant study reports that polysaccharides can promote the transformation of free water to non-flowing water in proteins products (Wang et al., 2015). Thus, it promotes protein structure unfolding and forms a more stable structure, so as to improve the properties and quality of protein products.

Based on the relevant studies and the present results, Fig. 6 might be

probably used to explain the action mechanism of NPGP in improving the quality of surimi. When the NPGP content in surimi increased, myofibrillar protein might completely stretch and expand with the decrease of folded state, and its internal reaction groups were opened (Dai et al., 2020). Meanwhile, NPGP exhibited the fine capability to to of adsorption, expansion, and cold denaturation to form a gel (Yuan et al., 2022)., and NPGP existed in the gel network in a physical filling way, which enhanced the firmness and hardness of gel (Zhang, Li, Shi, Zhu, & Luo, 2018). In addition, NPGP had a certain emulsifying capability and good gelation capability, and could bind with myofibrillar proteins and free water to form a stable gel network structure, thereby improving the quality of surimi.

4. Conclusions

The homogenous polysaccharides (NPGP) were identified from *N. physalodes* seeds with a high content of galacturonic acid and a low methoxylation degree. Tea polyphenols could induce the gelation of NPGP, and improve the gel properties of this polysaccharides gel. During the freeze-thaw cycles process of surimi, NPGP exhibited the fine capability to improve the properties and quality of surimi. All results suggest that NPGP has the good gelation with tea polyphenols, and it has the potential to be applied in food industry. However, the specific structure of the NPGP was not obtained in this study. In addition, the effects of the polysaccharidew on the structure of myofibrillar protein and the interactions between them were not observed. The further studies will focus on the effects of polysaccharidess on protein products under different processing or treatments.

CRediT authorship contribution statement

Qiu-Yue Ma: Writing – original draft, Software, Methodology, Investigation, Data curation. **Qian-Da Xu:** Methodology. **Nan Chen:** Data curation. **Wei-Cai Zeng:** Writing – review & editing, Project administration, Methodology, Investigation.

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

No data was used for the research described in the article.

Acknowledgments

References

Cao, Y., Zhao, L. Y., Huang, Q. L., Xiong, S. B., Yin, T., & Liu, Z. Y. (2022). Water migration, ice crystal formation, and freeze-thaw stability of silver carp surimi as affected by inulin under different additive amounts and polymerization degrees. *Food Hydrocolloids, 124*, Article 107267. https://doi.org/10.1016/j.foodhyd.2021

Cen, K. Y., Huang, C. X., Yu, X., Gao, C. C., Yang, Y. L., Tang, X. Z., & Feng, X. (2023). Quinoa protein Pickering emulsion: A promising cryoprotectant to enhance the freeze-thaw stability of fish myofibril gels. *Food Chemistry, 407*, Article 135139. https://doi.org/10.1016/J.foodchem.2022

Chen, X., Li, X. Z., Yang, F. J., Wu, J. H., Huang, D., Huang, J. L., et al. (2022). Effects and mechanism of antifreeze peptides from silver carp scales on the freeze-thaw stability of frozen surimi. *Food Chemistry, 396*, Article 133717. https://doi.org/10.1016/j.foodchem.2022

Cheng, J., Xiang, R., Tang, D., Zhu, M., & Liu, X. (2021). Regulation of protein oxidation in Cantonese sausages by rutin, quercetin and caffeic acid. *Meat Science, 175*, Article 108422. https://doi.org/10.1016/j.meatsci.2020

Dai, H. J., Chen, X. K., Peng, L., Ma, L., Sun, Y., Li, L., et al. (2020). The mechanism of improved myosin gel properties by low dose rosmarinic acid addition during gel formation. *Food Hydrocolloids, 106*, Article 105869. https://doi.org/10.1016/j.foodhyd.2020

Fu, Y., Cao, Y., Chang, Z. Y., Zou, C. J., Jiang, D. M., Gao, H. L., et al. (2023). Effects of *Flammulina velutipes* polysaccharide with ice recrystallization inhibition activity on the quality of beef patties during freeze-thaw cycles: An emphasis on water status and distribution. *Meat Science, 209*, Article 109420. https://doi.org/10.1016/j.meatsci.2023

Gao, M. R., Xu, Q. D., & Zeng, W. C. (2020). Effect of tea polyphenols on the tenderness of yak meat. *Journal of Food Processing and Preservation, 44*(5), Article 14433. https://ifst.onlinelibrary.wiley.com/abs/10.1111/jfpp.

Guo, C., Li, X. F., Gong, T., Yang, X. D., Wang, G. L., Yang, X., et al. (2021). Gelation of *Nicandra physalodes* (Linn.) Gaertn. Polysaccharide induced by calcium hydroxide: A novel potential pectin source. *Food Hydrocolloids, 118*, Article 106756. https://doi.org/10.1016/j.foodhyd.2021

Guo, Q. B., Xiao, X. Y., Lu, L. G., Ai, L. Z., Xu, M. G., Liu, Y., et al. (2022). Polyphenol-polysaccharide complex: Preparation, characterization and potential utilization in food and health. *Annual Review of Food Science and Technology, 13*, 59–87. https://doi.org/10.1146/annurev-food-052720-010354

Lee, Y., & Chang, H. Y. (2020). Microencapsulation of a maca leaf polyphenol extract in mixture of maltodextrin and neutral polysaccharides extracted from maca roots. *International Journal of Biological Macromolecules, 150*, 546–558. https://doi.org/10.1016/j.ijbiomac.2020.02.091

Li, J., Feng, R. N., Shen, J. D., Liu, C. K., Yu, D. W., Jiang, Q. X., et al. (2022). Influence of sodium chloride and sodium pyrophosphate on the physicochemical and gelling properties of silver carp myofibrillar proteins sol subjected to freeze-thaw cycles. *LWT, 170*, Article 114053. https://doi.org/10.1016/j.lwt.2022

Li, T., Zhao, J., Huang, J., Zhang, W., Huang, J., Fan, D., et al. (2017). Improvement of the quality of surimi products with overdrying potato starches. *Journal of Food Quality, 2017*, 1–5. https://doi.org/10.1155/2017/1417856

Liu, H., Dai, T., Chen, J., Liu, W., Liu, C., Deng, L., et al. (2022). Extraction, characterization and spontaneous gelation mechanism of pectin from *Nicandra physaloides* (Linn.) Gaertn seeds. *International Journal of Biological Macromolecules, 195*, 523–529. https://doi.org/10.1016/j.ijbiomac.2021.1012.1032

Lv, Y. F., Zhen, X. Y., Liang, Q. Q., Zhang, D. F., Yi, S. M., Li, X. P., et al. (2023). Effects of polyphenols on the gel and digestive properties of *Penaeus vannamei* myosin after freezing. *Food & Function, 14*(14), 6624–6635. https://doi.org/10.1039/D3FO00973D

Mohammed, H. H. H., He, L. C., Nawaz, A., Jin, G. F., Huang, X., Ma, M. H., et al. (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. *Meat Science, 175*, Article 108453. https://doi.org/10.1016/j.meatsci.2021

Tan, C., Xu, Q. D., Chen, N., He, Q., Sun, Q., et al. (2022). Effects of tea polyphenols on the quality of common carp (*Cyprinus carpio*) meat during the freezing process and its action mechanism. *Journal of Food Processing and Preservation, 46*, Article 16521. https://doi.org/10.1111/j.jfpp

Wang, F. X., Wang, W., Huang, Y. L., Liu, Z. W., & Zhang, J. (2015). Characterization of a novel polysaccharide purified from a herb of *Cynomorium songaricum* Rupr. *Food Hydrocolloids, 47*, 79–86. https://dio.org/j.foodhyd.2015.01.006.

Yang, X. D., Yuan, K., Zhang, H., Luo, S., Wang, S. D., Yang, X., et al. (2023). Regulation on gel textures of *Nicandra physalodes* (Linn.) Gaertn. Pectin by its synergistic interaction with sodium alginate. *Food Hydrocolloids, 134*, Article 108047. https://doi.org/10.1016/j.foodhyd.2022

Yekta, R., Assadpour, E., Hosseini, H., & Jafari, S. M. (2023). The influence of ionic polysaccharides on the physicochemical and techno-functional properties of soy proteins; a comprehensive review. *Carbohydrate Polymers, 319*, Article 121191. https://doi.org/10.1016/j.carbpol.2023

Yuan, K., Luo, S., Zhang, H., Yang, X. D., Zhang, S., Yang, X. G., et al. (2023). Physical modification on gelation of fish gelatin by incorporating *Nicandra physalodes* (Linn.) Gaertn. Pectin: Effect of monovalent and divalent cation ions. *Food Chemistry, 405* (PA), Article 134932. https://doi.org/10.1016/j.foodchem.2022

Yuan, K., Yang, X. D., Li, D. M., Wang, G. L., Wang, S. D., Guo, Y. R., et al. (2022). Incorporation of *Nicandra physalodes* (Linn.) Gaertn. Pectin as a way to improve the textural properties of fish gelatin gels. *Food Hydrocolloids, 131*, Article 107790. https://dio.org/j.foodhyd.2022.

Yuan, Y., & Macquarrie, D. (2015). Microwave assisted extraction of sulfated polysaccharides (fucoidan) from *Ascophyllum nodosum* and its antioxidant activity. *Carbohydrate Polymers, 129*, 101–107. https://dio.org/0.1016/j.carbpol.2015.04.057.

Zeng, W. C., Zhang, Z., & Jia, L. R. (2014). Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*). *Carbohydrate Polymers, 108*, 58–64. https://doi.org/10.1016/j.carbpol.2014.03.022

Zhang, H., Song, Z. C., Gong, T., Meng, Y. H., Wang, X. Y., Zhang, S., et al. (2024). Gelation behavior between *Nicandra physalodes* (Linn.) Gaertn. Pectin and Ca^{2+} is governed by degree and pattern of methyl esterification. *Food Hydrocolloids, 149*, Article 109640. https://doi.org/10.1016/j.foodhyd.2023

Zhang, L. T., Li, Q., Shi, J., Zhu, B. W., & Luo, Y. K. (2018). Changes in chemical interactions and gel properties of heat-induced surimi gels from silver carp (*Hypophthalmichthys molitrix*) fillets during setting and heating: Effects of different

washing solutions. *Food Hydrocolloids, 75*, 116–124. https://dio.org/0.1016/j.foodhyd.2017.09.007.

Zhang, X. H., Pan, H., Jiang, X., & Shi, W. Z. (2023). Study on the mechanism of soy protein isolate to improve quality of reduced-salt *Hypophthalmichthys molitrix* surimi gel: Focus on gel quality, protein structure, and in vitro digestibility. *Food Chemistry: X, 20*, Article 100878. https://doi.org/10.1016/j.fochx.2023

Zhu, Z., Zhou, Q., & Sun, D. W. (2019). Measuring and controlling ice crystallization in frozen foods: A review of recent developments. *Trends in Food Science and Technology, 90*, 13–25. https://doi.org/10.1016/j.tifs.2019.05.012