Food Hydrocolloids 159 (2025) 110671

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Improvement of the gel properties of curdlan gel by hydrogen bonding interaction with trehalose

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A R T I C L E I N F O

Keywords:
Trehalose
Curdlan gel
Hydrogen bond
Gel properties
Syneresis

A B S T R A C T

This study investigated the effect of trehalose addition (0.5–2.0%) on the gelation properties of curdlan gels were investigated. The results demonstrated that the optimal gelation performance of curdlan-trehalose composite gel was determined at trehalose concentration of 1.0% with preferable water holding capacity, water absorption rate, and freeze-thaw stability. The gelation behavior of the curdlan-trehalose composite gel showed a more intense network structure with increasing storage modulus (G′), loss modulus (G″), and deceasing tan δ at the trehalose concentration in the range of 0%–1%. And the highest gel strength was also observed in 1% trehalose addition. Moreover, the structures characterized by Fourier transform infrared (FT-IR), X-ray diffraction (XRD), Low field nuclear magnetic resonance (LF-NMR) and scanning electron microscope analysis (SEM) all revealed that the addition of trehalose strengthened the hydrogen bonding interaction with curdlan to create a much denser network of composite gel, which would provide a better mechanistic insight of the curdlan-trehalose gel in practical application potentials in food and medicine.

1. Introduction

Curdlan is a bacterial extracellular polysaccharide produced by *Alcaligenes faecalis* var. *myxogenes* with a linear homopolymer with β-(1 → 3)-glycosidic stranded helix (Yang et al., 2024). The single helix can transform into a triple helix structure under certain conditions, including temperature and pH (Gagnon & Lafleur, 2007), which are crucial for the gelation process. Because of its unique properties in aqueous suspension of forming gels under heating conditions, it is valuable in various industries including food, pharmaceuticals, and biomedicine (Al-Rmedh et al., 2023; Zhang & Edgar, 2014). In 1996, the U.S. FDA approved the use of curdlan in the food industry. Up to now, curdlan has been widely applied in the production of jelly, noodles, ham, edible fiber membrane, fried/frozen food, low-calorie food and so on as a gelling agent (Chen & Wang, 2020).

The gelation properties of curdlan are influenced by several factors including temperature (Tako & Hanashiro, 1997), molecular weight (MW) (Nobe et al., 2005), and the presence of ions or other additives (Chen et al., 2014). This indicates that any substance affecting the molecular arrangements or interactions within the polymer matrix could potentially alter its gel strength and texture. Recently, the influence of sugars on the gel characteristics of curdlan is a multifaceted topic that intersects with the fields of food science, material science, and biotechnology. A large number of studies have confirmed that monosaccharides, disaccharides, and polysaccharides can all accelerate the gelation of thermally reversible and thermally irreversible gels (Yan et al., 2020). Among them, disaccharides could play a crucial role in the gelation process of many polymers due to their ability to form hydrogen bonds and participate in hydrophobic interactions. The addition of sucrose can enhance the entire hydrogen bond structure to stabilize the curdlan gel, thereby preventing the separation of water and reducing syneresis after freezing and thawing, indicating an improvement of gel stability (Nakao et al., 1991). Yang et al. (2018) also reported the addition of sucrose accelerated the gelation of κ-carrageenan and enhances the formatting of fiber helical connections with tighter structures, which is explained by the helix-helix transition and subsequent double helix accumulation. However, the excessive use of sucrose will pose a certain threat to human health, including obesity, diabetes and

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https://doi.org/10.1016/j.foodhyd.2024.110671
Received 29 July 2024; Received in revised form 2 September 2024; Accepted 20 September 2024
Available online 23 September 2024

other chronic diseases.

Trehalose (α-D-pyranosyl-α-D-glucopyranoside) is a non-reducing disaccharide formed by the combination of two molecules of glucose through glycosidic bonds, where the intermolecular hydrogen bonding interactions form a stable three-dimensional network structure, giving trehalose good gel-forming ability and stability (Qiu et al., 2021). The biological activities of trehalose also include antiviral, antitumor, antioxidant, immune regulation, and other functions (Łopieńska-Biernat & Stryiński, 2019). These activities make trehalose a valuable compound for use in food, cosmetics, biomedicine, and agriculture (Li, Chen, et al., 2023). According to Xiang et al. (2023), the combination of trehalose with porous poly (vinyl alcohol) nano-clay hydrogels possessed excellent ability to accelerate bone defects repair, which proved that the hydrogel can be applied as a potential material for bone tissue engineering. This composite material enhanced mechanical properties and good biocompatibility, which can not only be absorbed by the human body, but also gradually degraded in the body, thus avoiding the risk of long-term retention in the body. The gel properties are significantly affected by their molecular structure. O'Shea et al. (2015) proved that covalently bound trehalose in hydrogels could enhance the long-term functional stability of the materials with controlling the release of the bioactive macromolecules. Moreover, the forming process of composite gel is affected by the concentration of components, temperature, pH and other factors (Tsupko et al., 2023), which indicates that the chemical composition and environmental conditions of trehalose have a direct impact on their gel properties.

In recent years, trehalose has been gradually applied to the improvement of frozen food quality as a natural anti-freeze protective agent. Whether the addition of trehalose will affect the gel properties of curdlan gel and its possible mechanism have not been reported. Therefore, this study intends to explore the effects of trehalose addition at different concentrations (0.5–2.0%, w/v) on the water holding capacity (WHC), gel strength, texture properties, rheological properties, microstructure and possible mechanism of curdlan gels through hydrogen bonding. This study will help to broaden the application of curdlan as a gelling agent in the frozen food industry.

2. Materials and methods

2.1. Materials and chemicals

Curdlan used in this study was purchased from Wako Pure Chemical Reagent Co. (Tokyo, Japan), and trehalose was produced by the Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All the other chemicals were laboratory pure and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of curdlan and curdlan-trehalose gels

The preparation of curdlan and curdlan-trehalose gels referred to Tao et al. (2021) with minor modifications. In short, 1.5 g of curdlan powder was accurately weighed and slowly added to 50 mL of distilled water under constant stirring at 25 °C for 15 min to obtain 3% (w/w) curdlan solution. Subsequently, different mass fractions of trehalose (0%, 0.5%, 1%, 1.5%, 2%, w/v) were added and magnetically stirred at 25 °C for 5 min. The obtained mixture was incubated at 90 °C for 30 min in a water bath with uninterrupted stirring. Finally, the gel was cooled promptly in ice and then reserved at 4 °C for 12 h to ensure sufficient gel hydration. Meanwhile, a part of the as-prepared curdlan and curdlan-trehalose gels was freeze-dried, ground into powder and placed in a sealed bag for later use.

2.3. Water absorption rate (AR) determination

The AR of the curdlan and curdlan-trehalose gels was calculated according to the

$$\text{AR (\%)} = (\text{A-B})/\text{A} \times 100 \qquad (1)$$

Where, A represents the mass of the initial gel (g) and B represents the mass of the gel after the water on the gel surface is absorbed by the filter paper (g).

2.4. WHC

The WHC represents the ability of a material to retain water, which was identified by high-speed centrifugation. Briefly, 10 g of the gel sample (W_0) was accurately weighed in a centrifugal tube, and then centrifuged at 10,000 *g* for 15 min (4 °C). The water after centrifugation was discarded and weighed as W_1 (deducting the weight of the tube). The WHC (%) is calculated as the percentage of the gel weight after centrifugation (W_1) relative to the initial gel weight (W_0), as shown in Eq. (2).

$$\text{WHC (\%)} = W_1/W_0 \times 100 \qquad (2)$$

2.5. Gel strength determination

Gel strength (g) refers to the ability of the gel to resist deformation and damage under the action of force, where the maximum force was determined by a texture analyzer equipped with a P/0.5R cylindrical probe (A-XT Plus, Stable Micro Systems Ltd, UK). The gel sample was cut into 10 mm high, 14.5 mm diameter, and then penetrated by a cylindrical probe with P/0.5 as the standard gel strength test probe (5 mm diameter, model P/0.5). The parameter settings were as follows according to Liu et al. (2017) with slightly modifications, where the pretest speed is 2.0 mm/s, test speed is 1.0 mm/s, posttest speed is 2.0 mm/s, test distance is 5.0 mm, and trigger force is 5.0 g.

2.6. Texture profile analysis (TPA)

Textural parameters of curdlan and curdlan-trehalose gels at different concentrations were measured by a Texture Analyzer (TAXT-plus/30, Stable Micro Systems, Surrey, UK) according to Yuan et al., 2019 with minor revision. All the gel samples were cut into cylinders with a stainless-steel circular blade (diameter of 20 mm, height of 10 mm), and analyzed by the texture analyzer. Briefly, samples were penetrated with a cylinder probe P/0.5 for the standard gel strength test. The samples were analyzed at following conditions: pretest speed 2.0 mm/s, test speed is 1.0 mm/s, posttest speed 2.0 mm/s, compression distance 3 mm, trigger force 5.0 g, and the type variable are 40%. The five parameters used for texture analysis including hardness, springiness, gumminess, cohesiveness, and chewiness, were recorded separately for analysis.

2.7. Freeze-thawing stability analysis

Freeze-thawing stability is the stability of emulsion system subjected to alternating freezing and melting changes, which was analyzed based on the previously described methods with slight revision (Yuan et al., 2019). In brief, all the gel samples were frozen at −18 °C for 24 h, and then thawed at 25 °C for 6 h Samples were then under high-speed centrifugation at 4000*g* for 5 min after thawing treatment, and the separated water was gathered and weighed for calculation. The syneresis was calculated through the following equation (Eq. (3)):

$$\text{Syneresis (\%)} = [(W_1 - W_2)/W_1] \times 100 \qquad (3)$$

where W_1 represents the weight of the sample before freezing; W_2 represents the weight of the samples after thawing and centrifugation.

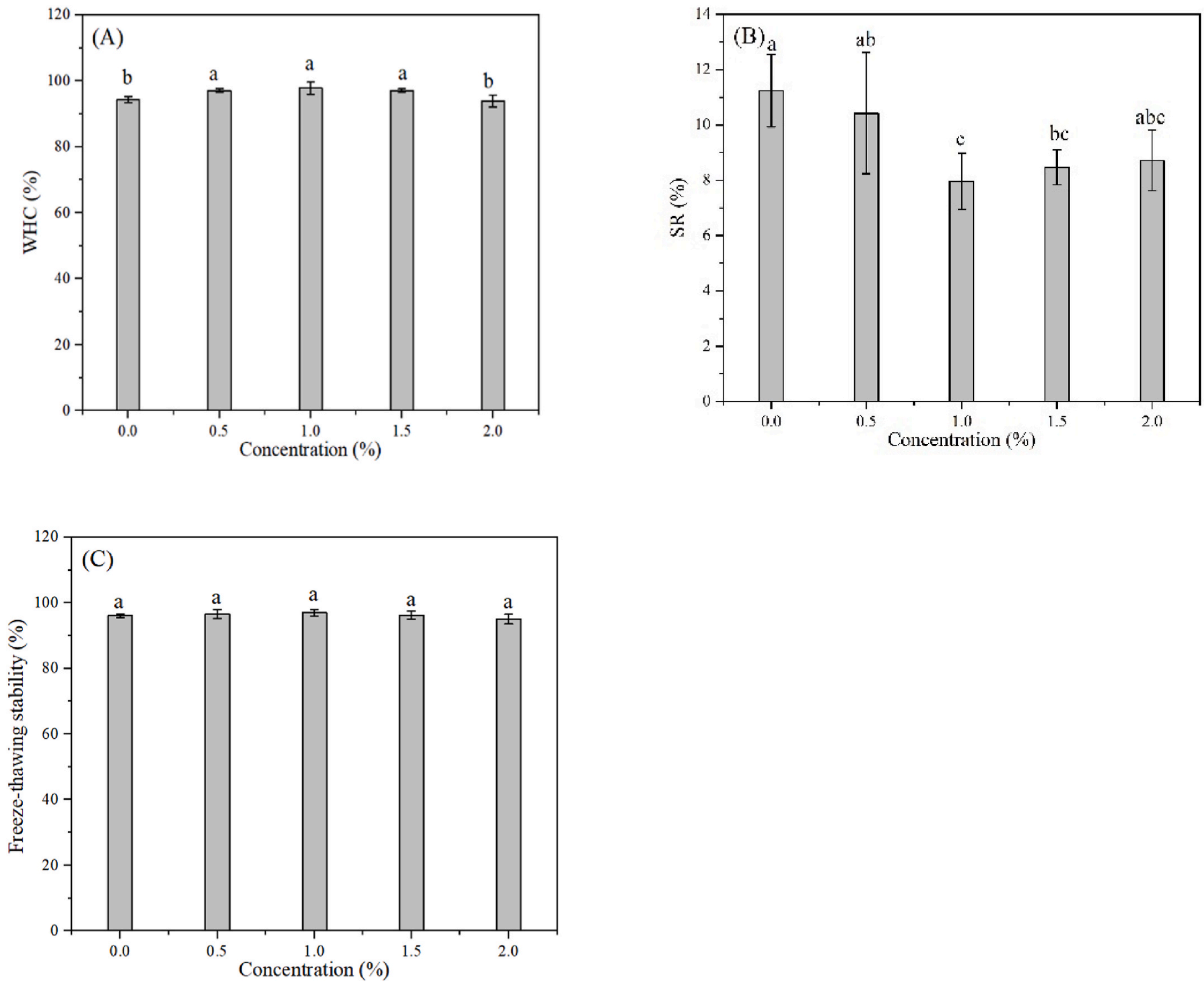

Fig. 1. Effect of trehalose addition (0–2.0%, w/v) on WHC (A), SR (B) and freeze-thawing stability (C) of curdlan gel.

2.8. Analysis of dynamic rheological properties

Dynamic rheological properties were conducted by a rotating Anton Paar MCR 102 stress-controlled rheometer (Anton Paar, Graz, Austria) with stress control (Yu et al., 2022). A stainless-steel parallel plate (diameter: 40 mm, gap: 1 mm) was selected to test the gel samples. The curdlan and curdlan-trehalose gel solutions were added dropwise to the bottom test platform of the rheometer, and dimethyl silicone oil was coated around to avoid sample evaporation. The solution was measured after equilibrium at 85 °C for 5 min. A temperature sweep with a 2 °C/min cooling and heating rate was at a constant angular frequency of 1 rad/s, and a fixed strain of 1%, carrying out from 85 °C to 25 °C and subsequently from 25 °C to 85 °C to acquire the temperature-dependent storage modulus (G′), loss modulus (G″) and phase angle (tan δ).

2.9. Fourier transform infrared (FT-IR) spectrum analysis

Gel samples were mixed with KBr powder at a mass ratio of 1:100 (w/w) and extruded into a transparent sheet. The FT-IR spectrum was measured using an FT-IR spectrophotometer (Nicolet 380, Thermo Nicolet Co., USA) in the wavenumber range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1} (Yan et al., 2020). OMNIC 8.2 software was used for data acquisition and processing.

2.10. Scanning electron microscope (SEM) observation

SEM is an efficient tool to observe the microstructure of gel samples. A small number of gel samples was cut into small cubes and freeze-dried after frozen in liquid nitrogen. The lyophilized samples were sprayed with gold for 3 min, and the microstructure was observed via SEM (S-3400, Dandong Bettersize Instruments Co., Ltd., China) at an accelerating voltage of 15 kV and a magnification of 1000×.

2.11. X-ray diffraction (XRD) analysis

The crystal structures of freeze-dried gel samples were analyzed by D8-Advance X-ray diffractometer (Bruker, Germany) with Cu-Kα radiation source (λ = 0.154 nm). The scanning range was 5–90° (2θ), and scanning speed was 5°/min.

2.12. Thermal stability analysis (DSC/TGA)

The thermal stability of the gel samples was analyzed by STA449C thermal analyzer (NETZSCH, Germany) according to Yan et al. (2020).

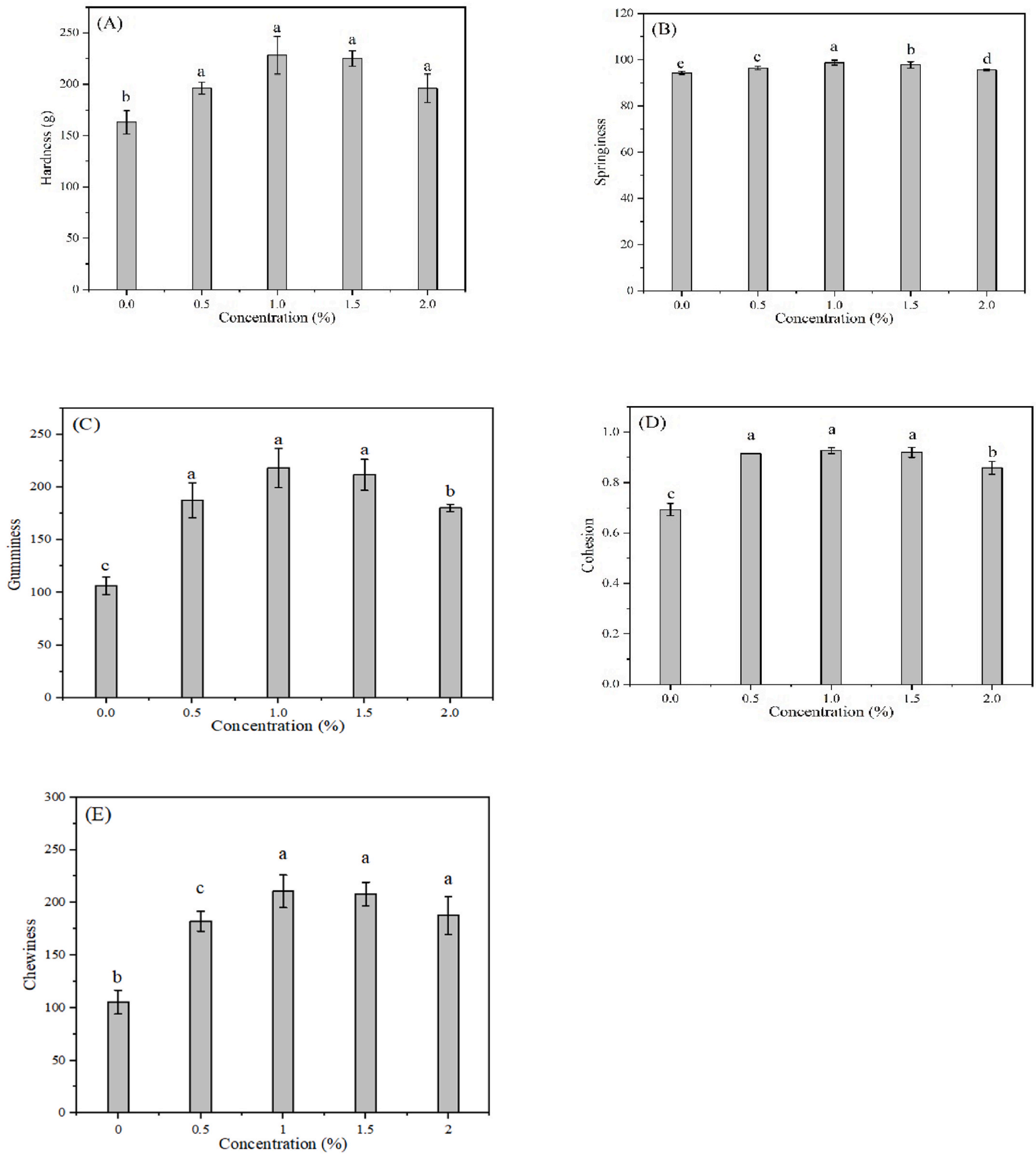

Fig. 2. Effect of trehalose addition (0–2.0%, w/v) on TPA of curdlan gel. (A) hardness, (B) springiness, (C) gumminess, (D) cohesion, and (E) chewiness.

In brief, 3 mg of freeze-dried samples were placed in a ceramic crucible, heated from 25 °C to 600 °C at a rate of 10 °C/min, and the nitrogen flow rate was 20 mL/min.

2.13. Low field nuclear magnetic resonance (LF-NMR) analysis

LF-NMR analysis was applied for the measurement of the transverse relaxation time (T_2) (MesoMR23-060H-I, Niumag Corporation, Shanghai, China). In short, 2.0 g of the samples were wrapped in a waterproof film and placed in a cylindrical nuclear magnetic glass tube (diameter 15 mm), which was inserted into an NMR analyzer and tested at a resonance frequency of 32 °C and 19 MHz. T_2 was analyzed by the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Song et al., 2024). The spectrum width (SW) was 200 kHz, the receiver gain (RG) was 20 dB, the echo number (NECH) was 6000, the relaxation decay time (T) was 0.3 ms, the scans (NS) was repeated for 4 times, and the repetition interval (TW) was 4000 ms.

2.14. Statistical analysis

All experimental data were repeated at least three times with standard deviation (SD). One-way analysis of variance (ANOVA) was performed using Origin software (version 8.0, OriginLab Corp., MA, USA) to identify the variation between samples.

3. Results and discussion

3.1. Effect of trehalose on the WHC, AR and freeze-thawing stability of curdlan gel

Fig. 1 reveals the WHC and AR of curdlan-trehalose composite gels changed with trehalose concentration. As shown in Fig. 1(A), the addition of trehalose (0%–1%) significantly increased the WHC of the curdlan-trehalose composite gels from 94.27% to 97.80% ($p < 0.05$). However, as the concentration further increased (1%–2%), the WHC increased to the maximum and then decreased significantly to 93.86% ($p < 0.05$). The SR results (Fig. 1(B)) was opposite to the WHC results. With the addition of trehalose (0%–1%), the AR of the curdlan-trehalose composite gels sharply decreased from 11.23% to 7.97%, but with the further increase of trehalose concentration from 1% to 2%, the AR slightly increased to 8.71%. This may be due to the addition of trehalose contributed to the raising of the collision probability between curdlan and trehalose, forming a dense network structure of curdlan-trehalose gel, conducing to the retention of water molecules in the gel (Tao et al., 2021). At high concentrations (1%–2%), although there was more hydrophilic trehalose in the composite gel, the aggregation of hydrogen bonds between trehalose molecules may change the hydration of curdlan (Oakenfull, 2000), leading to the difficulty of aggregation of condensed chains and hinder the formation of condensed structure. Therefore, the gradually decreasing hardness of curdlan-trehalose was affected by the further increasing of trehalose concentration, thereby reducing the WHC (Xiao et al., 2020; Yuan et al., 2019).

Curdlan is a flexible gel with strong stability at low (freezing) temperature. Since the quality of frozen food has close correlation to thermal fluctuation and water phase transition, the freeze-thawing stability of curdlan and curdlan-trehalose gels could be evaluated by freeze-thawing stability test (Verma et al., 2020). It can be observed from Fig. 1(C) that with the increase of trehalose concentration (0%–1%), the freeze-thawing stability of curdlan-trehalose composite gels gradually increased. When the trehalose concentration was 1% (w/w), the freeze-thawing stability reached to the highest at 96.91%, which was higher than the others. However, the addition of trehalose has no obvious effects on the freeze-thawing stability of the curdlan gel. Previous studies have proved that the addition of sugars and polyols can improve the gelation properties (Stenner et al., 2016). The addition of trehalose hence may affect the freeze-thawing stability of the curdlan gel by affecting the freezing of water molecules in the gel. The WHC of trehalose itself increases the bound water content in the gel. After the gel is frozen, a part of the water is not frozen but combined with trehalose molecules to form a high elastic state, which makes it resistant to form ice crystals, reducing the damage of ice crystals to the network structure, thereby during the thawing process, the amount of water leakage is greatly reduced. However, when the trehalose concentration exceeded 1% (ranging within 1%–2.0%), the SR of curdlan-trehalose composite gel was gradually enhanced, indicating that its WHC was weakened. This may be the enhanced effect of intramolecular hydrogen bonds of trehalose at a concentration higher than 1%, which led to the insignificance on the dehydration shrinkage of curdlan gel.

3.2. Influence of trehalose addition on the texture profile of curdlan gel

According to the specific properties of the gel system, five mechanical parameters were chosen in this study to explore the effects of various concentrations of trehalose on the texture characteristics of curdlan gel. Fig. 2(A) shows influence of trehalose addition on the hardness of the composite gel, which is defined as the force or maximum peak force required for a given deformation (Yuan et al., 2019). With the increase of trehalose concentration in the range of 0.0%–1.5%, the hardness significantly increased from 162.93 g to 228.19 g ($p < 0.05$), where the trehalose-curdlan mixed gels showed more pronounced hardnesses compared with the pure curdlan gel. The maximum hardness 228.19 g was obtained at the concentration of 1% trehalose. Studies have shown that the mutual repulsion between sugar molecules is one of the main driving powers for the formation of better gels in the process of gel formation (Stenner et al., 2016). At a low concentration, the addition of trehalose may form a tighter network structure by interrupting the water dispersion and weaken the molecular interaction between curdlan and water, which made the gel obtain higher strength. However, excessive trehalose filling between curdlan molecules will lead to difficulty in aggregation and impede the formation of curdlan gel network structure, leading to the reduction of hardness with the further increase of trehalose concentration (Stenner et al., 2016). This finding was accordance with the following microstructural analysis (Fig. 7). Natalie Russ et al. (2014) also reported that the high ability of trehalose to form hydrogen bonds and bind water, thus the addition of a small amount of trehalose molecules would enhance the residual water content of the hydrocolloids. These sugar molecules are limited within the network structure and hinder the diffusion of water molecules towards the surface, resulting in changing of the chewing characteristics (Russ et al., 2014).

Springiness represent the ability of a material to stretch and return to its original length, and gumminess is an indicator for measuring the energy requisite for crushing semi-solid foods before swallowing (Yuan et al., 2016). As shown in Fig. 2(B) and (C), the addition of trehalose significantly enhanced the springiness and gumminess values of the curdlan gel as the concentration increased from 0% to 1%, and then reduced within 1%–2% ($p < 0.05$). Amongst them, the lowest springiness value (94.33) was obtained in the pure curdlan gel, and the maximum elasticity value (98.74) was found in the curdlan-trehalose composite gel at the concentration of 1%. The springiness of the curdlan-trehalose composite gel is determined by its porous structure, and the addition of trehalose promotes its formation of a network structure, which may also prevent it from returning to its original length, enhancing the springiness of the curdlan-trehalose composite gel (Zhang et al., 2020). However, the presence of excessive trehalose molecules hinders the extension of the network structure may also affect the gumminess, which leading to the similar effect on hardness, springiness, as well as gumminess.

Fig. 2(D) shows the influence of trehalose with various concentrations on the cohesiveness of curdlan gel. The cohesiveness value showed a drastically upward trend with the growth of trehalose concentration (0%–1%), and then sharply decreased within 1.0%–2.0%, which may be connected with the skeleton structure of the curdlan-trehalose gel (Tao et al., 2021; Yuan et al., 2016, 2019). Trehalose strengthened the aggregation of curdlan chains, leading to an increase in cohesiveness. Similar to hardness, superfluous trehalose would impede the formation of curdlan gel structure, resulting in a reduction of cohesiveness with the further increase of trehalose concentration.

Chewiness refers to the chewing taste of food with lasting elasticity (Tao et al., 2021). From Fig. 2(E), the chewiness increased when the trehalose concentration was 0%–1% ($p < 0.05$), and then keep a downward trend without statistical significance. The slightly fluctuation in chewiness over 1.0% may due to the excessive trehalose prevented the clustering of curdlan gel chains and restricted the extension of the network structural wall, as well as had the negative effect on gel

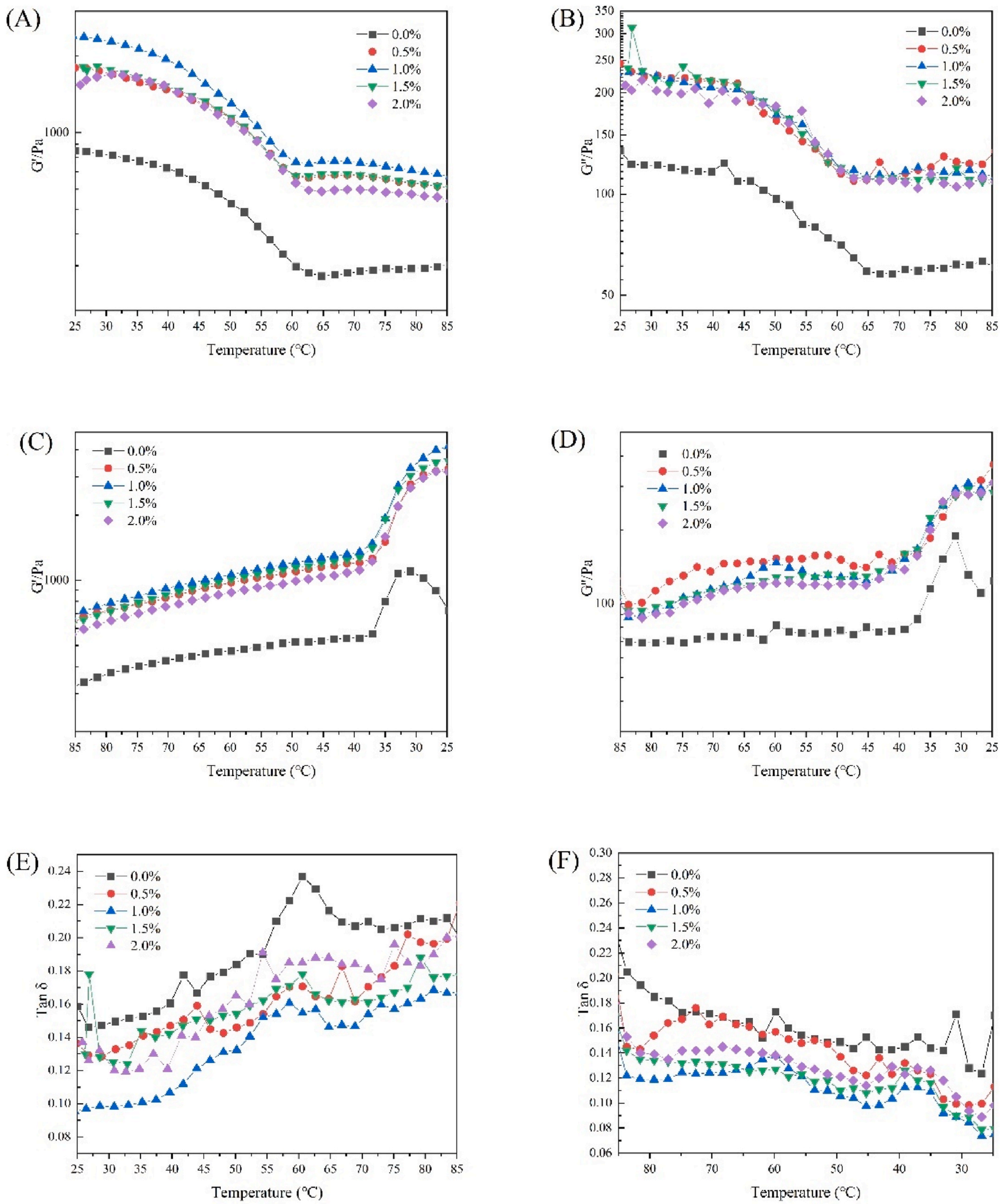

Fig. 3. The dynamic rheological behavior analysis (G′, the storage modulus; G″, the loss modulus; tan δ, loss tangent) of curdlan and trehalose-curdlan gels. (A), (B) and (E) represent heating the process; (C), (D) and (F) represent the cooling processes.

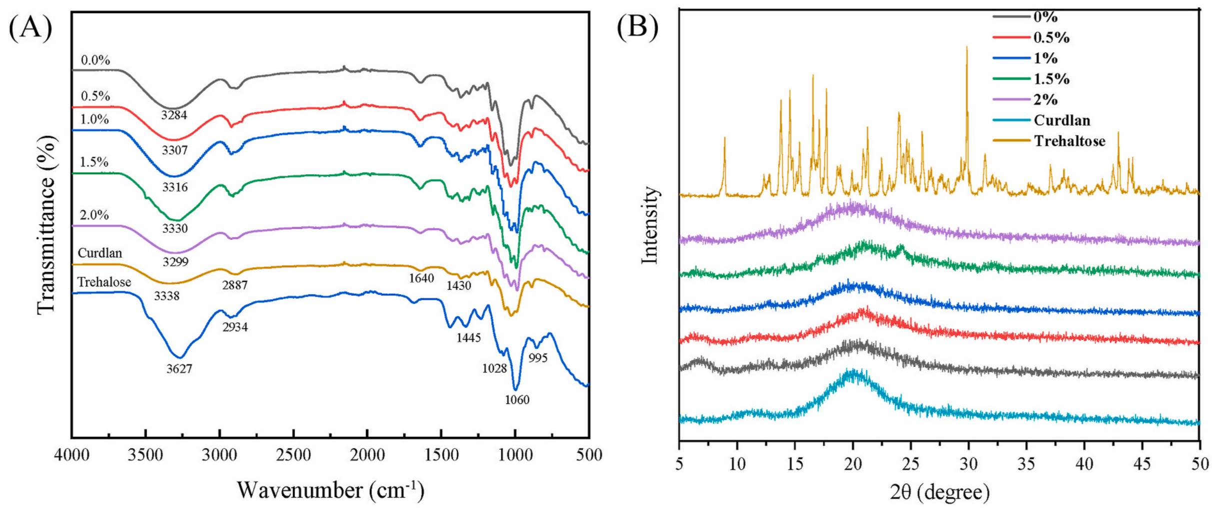

Fig. 4. (A) FT-IR spectra, and (B) XRD patterns of curdlan, trehalose, and curdlan-trehalose gels.

hardness.

3.3. Dynamic rheological behavior

The rheology behavior is a crucial indicator for gel material, representing the strength of the re-crosslinked gel at fracture can be expressed by G' (Yu et al., 2022). Herein, by measuring the storage modulus G′, loss modulus G″, and tan δ (G''/G′), the dynamic rheological behavior of curdlan and curdlan-trehalose gels during heating and cooling was evaluated and the results are shown in Fig. 3. The heating temperature was firstly increased from 25 °C to 85 °C, and then cooled back to 25 °C with a rate of 2 °C/min. In Fig. 3, the addition of trehalose extremely improved both G′ and G″ values of the curdlan-trehalose gels, and the G′ was always higher than G″ in the whole range in a gel state, which may be the curdlan gel was irreversibly formed by unwinding with the triple helix structure at a temperature greater than 80 °C. The single helix structure undergoes a sol to a strong gel transition, so G′ was greater than G'' (Verma et al., 2020). In Fig. 3(A) and (B), all the G′ and G″ were enhanced in curdlan-trehalose composite gels, indicating that trehalose could promote the hydrogen bonding formation to stabilize water molecules, thus enhance the deformation resistance of composite gel (Branca et al., 2001; Russ et al., 2014). In detail, the maximal values of G′ was obtained at 1% trehalose concentration with a corresponding value of 2397 Pa at 25 °C, which was apparently higher than curdlan gel (846.9 Pa). Therefore, these results indicated that addition of 1% trehalose is the optimal amount to affect the curdlan-trehalose synergy for a more elastic and tougher gel system. From Fig. 3(C) and (D), the fluidity of curdlan-trehalose composite gels also gradually declined during the cooling process, contributing to the gradual enhancement of G ′ and G''. The determination of both measured G′ and G″ were found to be influenced by the addition of trehalose. The reason may be attributed to the interaction between the trehalose and curdlan to strengthen the network structure of composite gel, where the cross-linked hydrogen bonds were formed between the equatorial -OH group of trehalose and curdlan molecules. This process led to the formation of a more ordered gel network structure, making a macroscopic performance of the hardened gel (Yang et al., 2018; Zhou et al., 2024). Our study presented a much higher value of G′ than G″, indicating G′ dominated the complex modulus. Therefore, the gel was more elastic. The data of the aforementioned springiness values are also consistent with this result in curdlan-trehalose composite gels, which are all higher compared with that of cohesiveness. In general, the favorable textural properties of curdlan-trehalose gel indicated a close correlation with the values of G′ and G''.

To further clarify the rheological behavior of curdlan-trehalose composite gels, the correlation analysis was reflected by tan δ (G''/G′). In the process of gel formation, the ratio of viscosity to elasticity was reflected by tan δ (G''/G′). Interestingly, the addition of trehalose can reduce the tan δ value of curdlan-trehalose composite gels. This may be because that trehalose is repelled on the surface of curdlan in the sol state at low concentrations. The repulsion of trehalose reduces the amount of water and makes the curdlan chains close to each other, thus forming a dense network structure with high gel strength (Li et al., 2024).

3.4. FT-IR spectrum and XRD analysis

FT-IR spectrum was to analyze the influence of trehalose addition on the structural characteristics of curdlan gel. As shown in Fig. 4(A), all the samples obtained a wide and strong hydroxyl stretching band at the absorption of 3338 cm^{-1}, the weak C-H stretching vibration and symmetrical deformation vibration was assigned near 2887 cm^{-1} and 1430 cm^{-1}, respectively. These vibrations are representative features of carbohydrates (Wang et al., 2019). The absorption at 1640 cm^{-1} may be induced by O-H stretching and bending vibration of associated water, and the absorption at 888 cm^{-1} indicates the existence of β-configuration (Zhang et al., 2018). The IR spectrum of trehalose showed the absorption of O-H (3267 cm^{-1}) and C-H (2934 cm^{-1} and 1445 cm^{-1}) also existed. The firm absorption at 1060 cm^{-1} and 1028 cm^{-1} may attribute to the stretching vibration of the C-O group, whereas the absorption at 995 cm^{-1} is contributed by the rocking vibration of C-H. No new absorption peaks appeared in the curdlan-trehalose composite gels comparing with the spectrum of curdlan gel or trehalose, indicating no generation of new chemical bonds. However, the addition of trehalose could enhance the hydroxyl stretching band at the absorption of 3338 cm^{-1}, which also attributed to the stretching vibration of free hydroxyl groups between and within the molecules (Jin et al., 2006). Overall, the red shift of curdlan and the blue shift of trehalose informed a new hydrogen bond was formed between curdlan and trehalose molecules.

XRD can be used to analyze the change of the crystallinity and the compatibility of the gel system. The XRD patterns of curdlan gels with different concentrations of trehalose are shown in Fig. 4(B). The pure curdlan gel showed a wide and low-intensity crystallization peak at about $2\theta = 20°$, and an additional crystallization peak at $2\theta = 6°$ and $2\theta = 12°$, which may relate to the triple helix structure of curdlan (Yan et al., 2020). The characteristic diffraction peaks of trehalose appeared at about 8.5°, 11.6°, 30.1° and other multiple crystallization peaks ranging from 10° to 35° and 40°–45° all indicating that trehalose had a highly crystalline structure. Similar research also proved that the addition of disaccharide (sucrose) could greatly improve the gel strength by

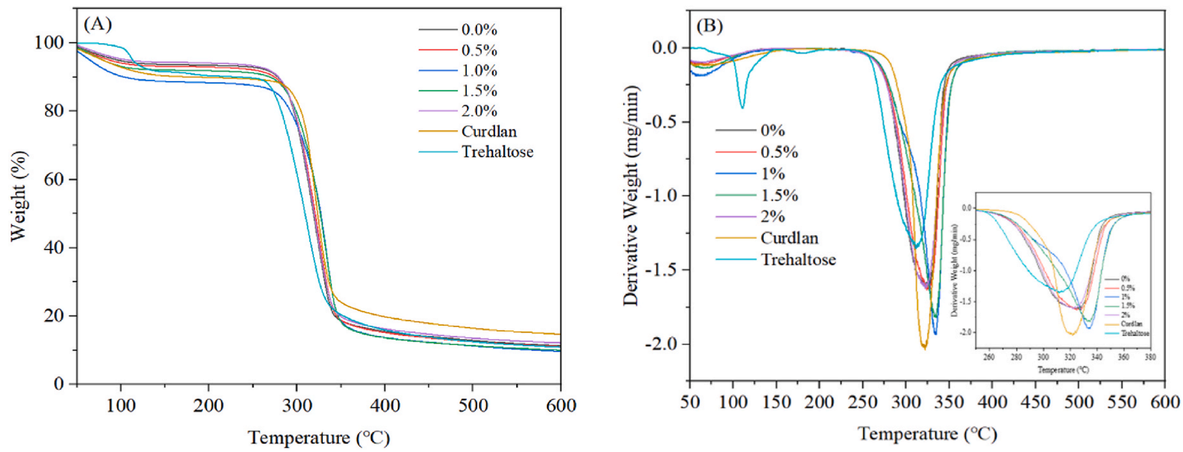

Fig. 5. (A)TG and (B)DSC curves of curdlan gels with different concentrations of trehalose.

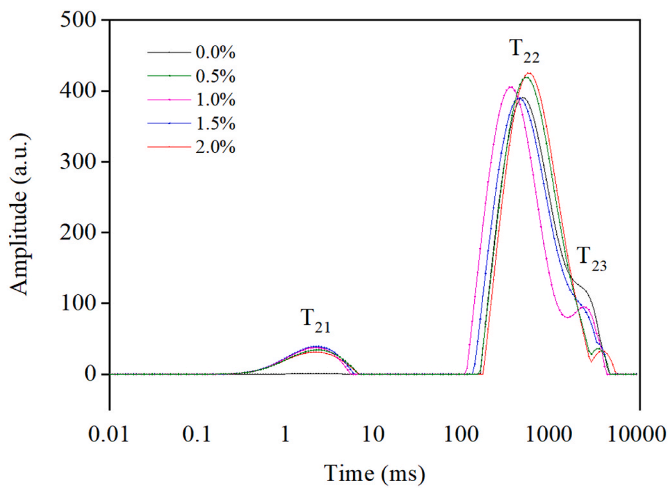

Fig. 6. LF-NMR curves of curdlan gels with different concentrations of trehalose.

intermolecular interaction of hydrogen bonds (Zhou et al., 2024). However, the diffraction peaks of curdlan gel with trehalose at 6.0°, 12.00°, 20.22° (2θ) were weakened. The crystallinity is proportional to the shape of the diffraction peak (Wang et al., 2019). Fig. 4(B) showed that the area of the diffraction peak of 1.0% curdlan-trehalose gel was the smallest and the crystallinity was the lowest, indicating that under this condition, it is easy to form multiple connection points and short distance network structure. The dense network structure makes 1.0% curdlan-trehalose gel have better TPA and higher WHC.

3.5. Thermal stability

Three different mass loss stages of curdlan, trehalose, and curdlan gels with different concentrations of trehalose were identified in Fig. 5 (A). At first stage (50 °C ~ 200 °C), the original water molecules and a large number of hydroxyl groups drive the water absorption in the air of the matrix. With the raising of temperature, both the free water and bounded water in the sample were evaporated, resulting in a starting decrease in mass about 9%. At the second stage (200 °C ~ 500 °C), the mass descends rapidly to 70 %, which may be related to the degradation of carbohydrates (Yuan et al., 2019). In summary, the quality of curdlan gels with different concentrations of trehalose decreased in the order of 0.0% (80.49%) > 0.5% (80.45%) > 2.0% (80.17 %) > 1.5% (79.84%) > 1.0% (77.06%). The results indicated that the addition of trehalose improved the thermal stability of the curdlan gel system, which was consistent with the increase of hydrogen bond interaction in the gel system. At the third stage ranging from 500 °C–600 °C, small molecules continue to carbonize to form simple compounds, including carbon dioxide, hydrogen, methane, aldehydes, etc., and the mass decline trend slows down to 7%. Meanwhile, Fig. 5(B) reveals the DTG curves of curdlan and curdlan-trehalose gels. From the diagram, it can be seen that the order of the maximum weight loss temperature of curdlan gels with different concentrations of trehalose is as follows: 1.0% (333.85 °C) > 1.5% (333.11 °C) > 0.5% (326.71 °C) > 2.0% (325.14 °C) > 0% (324.82 °C) > curdlan (322.34 °C) > trehalose (312.93 °C), which is consistent with the trend of TG loss rate. In summary, the curdlan-trehalose composite gel (1% trehalose) had the highest degradation temperature (333.85 °C) and the lowest weight loss rate (77.06%) compared with other gel samples, indicating that the addition of trehalose improved the thermal stability of curdlan gel.

3.6. Water distribution analysis

LF-NMR can be used for the water distribution and fluidity behavior of polysaccharide gels. Relaxation time T_2 reflects the chemical environment of hydrogen protons in samples (Song et al., 2024). In Fig. 6, three main peaks were observed in T_2 of curdlan gels with different concentrations of trehalose in the relaxation time of 0.1–10000 ms, which can be identified asT_{21}, T_{22}, and T_{23}, respectively, and the corresponding peak area ratio were labeled as S_{21}, S_{22}, and S_{23}, respectively, which attributed to the amount of different water component (Gudjónsdóttir et al., 2011). The first peak at 1–10 ms represented the bound water T_{21} by strong H-bonds in the gel, the range of 10–100 ms indicates that the gel retains water, T_{22} and T_{23} (>100 ms) can be identified as removable half-bounded water and free water in the gel, which incompactly bounded to the outside of the gel structure (Cheng et al., 2014). The measured relaxation time is positively correlated with the degree of freedom of water. According to Table 1 and Fig. 6, the T_{22} and T_{23} were the main populations of water present in curdlan-trehalose composite gels, while the population of T_{21} only on behalf of a minor part of the water present. Previous research has demonstrated that the T_2 had a positive relationship with the hydrogen protons bounding, leading to the more leftward of the peak on the T_2 spectrum (Han et al., 2009). At the addition of 0%–1% trehalose, T_{21} and T_{22} of curdlan-trehalose composite gels decreased with the addition of trehalose, along with S_{2b} gradually increased, while S_{22} and S_{23} gradually descended, indicating the enhancement of binding water leading to the closer binding with macromolecules, where the freedom water is weakened, indicating trehalose could enhance the WHC of the curdlan gel by raising the gel strength. With further increase of trehalose concentration (1%–2%), T_{21}, T_{22}, and T_{23} moved to the right, meanwhile the S_{2b} gradually decreased, and S_{22} and S_{23} gradually enhanced,

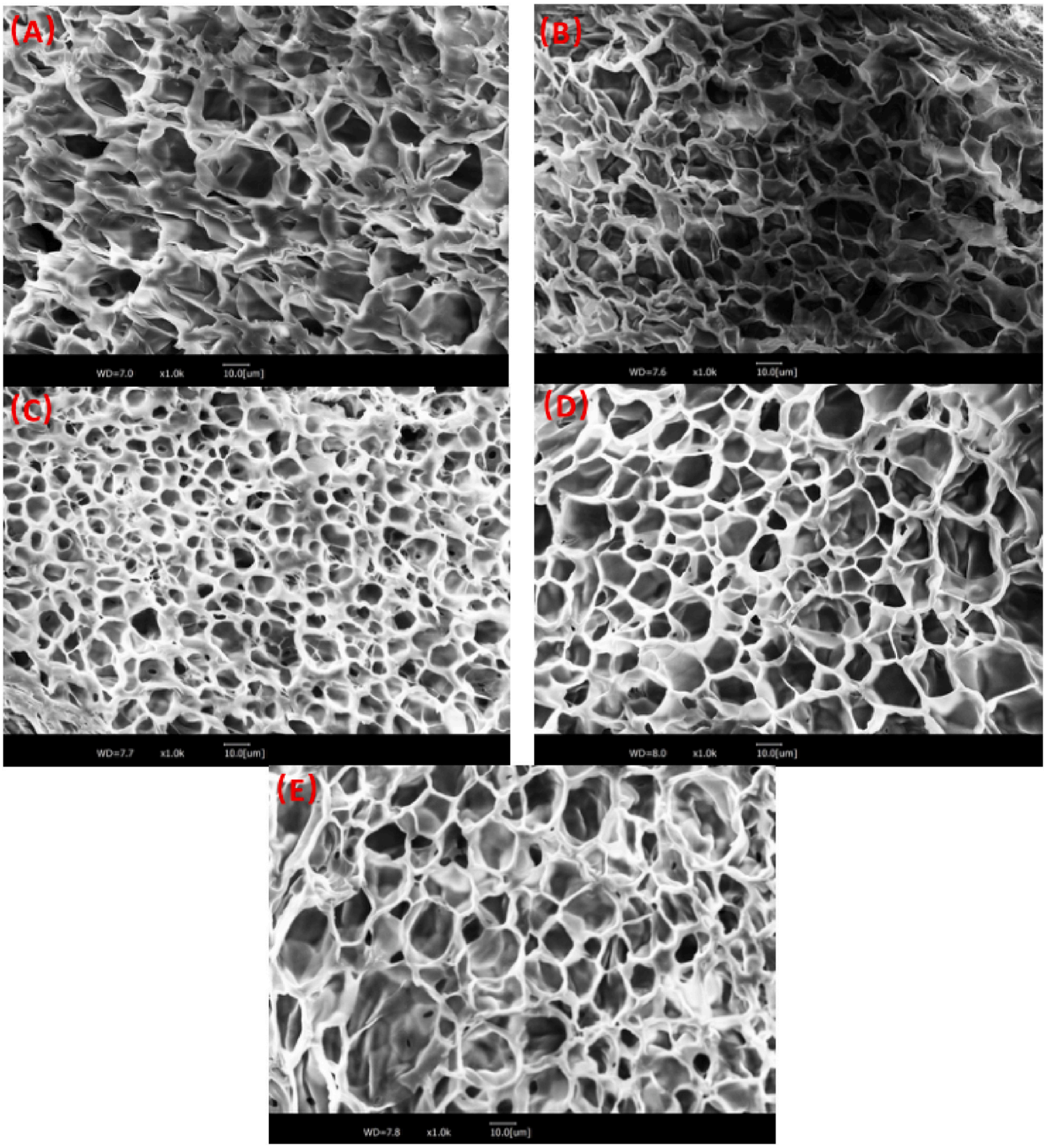

Fig. 7. SEM images of curdlan gels with different concentrations of trehalose. (A) 0%, (B) 0.5%, (C) 1%, (D) 1.5%, and (E) 2%.

Table 1
T_2 relaxation parameters of trehalose-curdlan gels.

Concentration (%)	T_{21}	T_{22}	T_{23}	S_{2b} (%)	S_{22} (%)	S_{23} (%)
0	2.41	505.26	2686.06	0.00	68.01	31.99
0.5	2.41	541.59	3583.47	0.02	62.03	37.94
1.0	2.10	357.08	2494.50	0.02	69.19	38.78
1.5	2.41	471.38	2673.84	0.02	92.91	7.06
2.0	2.41	580.52	4347.01	0.02	89.25	10.73

representing the reduce of bound water and the growth of half-bound and freedom water. This may due to the fact that excessive trehalose filling between curdlan chains will prevent the aggregation of curdlan chain and impede the construction of gel network, leading to a reduction in hardness with the further increase of trehalose concentration. These findings illustrated that the addition of trehalose could change the proportion of the different forms of water, leading to the different structural characteristics of curdlan-trehalose composite gels, which was consistent with the results of WHC and SR (Tong et al., 2022).

3.7. Microstructure

The SEM image of lyophilized curdlan gels with different concentrations of trehalose are showed in Fig. 7 to visually examine their microstructures. It can be clearly observed that the pure curdlan gel presented a porous, semi-transparent sheet structure with (Fig. 7(A)) (Verma et al., 2020), while the curdlan-trehalose composite gels showed relatively homogeneous and compact structures in Fig. 7C–E, as expected.

Curdlan exhibits different layers of gel network connected by weak and thin hydrogen bonds, which may be the crucial reason for its low hardness, springiness and cohesiveness. The highly ordered helical

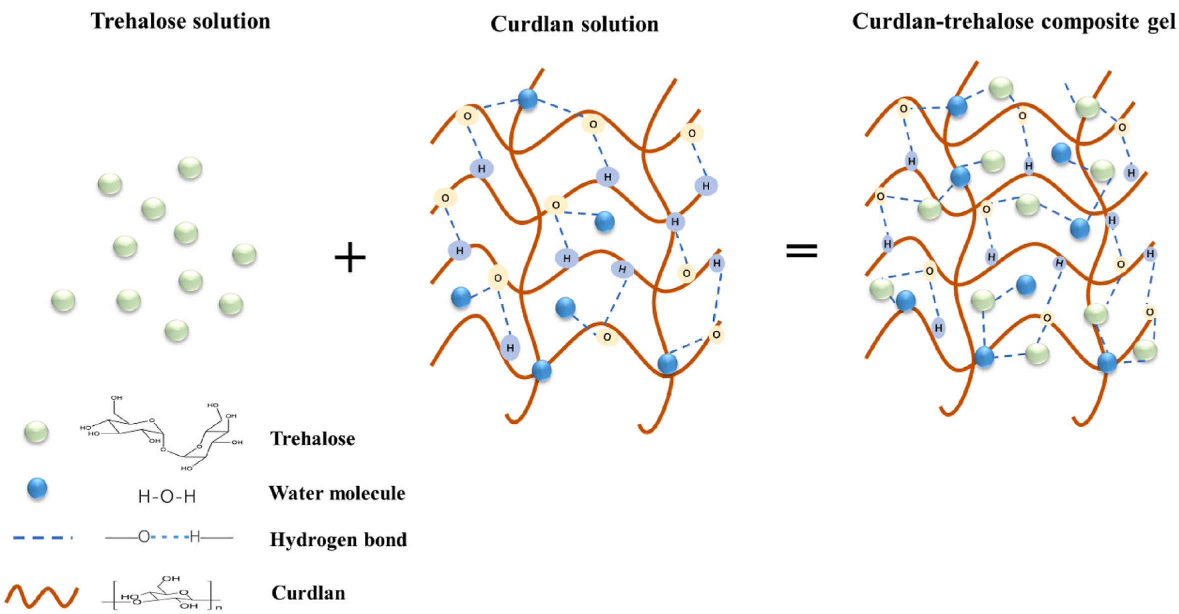

Fig. 8. Proposed mechanisms for the formation of network of curdlan-trehalose composite gels containing 1% (w/v) trehalose.

structure is mainly formed by the stability of hydrogen bonds between and within the chains (Qin et al., 2017). The structure of curdlan gel was more compact after the addition of trehalose. Trehalose addition could maintain the structural integrity of the curdlan-trehalose composite gels. Compared to the pure curdlan gel, the microstructure of the curdlan gel include 1% trehalose concentration could obtain higher density and more uniform structure (Fig. 7(C)). This phenomenon indicated that the combination of trehalose could reduce the water mobility in the curdlan gel system, generating smaller and more compact porous structures. These differences in the pore structure size of the gel network may be the main reason for the difference in the physical properties (including hardness, elasticity, strength, cohesiveness and rheological properties) of the curdlan gel (Xiao and Li et al., 2020). It may be that the addition of trehalose strengthens the interaction of hydrogen bonds between trehalose and curdlan, thus forming a denser porous network structure (Zhang and Zhu et al., 2020). The changes in the surface morphologies of the curdlan gels are accordance with the previous texture property results (Fig. 2). Except for the enhanced texture properties, the improvement of the dense structure can offer a modified combination with food or drug components for targeted delivery, environmental adsorption, and so on.

3.8. Possible mechanism for the improvement in gelation characteristics of curdlan gel after trehalose addition

According to the above-mentioned findings, the hydrogen bonding interaction enhanced the gel quality of the curdlan gel at a low trehalose concentration (0–1%). The addition of trehalose is conducive to the retention of water molecules in the curdlan-trehalose composite gel by forming a dense network structure, thus affecting gel properties, the potential mechanism is shown in Fig. 8. For curdlan-trehalose composite gel, the best concentration of trehalose was reached at 1% to entangle and interpenetrate with curdlan, forming an interpenetrating network structure. At higher concentration from 1% to 2%, the excessive trehalose molecules may lead to the difficulty of aggregation of condensed chains, thus hinder the formation of condensed structure of curdlan-trehalose composite gel. Overall, a higher-strength gel was produced after adding 1% trehalose because the cross-linking hydrogen bond between trehalose and curdlan stabilized the gel structure, allowing them to perform a moderate bonding function and yield a higher strength performance after gelatinisation. Additionally, after trehalose inclusion, hydrophobic interactions to improve the quality of curdlan-trehalose composite gels.

4. Conclusions

In summary, trehalose can enhance the texture characteristics of curdlan gel by hydrogen bonding interaction. The enhancing effect was pronounced when 1% trehalose was added in combination with curdlan by improving WHC and freeze-thaw stability with a uniform and dense network structure. Dynamic rheological behavior indicated that the addition of 1% trehalose helps to form a dense network structure with high gel strength by increasing the G′, and G″ values affected by hydrogen bonding. Together with the descended LF-NMR, the enhanced water binding capacity of curdlan-trehalose composite gel (1% trehalose) was also evidenced. The FT-IR spectra and XRD revealed that the favorable crystallinity of trehalose could enhance the crystallinity of the composite gel. The SEM observation proved that the addition of trehalose strengthened the hydrogen bond interaction to build a much denser network. In conclusion, the novel curdlan-trehalose composite gel formatted in this research with good textural characteristics will broaden the application of trehalose in food and pharmaceutical applications in future.

CRediT authorship contribution statement

Long-Qing Li: Writing – review & editing, Writing – original draft. **Le-Yi Pan:** Methodology, Investigation. **Tong-Xin Liang:** Investigation. **Mingyu Jin:** Methodology. **Ya-Hui Yu:** Methodology. **Xiangying Yu:** Investigation, Formal analysis. **Xiaozhen Liu:** Supervision, Investigation. **Fengyuan Liu:** Software, Conceptualization. **Yuting Li:** Data curation. **Jing-Kun Yan:** Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

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

Acknowledgments

This work was supported financially by the Foundation for Innovation Team in Higher Education of Guangdong, China (2021KCXTD035), and ISTI, Dongguan University of Technology, China (KCYCXPT2017007), the Foundation of Young Innovative Talents Project of Universities of Guangdong province (2022KQNCX087), and the Foundation and Applied Basic Research Fund Project of Guangdong province (2022A1515110715). In addition, the authors acknowledge the assistance of Dongguan University of Technology Analytical and Testing Center.

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