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Effects of hawthorn addition on the physicochemical properties and hydrolysis of corn starch

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ABSTRACT

Hawthorn powder were mixed with corn starch and heated in water to make corn starch-hawthorn mixtures (CS-Haw) and then the physicochemical properties and hydrolysis characteristics of the mixtures were measured. Results showed that the addition of hawthorn powder decreased the viscosity of corn starch, and prolonged the pasting temperature, while the microstructure analysis indicated that hawthorn particles aggregated on the surfaces of starch granules, reducing the chance of starch contacting with water, then delayed the starch gelatinization. The presence of hawthorn powder also reduced the G' value to varying degrees and the loss tangent of CS-Haw was significantly higher than that of corn starch. The addition of hawthorn powder in large amounts also increased the rapidly digestible starch, while decrease the slowly digestible starch and resistant starch. The present research will provide basic theoretical support for the application of hawthorn in healthy starch food processing.

Introduction

Hawthorn (*Crataegus pinnatifida Bunge*) is a plant of the genus *Crataegus* in the Rosacea (Kwok et al., 2013), which is widely planted in China, North America and Europe (Wen et al., 2015). Attributing to its high content of phytochemicals, the fruits of hawthorn has been regarded as a kind of medicinal and edible foodstuff since ancient times around the world (Roman et al., 2021; Ye, Yang, Liu, & Cao, 2021). Particularly, the sweetness and sour taste makes it popular for all ages, and it is often used in food to increase the appetite. As commonly known, the function of the gastrointestinal tract will gradually decline with age. Undoubtedly, the rate of digestion and the degree of absorption of food will also decline (Pilotto and Di Mario, 2007). Gastrointestinal diseases, such as indigestion, flatulence, etc., are commonly occurred in the elderly (Kumar, Sudha, Bennur, & Dhanasekar, 2020). This not only makes the elderly mental and physical exhaustion, but also adds a lot of burden to social medical care. One of the most important factors leading to indigestion is the digestion rate of food. The food with compact structure (e.g., whole grain, spaghetti, etc.) will bring burden to the stomach and intestine, while food with puffy structure are not. Therefore, for people with poor gastrointestinal conditions, an easy digested diet is particularly important.

Starch is an easy-to-obtain and abundant polysaccharide, which is the main storage carbohydrate in plants and the main energy source for humans (Ning et al., 2020). It has been reported that more than half of the total energy of human diet were provided by starch, and the digestibility of starch is closely related to human metabolic health (Zhang et al., 2021). Thus, controlling the starch digestion speed might be a protentional way to relieve the burden of gastrointestinal diseases. Different from previous studies that try to reduce the starch digestion by adding edible gels, phenolic acids, the promotion of starch digestion might also important, but few works have focused on this. Considering the hawthorn fruits has been used as an appetite-stimulating and peptic material around the world since ancient time, we hypothesized that the addition of hawthorn fruits in starchy food might regulate the digestion of starch and be beneficial for the people with poor gastrointestinal

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conditions.

To investigate how the addition of hawthorn fruits in starch affect its digestibility and physicochemical properties, the hawthorn powder was mixed with corn starch at different proportions and heated, the effect of hawthorn fruits powder on the gelatinization, thermal property, ordered structure, rheological properties of corn starch were investigated with FT-IR, X-ray, DSC, etc., and the digestibility were also evaluated with an enzyme hydrolysis method. The present study might provide new ideas to produce health care products for patients with dyspepsia as well as basic theoretical support for the using of hawthorn in functional foods.

Materials and methods

Materials

Corn starch, porcine pancreatic α-amylase, glucoamylase, invertase were purchased from Sigma-Aldrich Co., ltd (Milwaukee, Wisconsin, USA). Hawthorn was obtained from Huixian, Henan, China. The glucose determination kit was purchased from Megazyme International Ireland ltd. (Wicklow, Ireland). Other analytical reagents were brought from Aladdin Co., ltd. (Shanghai, China).

The hawthorn-corn starch mixtures preparation

The preparation of hawthorn-starch mixtures was performed according to Zheng et al. (2020). Briefly, 20 g corn starch was accurately weighed and 0.0 %, 2.0 %, 4.0 %, 6.0 % and 8.0 % (W/W%) hawthorn powder was added respectively. After mixing thoroughly, distilled water was used to make 10 % (W/W %) starch suspension. Then, the suspensions were stirred magnetically at 95 °C for 20 min. The prepared mixtures were cooled to room temperature and then placed at −80℃. The freeze-dried samples were ground through, sealed and stored for further use.

Semi crystallinity analysis of the mixtures

The crystallinity property of corn starch and hawthorn-starch mixtures were analyzed with an X-ray diffractometer (X'Pert3 Powder) at room temperature. The parameters of the obtained X-ray diffraction pattern are as follows: the radiation source of Cu-Ka target rays at a wavelength of 0.15406 nm was 40 kV and 100 mA. The diffraction pattern was obtained by scanning the scattering range (2θ) from 4° to 40° at a speed of 2 min^{-1} and scanning step of 0.04° (2θ). The crystallinity degree of the mixtures was calculated and analyzed with JADE 5.0 software (Material Data Corporation, Livermore, California, USA) according to Chen et al. (2017).

Short-range ordered structure analysis

To analyze the short-range ordered structure changes of the CS-Haw mixtures, the FT-IR spectra of the samples were measured with Nicolet tar 370 (Thermo Nicolet co., Waltham, USA) spectrometer at room temperature according to Wang et al. (2014). Before the measurement, the composite was kept at room temperature for 24 h, then it was mixed with KBr at a ratio of 1:100 (w/w) thoroughly and pressed into a sheet, and scanned under the condition of a resolution of 4 cm^{-1} and a wavelength range of 400–4000 cm^{-1}. With the spectral grade KBr as the blank, the infrared spectrum was obtained after scanning 32 times and averaging.

The analysis of thermal properties

The thermal properties of the mixtures were determined by a differential scanning calorimetry (Mettler, Toledo, Switzerland) according to Tian et al. (2019). Briefly, 3 mg corn starch or mixtures were weighed into the DSC dish accurately, distilled water was added at the ratio of 1:3 (w/V) and balanced overnight at 25 ℃. Then, the pan was first heated to 111℃ at 10 ℃/min and then cooled to 30 ℃. The blank pan was then scanned with the same settings as the control group.

The analysis of the pasting properties

For the analysis of pasting properties, 2.58 g corn starch (DW, dry weight) was mixed with distilled water to a total weight of 28 g (Yang et al., 2019). And then the slurry was measured with a rapid viscosity analyzer (RVA TecMaster, Perten Instruments, Inc., Hägersten, Sweden) under the following conditions: running at 50 ℃ for 1 min, heating to 95 ℃ (6℃/min heating rate) for 5 min, cooling to 50 ℃ (6℃/min cooling rate) for 2 min, then the gelatinization curve of starch was obtained (Luo et al., 2017).

The analysis of rheological properties

To analyze the **r**heological properties of the mixture, the static rheological properties of hawthorn-starch complexes were determined. Briefly, the gelatinized hawthorn-starch mixtures (1 mL) were uniformly added to a rotational rheometer plate (diameter 60 mm, plate spacing 1 mm) for static rheological experiments (25 °C). The shear rate was increased in the range of 0.1–100 s^{-1} and the corresponding shear stress changes were recorded (Qiu et al., 2015).

The observation of gel structure morphology

Scanning electron microscope was used to investigate the gel morphology of corn starch with different proportions of hawthorn powder (2, 4, 6 and 8 %, w/w) after gelatinization. The gelatinized hawthorn-corn starch mixtures were immediately added to a 2.5 % glutaraldehyde solution and soaked for 2 h to fix the gel structure and then dehydrated with ethanol. The dried gel was put on the conductive glue to observe the microstructure after gold spraying according to Yin et al. (2021).

The hydrolysis analysis of hawthorn-starch mixtures

The hydrolysis of the corn starch and hawthorn powder in different proportions (2, 4, 6 and 8 %, w/w) was carried out. Briefly, the mixture (1 g) was suspended in 50 mL of sodium acetate buffer (pH 5.2), stirring in a water bath at 90 °C for 20 min to allow it to gelatinize sufficiently. Then, the system was placed at 37 °C for 20 min, and 3 mL enzyme solution (120 U/ml, α-amylase; 240 U/ml, α-1,4-Glucanglucohydrolace) was added. The mixed enzyme solution was incubated at 37 °C for 5 min in advance to keep the temperature of the reaction system stable. The reaction system was kept at 37 °C and vortexed for 3 h, 0.5 mL was sampled at 0, 20, 40, 60, 90, 120, and 180 min, and 2.5 mL absolute ethanol was immediately mixed to inactivate the enzyme activity. After centrifuged at 2000 rpm for 10 min, 0.1 mL supernatant was mixed with 0.5 mL invertase solution, and reacted in a 37 °C for 10 min. Finally, the GOPOD kit was used to detect the glucose content. The content of rapid digested starch (RDS), slow digested starch (SDS) and resistant starch (RS) was calculated according to Yang et al. (2020).

Statistical analysis

SPSS 20.0 software (IBM, Chicago, USA) was applied to determine whether there are significant differences. The Origin 2018 (Origin Lab, California, USA) software was used to draw the chart of this article. At least three parallels were set for each test to ensure the accuracy and credibility of the experiment.

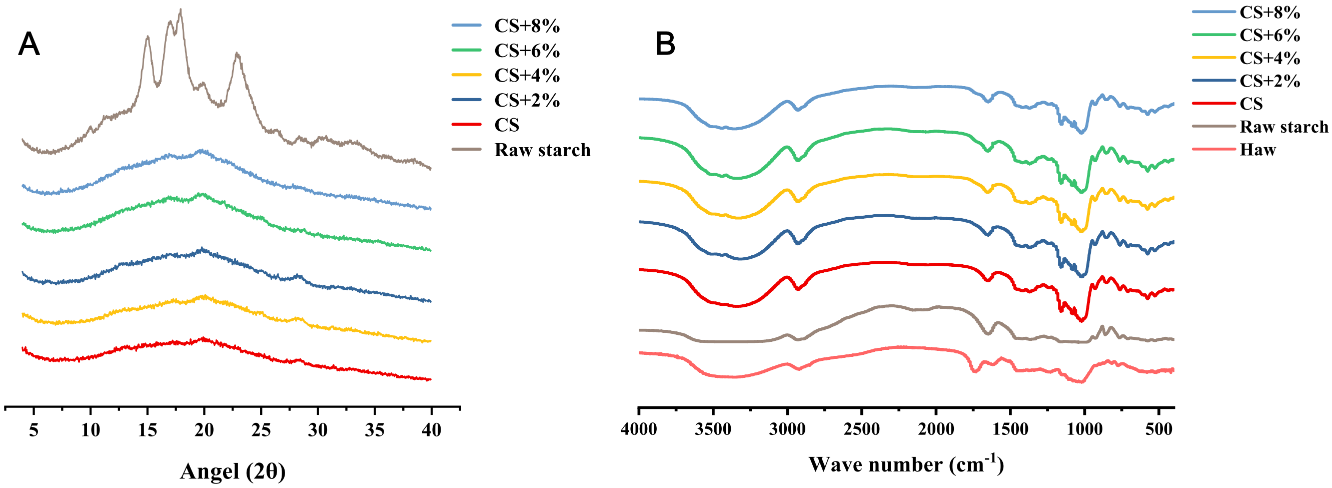

Fig. 1. XRD patterns (A) and FT-IR spectra (B) of Raw corn starch and corn starch–hawthorn complexes. Raw starch, CS, CS + 2 %, CS + 4 %, CS + 6 %, CS + 8 % represent Raw corn starch, corn starch with 0.0 %, 2 %, 4 %, 6 % and 8 % Hawthorn (w/w) respectively.

Results and discussion

Semi crystallinity of hawthorn-corn starch mixtures

The effect of hawthorn on the crystallinity of corn starch was studied by XRD test. As shown in Fig. 1A, raw corn starch showed a A-type diffraction pattern (Zhu et al., 2021). It could be seen from the figure that the gelatinized corn starch lost its natural A-type XRD pattern (Zheng et al., 2021). Different from the control group, the gelatinized corn starch and compound had typical reflection peaks at 20.1° and 17° respectively, demonstrating the crystal form of the mixture was B + V (Chen et al., 2017). Additionally, no significant difference between the peak shape and RC of the samples at all concentrations were observed, indicating that the influence of hawthorn powder additives on the crystal structure of corn starch might not be directly related (Tian et al., 2018).

Short-range ordered structure changes of the mixtures

The FT-IR spectra of the corn starch–hawthorn mixtures were showed in Fig. 1B. All samples showed broadband characteristics in the spectral region of 3200–3400 cm^{-1}, which represents the vibrational stretching of –OH in carboxyl and hydroxyl groups. The peaks at 2925 and 1654 cm^{-1} suggested the tensile vibrations of C—H and carbonyl, respectively. When the spectral range was 927–1010 cm^{-1}, 1140–1171 cm^{-1} and 1316–1416 cm^{-1}, the corresponding peaks separately showed C—O—H bending, C—H, C—H tensile vibration. The band at 1082 cm^{-1} was mainly contributed by the C—O stretching of a hydro glucose ring of C—O—H (Dangi, Yadav, &Yadav, 2020). It is known that the FT-IR spectral bands at 1047 cm^{-1} and 1022 cm^{-1} were strongly correlated with the amorphous and ordered structures of starch, respectively. Therefore, the number of ordered structures in starch could be represented by the ratio of $R_{1047/1022}$ (Ding, Luo, &Lin, 2019). With the increasing of hawthorn addition, the ratio of $R_{1047/1022}$ increased, indicating that the amorphous structure increased in the mixtures.

However, although there was no new covalent bond formation, as the proportion of hawthorn powder increased, the band in the 3200–3400 cm^{-1} spectral region became wider. Studies had shown that wider O—H stretch band indicating stronger hydrogen bonds (Gao et al., 2021). Therefore, it could be explained that hawthorn powder and corn starch granules interacted through non-covalent bonds (e.g. hydrogen bonds) during the starch gelatinization.

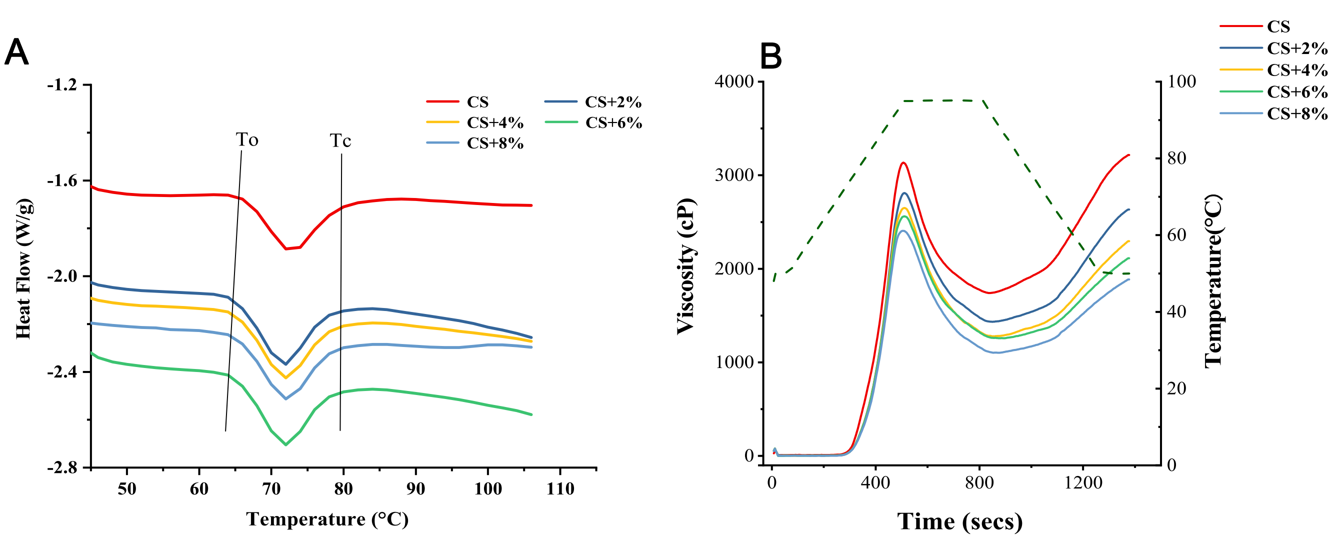

Fig. 2. DSC heat flow curves (A) and pasting curves (B) of corn starch and corn starch–hawthorn complexes. Raw starch, CS, CS + 2 %, CS + 4 %, CS + 6 %, CS + 8 % represent Raw corn starch, corn starch with 0.0 %, 2 %, 4 %, 6 % and 8 % Hawthorn (w/w) respectively.

Table 1
Rheological properties of corn starch, corn starch-Hawthorn complexes from RVA analysis.

Samples	PV(cP)	TV(cP)	BD(cP)	FV(cP)	SB(cP)	PT (cP)
CS	3126 ± 9.9^{a}	1752.5 ± 21.92^{a}	1373.5 ± 31.82^{ab}	3215 ± 1.41^{a}	1462.5 ± 20.51^{a}	74.68 ± 0.04^{b}
CS + 2 % Haw	2842.67 ± 35.53^{b}	1432.67 ± 6.35^{b}	1410 ± 30.05^{a}	2651.67 ± 17.21^{b}	1219 ± 14.18^{b}	75.65 ± 0.17^{a}
CS + 4 % Haw	2659.67 ± 13.2^{c}	1281.33 ± 7.51^{c}	1378.33 ± 5.86^{ab}	2301 ± 14.73^{c}	1019.67 ± 17.01^{c}	75.93 ± 0.03^{a}
CS + 6 % Haw	2538.67 ± 36.09^{d}	1235.67 ± 19.86^{c}	1303 ± 18.68^{bc}	2102.33 ± 35.13^{d}	866.67 ± 23.07^{d}	75.92 ± 0.03^{a}
CS + 8 % Haw	2410.67 ± 8.02^{e}	1127.67 ± 26.08^{d}	1283 ± 19.05^{c}	1896 ± 11.79^{e}	768.33 ± 16.5^{e}	76.08 ± 0.23^{a}

Note: Lower letters in the same column of one cultivar followed were significantly different at the $p < 0.05$.

The thermal properties of hawthorn-corn starch mixtures

The obtained DSC heat flow curve was shown in Fig. 2A, with the addition of hawthorn, the *To* value of the hawthorn–corn starch decreased, and the gelatinization temperature range (*Tc-To*) increased, but little changes were observed in the values of *Tp* and *Tc*, indicating that the hawthorn reduced the thermal stability of the corn starch and increased the degree of heterogeneity of starch crystals. In addition, the endothermic peak of the hawthorn-starch samples increased and the *ΔH* value became larger, indicating that hawthorn changed the original starch intermolecular bond connection and its crystal structure.

The DSC results were consistent with Zhu et al. (2009), in their study, they reported that after adding the hawthorn extract, the thermodynamic properties of wheat starch changed significantly, and the enthalpy change value decreased. Before the DSC test, the samples were equilibrated at room temperature for 24 h, and during this period, the natural active substances in the hawthorn powder such as acid molecules might attack the amorphous area of the particles, which made them disappear partially. In addition, phytochemicals with different conformational flexibility and hydroxyl groups might bind to the amorphous regions of starch granules, re-arranging the structure of the starch chain, thus changed the coupling force between the two substances.

The pasting behavior of hawthorn-corn starch mixtures

The gelatinization parameters and gelatinization curve were shown in Table 1 and Fig. 2B. Gelatinization parameters including peak viscosity (PV), breakdown viscosity (BD), pasting temperature (PT), final viscosity (FV), through viscosity (TV) and setback viscosity (SB), were listed in Table 1. As shown, when the proportion of hawthorn powder increased from 2.0 % to 8.0 %, the values of PV, BD, TV, FV and SB gradually decreased, with the prolonging of gelatinization time. PV

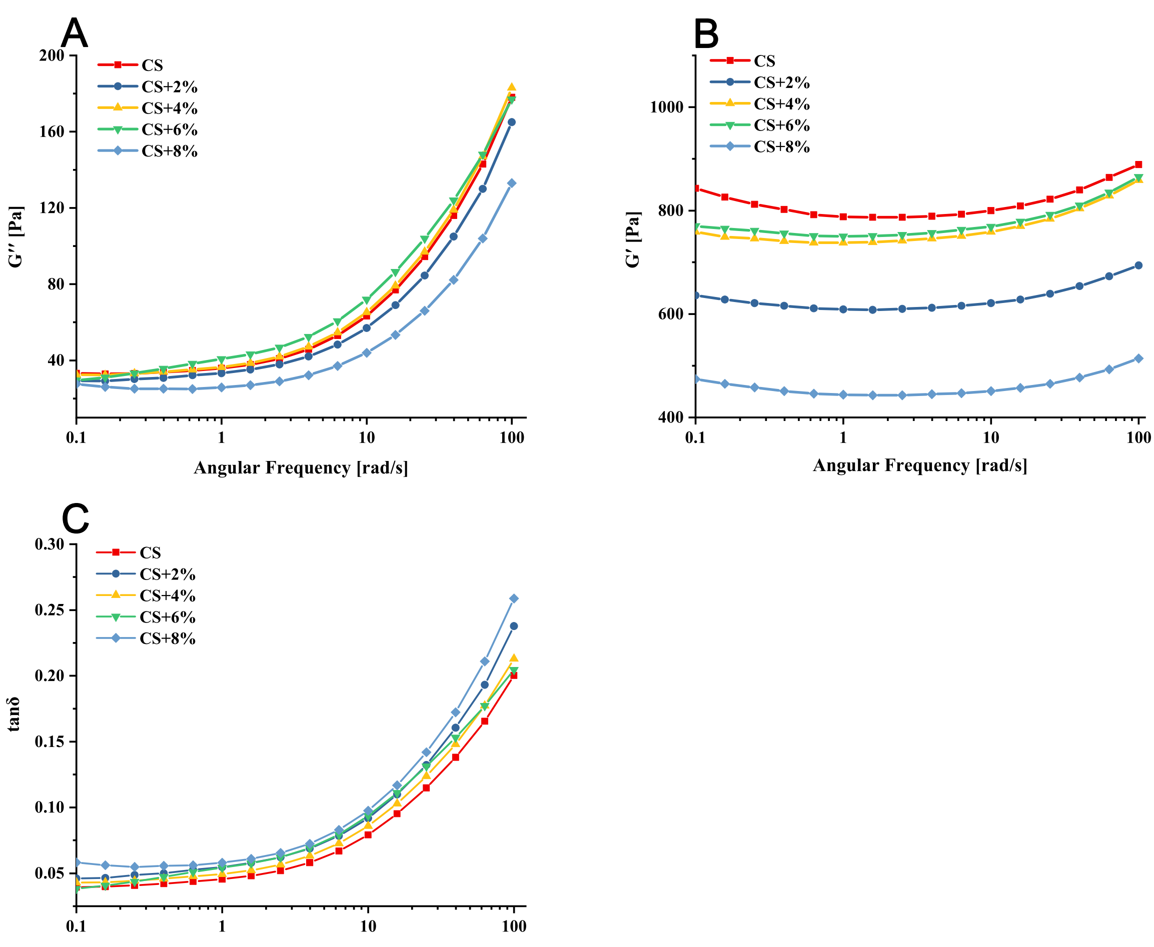

Fig. 3. Dynamic rheological properties of gelatinized corn starch (CS) with corn starch–hawthorn complexes. CS, CS + 2 %, CS + 4 %, CS + 6 %, CS + 8 % represent corn starch with 0.0 %, 2 %, 4 %, 6 % and 8 % hawthorn (w/w), respectively.

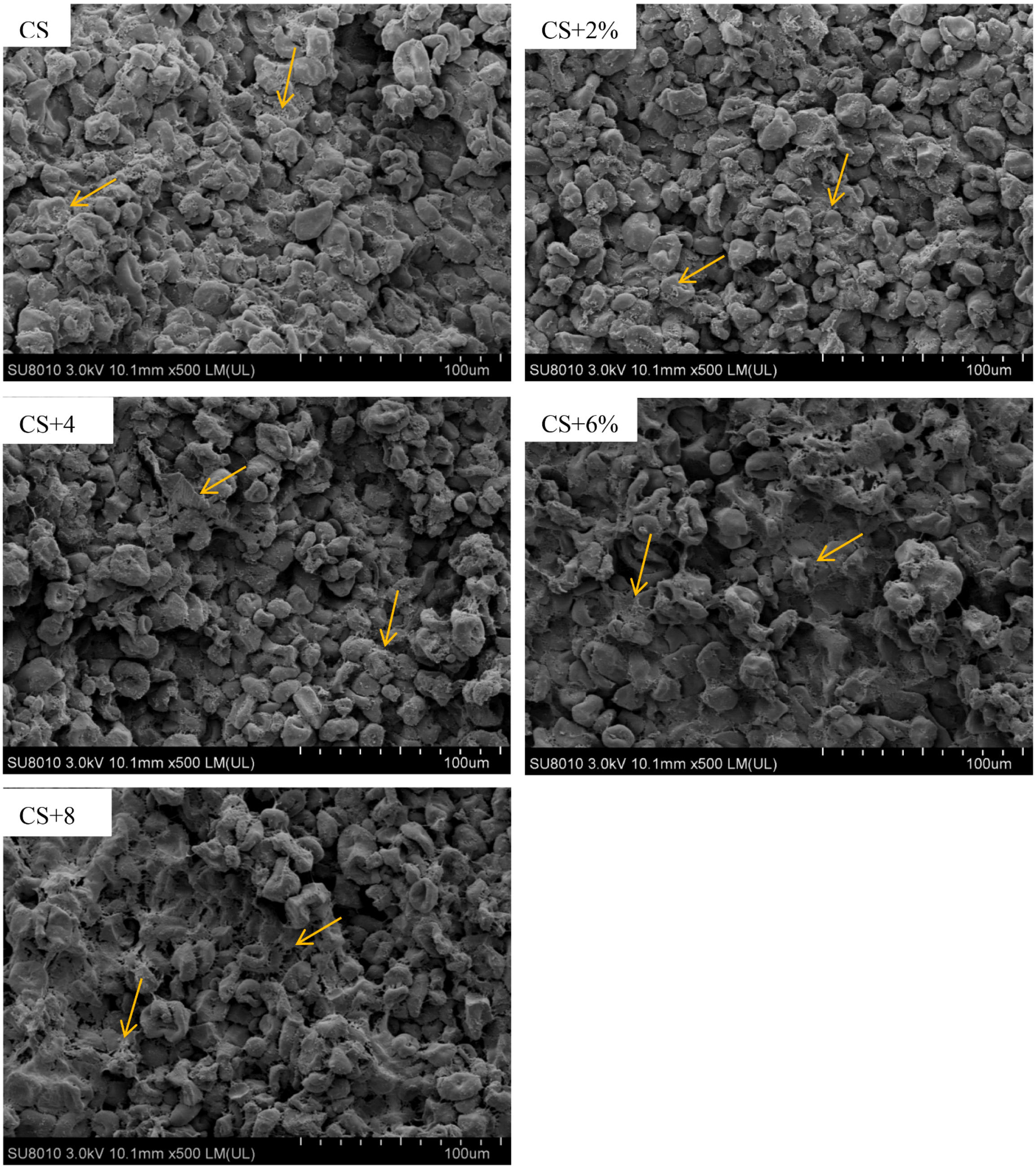

Fig. 4. Morphology of gelatinized corn starch (CS) with corn starch–hawthorn complexes. CS, CS + 2 %, CS + 4 %, CS + 6 %, CS + 8 % represent corn starch with 0.0 %, 2 %, 4 %, 6 % and 8 % hawthorn (w/w) respectively.

represented the peak viscosity that the starch could reach before it was gelatinized to cooling. The PV of corn starch was the highest. As the amount of hawthorn powder increased, the PV gradually decreased. It might be explained that hawthorn powder aggregated on the surface of the starch granules, avoiding the chance of contact between starch and water, delaying gelatinization, and then resulting in a decrease in PV, TV, and FV, and changing the gelatinization characteristics of corn starch (Zheng et al., 2020). The chemical substances contained in hawthorn could interact with water molecules, thereby changing the ionic strength and pH of the water and changing the "external environment" of the corn starch granules (Bao and Corke, 2002).

BD could reflect the stability of starch against heat and shear. The higher the BD was, the more severe the starch cracking during the gelatinization process. Different from the control group, the addition of hawthorn powder reduced the BD values of corn starch, which illustrated that the structure of corn starch was more compact after the mixing of hawthorn powder. Starch retrogradation seriously affected the nutritional value and shelf life of corn starch and its products, and hindered the development of the food industry. The SB value reflects the short-term retrogradation of starch and the molecular rearrangement of amylose. The results showed that the SB value of starch decreased after adding hawthorn powder, which indicated that hawthorn powder could inhibit the retrogradation of starch.

The rheological properties of hawthorn-corn starch mixtures

As showed in Fig. 3, the storage modulus (G') represented how much energy the starch has stored through elasticity, while loss modulus quantity (G'') indicated the ability of starch to dissipate energy. The loss factor tan δ (G''/G') referred to the relative importance of starch

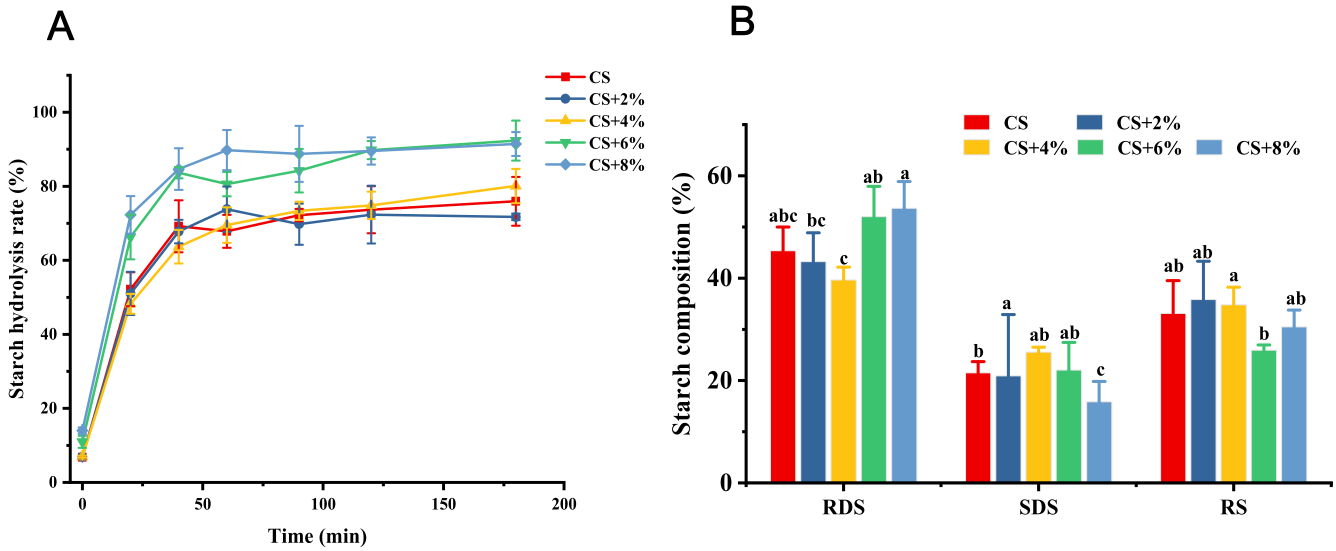

Fig. 5. Hydrolysis of gelatinized corn starch (CS) with corn starch–Hawthorn complexes. (A), RDS, SDS and RS contents of corn starch and the complexes (B). CS, CS + 2 %, CS + 4 %, CS + 6 %, CS + 8 % represent corn starch with 0.0 %, 2 %, 4 %, 6 % and 8 % Hawthorn (w/w) respectively.

viscosity and elastic behavior. As shown in Fig. 3A and 3B, with the increasing angular frequency, both *G'* and *G"* increased in varying degrees, and the addition of hawthorn powder affect the dynamic modulus of corn starch. It is easy to refer that the storage modulus was higher than the loss modulus. These results indicated that in the entire frequency range, the elastic behavior dominated the viscous component, and the CS-Haw composite could be classified as a weak gel structure (Zhang, Li, Tian, Song, & Ai, 2019).

After the addition of hawthorn powder, *G'* value decrease to different degrees. The tan δ (G"/G') of CS-Haw mixtures was significantly higher than that of corn starch gel (Fig. 3C). This indicated that the addition of hawthorn powder reduced the gelling ability of corn starch. It was possible that hawthorn compounds would compete with starch for water, which limited the gelatinization of starch and reduced the gelling of corn starch. This result was consistent with the conclusion of the pasting analysis, where adding different proportions of hawthorn powder altered the gelatinization characteristics of corn starch.

The microstructure of hawthorn-corn starch gel

As shown in Fig. 4, due to partial gelatinization, the shape of corn starch granules was irregular and the surface was relatively rough. When the addition amount of hawthorn powder was less than 4 %, the starch granules became easy to distinguish, hawthorn powder did not have a significant effect on the microstructure of starch. But when the addition amount was greater than 6 %, the hawthorn powder gathers on the surface of the starch granules, forming a layer of network, which was connected and wrapped among the starch granules.

Effects of hawthorn powder on the hydrolysis of corn starch

The corn starch with different proportions of hawthorn powder were gelatinized and the hydrolysis rates of corn starch and corn starch mixed with different ratio (2–4 %) of hawthorn powder was evaluated, and results were showed in Fig. 5A. The addition of hawthorn accelerated the starch hydrolysis, particularly at the addition of 6 and 8 %. When the amount of hawthorn powder addition was 2 %–4%, the initial hydrolysis rate was not much different from that of the corn starch. However, when the hawthorn powder addition was increased to 6 % and 8 %, the hydrolysis rate was significantly faster than that of corn starch, and the final hydrolysis rate of starch was also significantly increased ($p < 0.05$).

As shown in Fig. 5B, corn starch without hawthorn powder contains 45.38 % RDS, 21.52 % SDS and 33.1 % RS. Different from the control group, when the addition of hawthorn was 2 %–4%, the content of RDS in corn starch was reduced and the content of SDS and RS was increased, but those were not significant. This might be due to the little amount of hawthorn added, which was not enough to break the starch network structure, and the low concentration of hawthorn inhibited the swelling of corn starch and kept the starch granules intact. Hawthorn powder surrounded on the surface of the particles and became a natural barrier between starch and enzymes (Yin et al., 2021).

However, when the amount of hawthorn powder addition was higher than 6 %, the content of RDS increased, while SDS and RS content decreased. It may be the large amount of hawthorn broken the gel network of corn starch and increased the fluidity. On the other hand, hawthorn particles gathered around the starch granules, making the surface of the starch gel structure rough, reducing the gel density, increasing the specific surface area of the enzyme to contact, then increasing the RDS content, and accelerating the hydrolysis rate (Iqbal, Wu, Kirk, & Chen, 2021).

Conclusion

The changes in the structural characteristics and hydrolysis of corn starch with different proportions of hawthorn powder were analyzed. The results showed that the peak viscosity and setback of corn starch were reduced after adding hawthorn powder, which inhibited starch retrogradation and better retained the nutritional value of starch products. The chemical substances contained in hawthorn powder changed the ionic strength and pH of water, thereby changing the "external environment" of corn starch granules. XRD results showed that the crystal form of corn starch-hawthorn complex was B + V. Compared with the control group, when the addition amount of hawthorn powder was higher than 6 %, the RDS in the starch increased, and the SDS and RS decreased. At the beginning of the digestion process, the hydrolysis rate of the corn starch-hawthorn mixtures was significantly higher than that of the control group, and the final hydrolysis rate of starch was also significantly improved. It might be because when the proportion of hawthorn powder involved in gelatinization was higher than 6 %, the gel network of corn starch would be destroyed. In addition, hawthorn powder gathered around starch granules to compete for water and inhibited starch granules from swelling, and the presence of hawthorn powder made the surface of the starch gel structure rough, reducing the density of the gel and enhanced the contact between starch and water, therefore increasing the specific surface area of the enzyme to contact, and speeding up the digestion rate.

Declaration of Competing Interest

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

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