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Structural and functional properties of two phenolic acid-chitosan derivatives and their application in the preservation of Saimaiti apricot fruit

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Abstract

In this study, we incorporated gallic acid (GA) and salicylic acid (SA) onto chitosan (CS) using free radical grafting initiated by hydrogen peroxide/Vitamin C (H_2O_2/Vc) redox system. We characterized the structural properties of the GA (CS-GA) and SA (CS-SA) derivatives using UV–vis absorption, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), nuclear magnetic resonance (NMR), and thermal stability analysis. We proved that these phenolic acids were successfully grafted onto the molecular skeleton of CS. We investigated the antioxidant and antibacterial properties of CS-GA and CS-SA and found that they had significantly higher antioxidant and antibacterial properties than native CS. Furthermore, the antioxidant ability increased with the increase in grafting ratios. Finally, in vivo tests showed that CS-GA could maintain the firmness and the content of soluble solids and titratable acids in Saimaiti apricots at cool storage. The storage period was prolonged by inhibiting the respiratory intensity of apricots. Our results suggest that CS-GA can be potentially used as an edible coating material to preserve apricots.

Keywords: chitosan; phenolic acids; antioxidant activity; antimicrobial activity; apricot.

Practical Application: Chitosan - phenolic acid derivatives were applied to the preservation of apricot, Xinjiang's characteristic fruit, to solve the problems of short storage period and small market scope of Xinjiang fruits, and prepare to develop a new type of fruit and vegetable preservative.

1 Introduction

Chitosan (CS) is a natural cationic polysaccharide composed of β-(1-4)-2-amino-D-glucose and β-(1-4)-2-acetylamino-D-glucose. CS is a deacetylated product of chitin and has unique biological properties such as antioxidant activity, antibacterial activity, biocompatibility and biodegradability (Gao et al., 2021). As a natural amino polysaccharide, CS's non-toxic and non-hazardous properties have received widespread attention from researchers, and is often used as a new material in food and pharmaceutical packaging, etc (Koshy et al., 2015; Ngoc et al., 2022). However, the special spatial structure of CS makes it poorly soluble only in dilute organic acid solutions (Hafsa et al., 2014), while the lack of hydrogen atom donors in the molecular structure leads to the poor antioxidant properties of CS, and these disadvantages largely reduce the application of CS (Lee et al., 2014; Liu et al., 2013b). It is worth investigating that the presence of large amounts of amino and hydroxyl groups in CS molecules can provide effective reactive sites for chemical modifications that substantially improve functional properties (Li & Zhuang, 2020; Torkaman et al., 2021). Therefore, in order to improve their intrinsic physical structure and chemical properties and to obtain new derivatives with enhanced solubility and biological activity (Dandan et al., 2008; Chung et al., 2011), one needs such biopolymers to introduce external functional groups through certain chemical modifications (Hu & Luo, 2016). At present, there are many methods in the modification of CS, such as carboxymethylation, esterification, acylation, quaternary ammonium, alkylation and graft copolymerization (Dash et al., 2011; Wu et al., 2016b).

Regarding phenolic compounds, they are often considered as natural antioxidants with antioxidant, anti-inflammatory, anti-mutagenic and anti-cancer properties (Brewer, 2011; Yun et al., 2008). Polyphenols are classified into four groups, namely phenolic acids, flavonoids, stilbene and lignans (Hu & Luo, 2016). Gallic acid (GA) is a phenolic acid that can be extracted from plants and is the more common phenolic acid used in CS grafting studies because of its wide distribution and various biological activities (Mahindrakar & Rathod, 2020). Salicylic acid (SA) is a phenolic compound with the related properties of phenolic compounds and also contains active groups such as hydroxyl carboxyl groups on the molecule, but the literature on salicylic acid grafted CS is less studied (He et al., 2011). Due to the novel properties of polyphenol-chitosan complexes, their preparation methods have attracted many scholars to study, so far, there are enzyme-catalyzed polymerization, carbodiimide based coupling method, free radical-induced grafting method and electrochemical methods (Liu et al., 2017). As an emerging modification method, graft modification has been rapidly and widely used in CS modification (Pasanphan & Chirachanchai,

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2008), mainly by introducing some functionalization factors such as natural antibacterial or antioxidant factors on the amino and hydroxyl reaction sites of CS molecules, thus giving new properties to the polymer (Prodpran et al., 2012). Derivatives formed by CS and different biomolecules have received increasing attention in grafting reaction studies (Hu & Luo, 2016), especially polyphenol-chitosan combined with phenolic compounds such as gallic acid (Lunkov et al., 2020), chlorogenic acid (Rui et al., 2017), caffeic acid (Liu et al., 2018) and ferulic acid (Wang et al., 2017), which show potential applications in the biomedical and food industries (Aljawish et al., 2015) .

Apricot (Prunus armeniaca L.) belongs to Rosaceae and Plum. Xinjiang is an important origin and cultivation center of apricot, with rich germplasm resources (Rai et al., 2015) . Samiti apricots, originally produced in Xinjiang, China, are popular among consumers for their high nutritional value and unique sensory quality. However, apricot is a typical respiratory jump fruit that rapidly softens and deteriorates after the respiratory peak, greatly limiting its distribution and shelf life (Liu et al., 2019) . In order to better maintain the quality of apricots after harvest, low temperature storage is often required, but long-term low temperature storage usually leads to cold injury symptoms, such as pulp fibrosis, loss of flavor or internal Browning (Stanley et al., 2013). To reduce postharvest losses, chemical treatments can be used to improve the quality and shelf life of apricots. Apricots can be preserved using a novel edible coating containing chitosan, phenolic acid, protein and other materials, which form a semipermeable protective barrier on the fruits' surface (Cui et al., 2020).

The aim of this study was to prepare CS derivatives with SA (CS-SA) and GA (CS-GA), and characterize their structures. We also evaluated the antioxidant and antimicrobial properties of these two CS derivatives, and investigated their effect on the storage quality of Saimaiti apricots.

2 Materials and methods

2.1 Materials and reagents

We purchased CS (average molecular weight = 130 KDa, degree of deacetylation ≥90%) from Bozhi Huili Biotechnology Co. Ltd. (Qingdao, China). GA, SA, Folin–Ciocalteau reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6)-sulfonic acid (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Maclin Biotechemical Co. Ltd. (Shanghai, China). The bacterial strains *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* were provided by the Microbiology Laboratory of Shihezi University. All other reagents were of analytical grade.

2.2 Synthesis of CS-SA and CS-GA

The synthesis of gallnut and salicylic acid graft CS was performed according to the previous method with modifications (Curcio et al., 2009): Briefly, 1 g of CS was dissolved in 100 mL of 1% acetic acid (v/v) in a 250 mL three-necked round bottom flask. After stirring continuously for 8 h (500 rpm), 4 mL of 1M H_2O_2 and 1ml of 0.3mM Vc were added and constantly stirred for 30 min. Then, phenolic acid (SA or GA) was added and reacted for 24 h. The whole reaction takes place under N_2 protection. After the reaction, the solution was dialyzed using an 8–14 kDa dialysis bag to remove the unreacted phenolic acid, ascorbic acid, and other small molecular compounds. CS derivatives were prepared by freeze-drying after dialysis for 72 h (dialysis water was changed every 12 h).

2.3 Determination of phenolic content

The Folin-Ciocalteau reagent method was used to determine the amount of grafting in chitosan-phenolic acid derivatives (Liu et al., 2008). Briefly, 80 μL of CS derivative (1 mg/mL) was mixed with 400 μL of Folin-Ciocalteau reagent and 2320 μL of distilled water, incubated at 30 °C for 3 minutes, then 1200 μL of Na_2CO_3 (20%, w/v) was added, and the mixture was allowed to stand for 2 h before reading the absorbance at 760 nm. The ratio of the phenolic acid content in chitosan-phenolic acid to the amount of added phenolic acid was determined as the grafting amount, and the grafting rations of CS-SA and CS-GA were expressed as mg of SA equivalents per g (mg SAE/g) and mg of GA equivalents per g (mg GAE/g), respectively.

2.4 Characteristic of CS-GA and CS-SA

To ensure the successful grafting of SA and GA onto CS, the structures of SA- and GA-CS were characterized using UV-visible spectrum, Fourier transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (1H NMR and 13C NMR) spectroscopy. The UV-vis spectra of CS, CS-SA and CS-GA were recorded using an UV-vis spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). FT-IR spectral analysis was performed using a continuous scan FT-IR spectrometer (Nicolet IR200, Thermo, USA) in the frequency range of 4000-400 cm^{-1}, using the KBR-disks method. The samples were dissolved in CF3COOD/ D2O solution (1%, v/v) and 1H NMR analysis was determined by 400 MHz NMR spectrometer (Ascend 400, Bruker, Switzerland). The crystal behavior of phenolic acid -g- cs was determined by X-ray diffraction (XRD) on an X-ray diffractometer at 2θ = 10°-80° on Bruker AXS D8 Advance (Bruker Inc., Germany). Thermogravimetric (TG) and differential thermogravimetric (DTG) analysis of CS-SA and CS-GA was using TGA (NETZSCH STA 449F3, USA) and heating from 25 °C to 800 °C at 10 °C/min increments under nitrogen protection.

2.5 Determination of antioxidant activity in vitro

The antioxidant activities of the conjugates were evaluated using a series of concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL by several methods as follows. The determination of DPPH radical scavenging activity was performed according to the reported literature with modifications (Liu et al., 2010; Wootton-Beard et al., 2011). The ABTS scavenging activity was measured using the method by Liu et al. (2009a). The reducing power and hydroxyl radical scavenging activity was evaluated according to the methods of Liu et al. (2009b) and Zhang (Zhang et al., 2020a), respectively.

2.6 Determination of antibacterial activity

The antibacterial properties of CS derivatives were studied using the inhibition circle method and the minimum inhibitory

concentration. *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* as typical pathogenic bacteria were incubated in a constant temperature incubator at 37 °C for 18h, and then diluted to a live bacterial concentration of 1×10^6-1×10^7 CFU/ mL, respectively. the LB medium was heated and mixed evenly with samples of different concentrations, poured into a sterilized surface dish of 9cm diameter, and after the medium cooled and solidified, 200 μL of each the diluted bacterial solution evenly coated on the surface of the solid medium, the constant temperature incubator to observe the growth of colonies after 24 h, the concentration of cell growth is not visible to the naked eye is defined as the minimum inhibitory concentration. Regarding the inhibition circle, LB medium was heated and poured directly into sterilized Petri dishes, after cooling and solidifying, 200 μL of bacterial solution was evenly applied to the surface of the medium, 3 oxford cups were placed on the Petri dishes, and CS derivative aqueous solution was injected into each oxford cup, and then the petri dishes were placed in a constant temperature incubator at 37 °C for 24h, and the width of the inhibition circle around each sample on the medium was measured with vernier calipers. 0.85% saline was used as the control group.

2.7 In vivo assay

The apricots variety tested in this study was 'Saimaiti' apricot, which was harvested from Kashi, Xinjiang, China, on June 29,2022. Fruits free from diseases and pests, free from mechanical damage, and of uniform size were selected at harvest for the experiment. In this study, these fruits were randomly divided into 5 groups (n = 200/group) and three replicates were used per group. The fruits were immersed in 0.1% CS/CS-GA, 0.5% CS/CS-GA, and distilled water. Distilled water treatment was used as the control group. After treatment, the fruits were dried at 20°C and refrigerated in a freshness store (0 ± 1 °C, 90-95% relative humidity) for a storage period of 35 d. Test sampling and determination of weight loss, hardness, soluble solids content(SSC), titratable acid(TA) (Azam et al., 2021), relative conductivity, and respiration rate were performed every 7 d. Each experiment was performed at least twice.

3 Results and discussion

3.1 Characteristic of phenolic acid-g-CSs

In this study, phenolic acid was successfully grafted onto chitosan by free radical-induced (H_2O_2/Vc) grafting method, and the grafting amount of phenolic acid in CS derivatives was determined by Folin-Ciocalteu method. The grafting amount of CS-GA (112.14 mg GAE/g) was higher than that of CS-SA (83.04 mg GAE/g), and there was no significant difference between this paper and Wu et al. (2016a).

The UV spectra of CS and CS-phenolic acid derivatives were characterized by UV spectrophotometer as shown in Fig.1. CS showed no obvious absorption peaks in the range of 200-500 nm, while the UV absorption spectra of GA and CS-GA and SA and CS-SA were similar, which could tentatively indicate that both have similar chromogenic groups. GA showed two UV absorption peaks at 205 and 258 nm, respectively, where the stronger absorption peak at 258 nm was due to the edge of the benzene ring of GA and the weaker absorption peak at 205 nm, which was related to the carboxyl group in GA. The CS-GA showed two absorption peaks at 212 and 261 nm, respectively, presumably due to the possible presence of the benzene ring. These phenomena suggest a reaction between CS and GA. This has a slight similarity to previous studies (Wu et al., 2016). SA has a strong absorption peak at 298 nm, and the CS-SA derivative has a broad absorption band at 230 and 298, indicating that CS has grafted on SA (He et al., 2011).

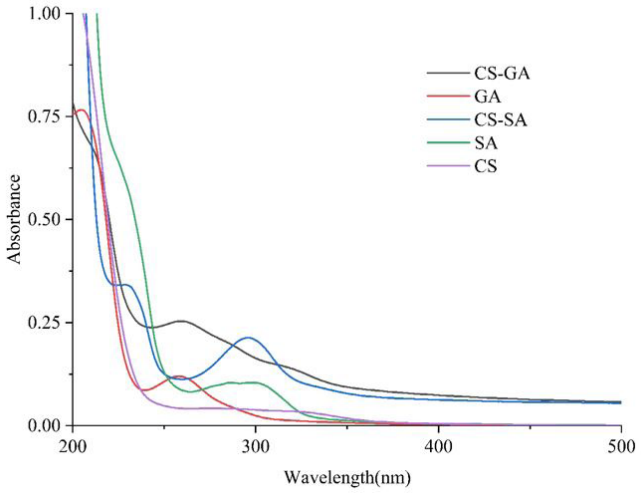

Figure 1. (A) UV–vis spectra of CS, GA, SA, CS-GA and CS-SA.

The FT-IR spectra of CS and CS-phenolic acid derivatives are shown in Figure 2A. CS has a strong absorption band at about 3400 cm^{-1} attributable to O-H and N-H stretching, with absorption bands at 1658, 1590, and 1323cm^{-1}, respectively, for C=O stretching of residual N-acetyl groups (amide I), N-H bending (amide II), and C-N stretching (amide III), typical of the amide bond of several peaks, the 1590 cm^{-1} band corresponds to the N-H bending of the primary amine (Lian et al., 2022). At 1421 cm^{-1} and 1379 cm^{-1} can be attributed to CH_2 bending and CH_3 symmetric deformation, respectively. The absorption band is located at 1157 cm^{-1} (C=O=C bridge asymmetric stretching) and 1089 and 1031 cm^{-1} (C=O stretching) are characteristic of its sugar structure (Lim & Hudson, 2004). In contrast, the graft product monoamide has a reduced N-H bend at 1590 cm-1, indicating a transition from primary to secondary amide, and the covalent coupling reaction may occur at the -NH_2 site of CS.

The crystal structures of CS and CS-phenolic acid derivatives were determined by XRD, and as shown in the Figure 2B. CS showed the main peaks associated with crystal form I crystal form II at 2θ≈11.4° and 20.28°, respectively. The crystal morphology of CS is mainly formed by inter- and intramolecular hydrogen bonding arising from hydroxyl and amino groups, however, after the introduction of phenolic acid, the derivatives show wider and weaker peaks, which may be due to the fact that the grafted phenolic acid weakens or breaks the original hydrogen bonding of CS and reduces the crystallinity of CS. Notably, the main peaks of CS-phenolic acid derivatives at 21.67 and 21.55 also verified the results of the reaction of SA and GA with CS.A

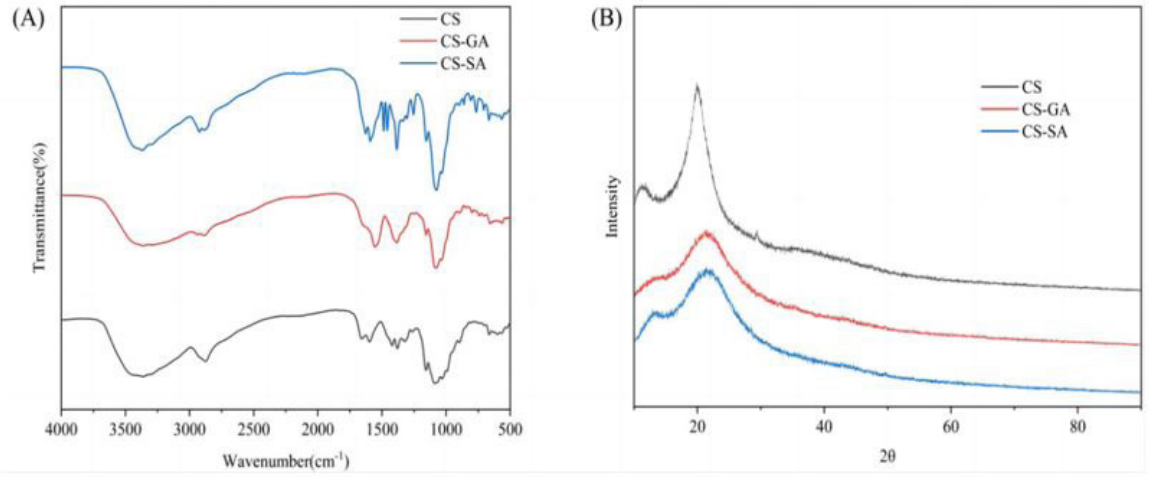

Figure 2. (A) FT-IR spectra and (B) X-RD spectra of CS, CS-GA and CS-SA.

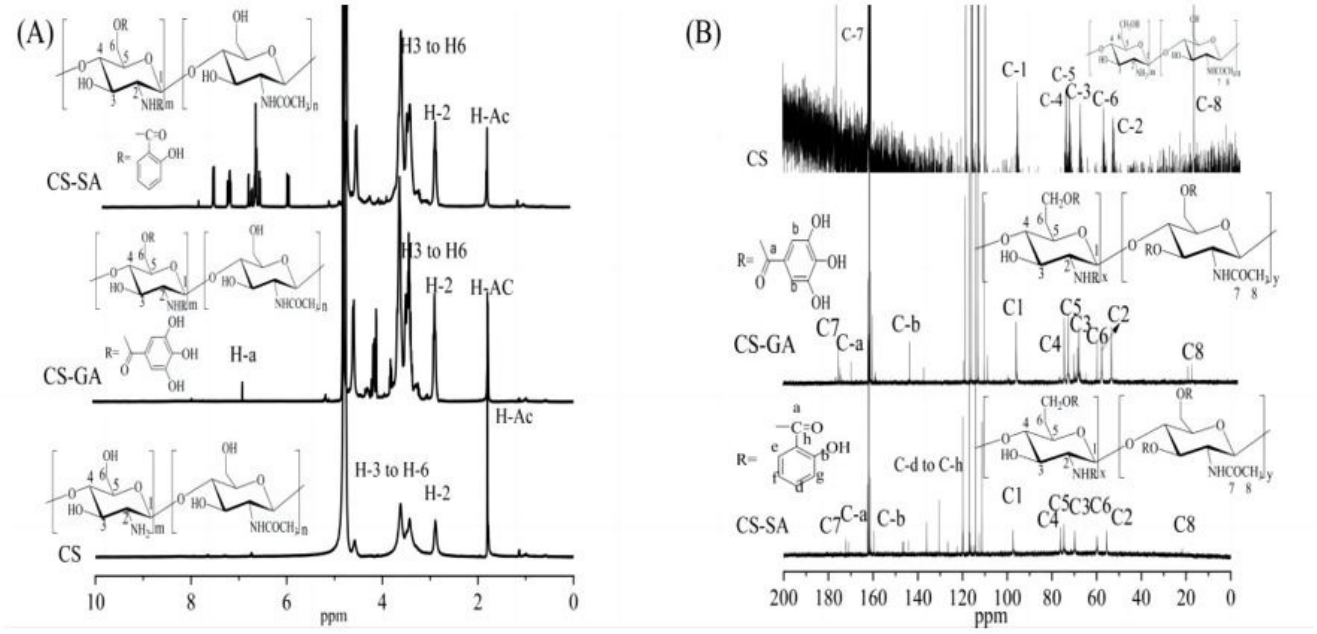

Figure 3. (A) 1H NMR spectra and (B) 13C NMR spectra of CS, CS-GA and CS-SA.

similar phenomenon has been reported previously, but with slight differences (Wang et al., 2019) .

To further verify the skeletal structure of CS and its derivatives, ^{1}H NMR and ^{13}C NMR of CS and its derivatives were analyzed. The results are shown in Figure 3. The pure CS showed typical H-2 absorption peaks at 1.95 ppm and 2.93 ppm, the peak around 2.0 ppm showed the hydrogen protons in the n -acetyl residue, and the multiple peaks at 3.5-3.9 ppm were the signal peaks of H-3, H-4, H-5 and H-6. Both derivatives retain the specific absorption peaks of CS, while a new absorption peak (caused by protonation of the benzene ring on the phenolic acid chain) appears around 7.00 ppm for both derivatives (He et al., 2011; Xie et al., 2014). These results indicate that SA and GA have been successfully grafted onto the CS molecular chain. From the ^{13}C NMR spectrum, it can be seen that CS exhibits typical structural features, with C-2, C-6, C-3, C-5, C-4 and C-1 signal peaks at 55.58, 59.86, 69.59, 74.33, 76.20 and 97.36 ppm, respectively. the carbonyl (-C = O) and methyl (-CH3) of the CS molecular chain acetylglucosamine signal peaks belonged to C-7 and C-8, they appeared at 176.47 and 20.19 ppm, respectively (Liu et al., 2013a). Compared with the C spectrum of pure CS, some signal intensities on the molecular backbone of the derivatives changed to different degrees, and also new signal peaks appeared. the carbon spectra of the derivatives all showed new signal peaks at around 145 ppm, corresponding to the C = C bond in phenolic acid, thus inferring that the phenolic acid was successfully grafted with CS.

The thermal properties of CS and derivatives were analyzed using TG and DTG, and the results are shown in Figure 4. In the TG analysis, CS and derivatives showed similar behavior, i.e., there were two stages of weight loss. The first stage has a small weight loss, mainly residues and bound water on the material. The second phase of major weight loss is due to the decomposition of the polymer (Zhang et al., 2020b). In this case, the second stage of CS starts at 271.1 °C and can be considered as the initial temperature of CS decomposition. In comparison, the initial temperature of decomposition of CS-SA is 215.7 °C and the initial temperature of decomposition of CS-GA is only 168.3 °C. Both showed a significant decrease in the decomposition onset temperature, indicating that the thermal stability of the derivatives was lower than that of CS. The stability of polymers is generally related to their inter- and intramolecular hydrogen bonds. The lower thermal stability of derivatives may be due to the above-mentioned disruption of inter- and intramolecular hydrogen bonds, and also indicates that derivatives are more susceptible to degradation than CS. In the DTG analysis, it was seen that the fastest rate of mass depletion of CS was 12.27% at 303.7 °C, the fastest rate of mass decomposition of CS-GA derivative was 4.94% at 282.9 °C and the fastest rate of mass decomposition of CS-SA derivative was 5.25% at 248.3 °C, which corresponded to the three temperature intervals where the substances lost the most weight, respectively.

3.2 Evaluation of antioxidant activity in vitro

Antioxidant activity is an important parameter to evaluate the biological activity of chitosan/phenolic acid graft copolymers. ABTS radical scavenging assay, DPPH radical scavenging assay, hydroxyl radical scavenging assay and reducing ability were used to determine the antioxidant capacity of chitosan and its graft products, and the results are shown in Figure 5. It is obvious that the antioxidant properties of the graft products showed a certain concentration dependence after the grafting reaction, i.e., the scavenging effect increased with increasing concentration, and the antioxidant activities of both derivatives were significantly higher than those of chitosan, and the difference between the scavenging ability of GA derivatives for ABTS radicals (97.55 ± 0.13%) and ascorbic acid at a concentration of 2.0 mg/mL was was negligible. This phenomenon is caused by the phenolic acid substitution in the graft copolymerization reaction, and the antioxidant activity of phenolic acid is related to the number and distribution of phenolic hydroxyl groups and the subclasses of phenolic acid, so polymers with high phenolic acid substitution can have high antioxidant activity at certain concentrations (Sroka & Cisowski, 2003). In general, the antioxidant activity of CS-GA was significantly better than that of CS-SA as well as CS at the same concentration.

3.3 Antimicrobial activity

CS has good antibacterial activity, non-toxicity and excellent physicochemical properties, therefore, CS has been widely used in the field of antibacterial applications. CS and its derivatives have antibacterial activity against fungi, Gram-positive bacteria and Gram-negative bacteria (Li & Zhuang, 2020). Bacterial inhibition analysis of chitosan and graft products was performed and the results are as follows. As shown in the Figure 6, the inhibitory effect of chitosan and its derivatives on *E. coli, S. aureus* and *B. subtilis* increased with increasing concentration, showing a certain concentration dependence, and the inhibition effect of both derivatives on bacteria was stronger than that of CS, and the diameter of the inhibition circle of CS-GA was larger than that of CS-SA at the same concentration. It was also found that chitosan and derivatives had the strongest inhibitory ability against *S. aureus*, followed by *E. coli* and *B. subtilis*. As shown in the Table 1, the lowest inhibitory concentration of CS-GA against *E. coli*, *S. aureus* and *B. subtilis* was 4.0 1.0 2.0 mg/mL respectively, which were significantly lower than CS, and in contrast, CS-SA were significantly less effective than CS-GA.

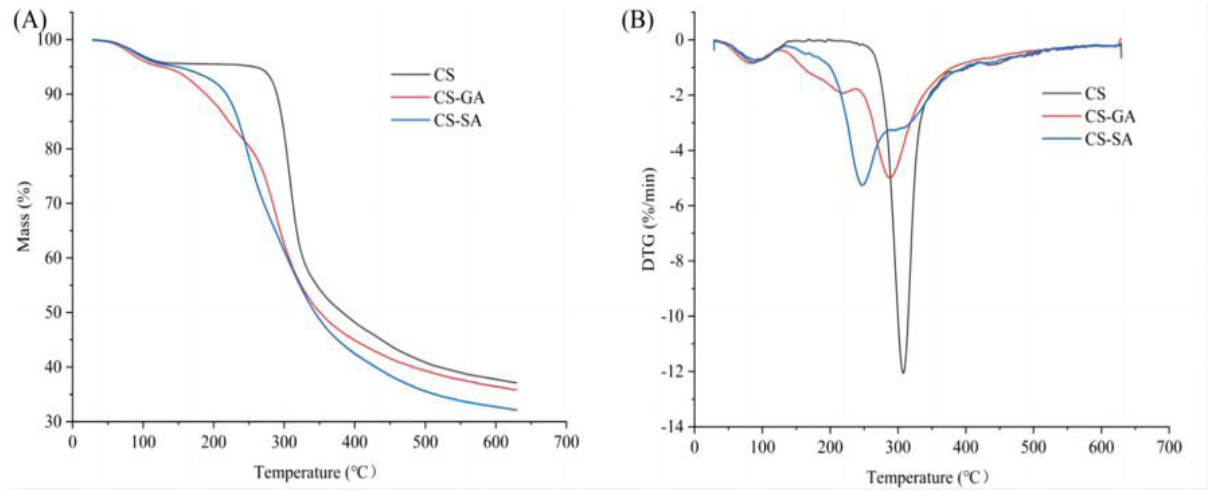

Figure 4. (A) TG and (B) DTG curves for CS, CS-GA and CS-SA.

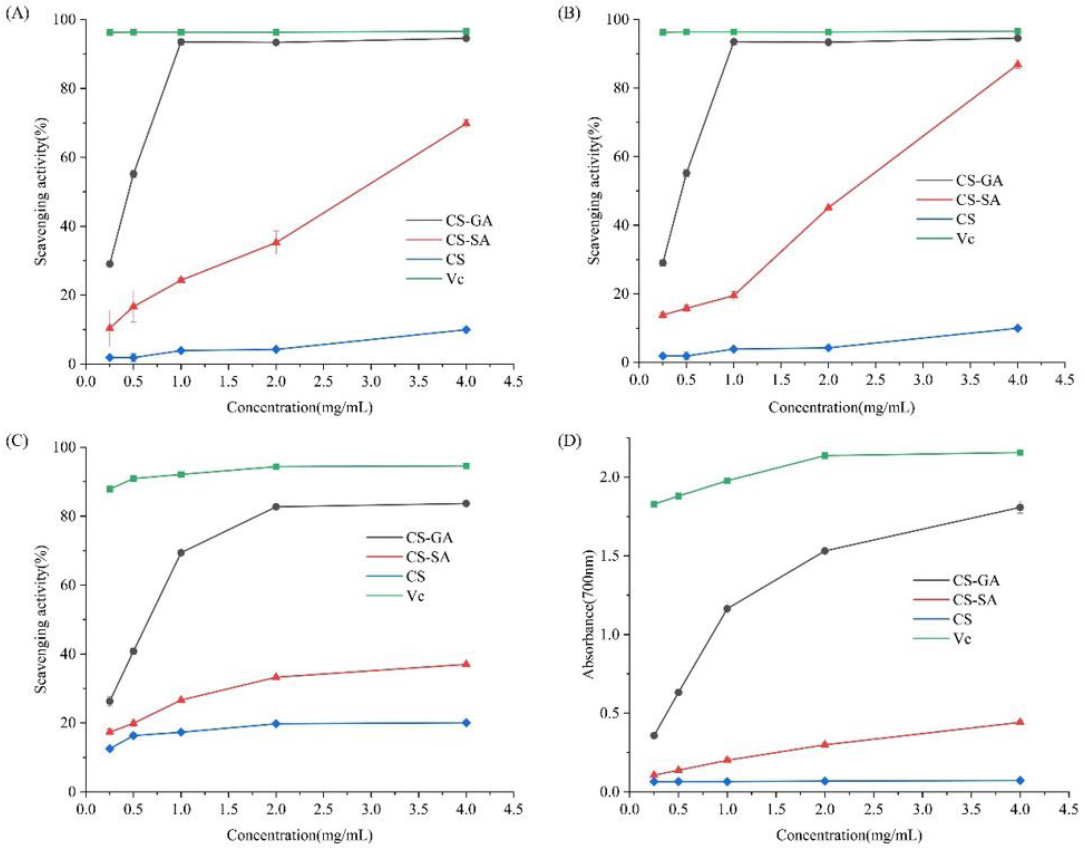

Figure 5. Antioxidant assay (A) ABTS (B) DPPH (C) hydroxyl radical and (D) reducing ability The ABTS radical (A), DPPH radical (B), hydroxyl radical (C), and reducing power (D) of CS (♦), CS-GA (•), CS-SA (▲) and Vc (▪). Data are presented as means ± SD of triplicates.

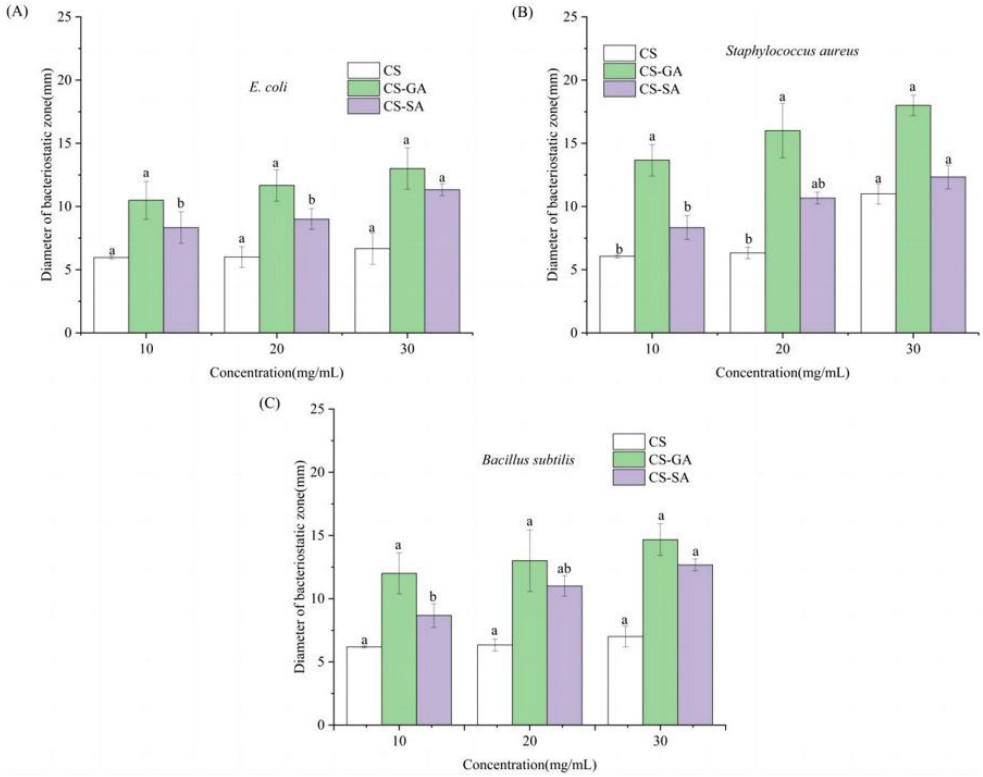

Figure 6. Inhibitory zone of CS, CS-GA and CS-SA. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

Table 1. Minimum inhibitory concentration (MIC) of CS, CS-GA and CS-GA.

Bacterial strain	Sample	Concentration(mg/mL)					
		0.125	0.25	0.5	1.0	2.0	4.0
E.coli	CS-GA	+	+	+	+	+	-
	CS-SA	+	+	+	+	+	+
	CS	+	+	+	+	+	+
Staphylococcus aureus	CS-GA	+	+	+	-	-	-
	CS-SA	+	+	+	-	-	-
	CS	+	+	+	+	+	+
Bacillus subtilis	CS-GA	+	+	+	+	-	-
	CS-SA	+	+	+	+	+	-
	CS	+	+	+	+	+	+

Note: "-" means no colony growth, "+" means colony growth.

3.4 In vivo assay

Weight loss affects the appearance and quality of the fruit and is an undesirable phenomenon (Anastasiou et al., 2014). Figure 7A shows the weight loss rate of apricot fruits under different treatments, from which we can see that the weight loss rate of apricot fruits showed an increasing trend, which gradually increased with the storage time throughout the storage process. During the storage period, the weight loss rate of apricot fruits in all treatment groups was significantly lower than that of the control group. In the early stage of storage, the weight loss rates of the treatment groups were not significantly different ($P > 0.05$). At 35 days of refrigeration, the weight loss rates in the CS-0.1%, CS-0.5%, CS-GA-0.1%, and CA-GA-0.5% treatment groups were 7.05%, 6.05%, 5.13%, and 3.98%, significantly lower than the control group ($P < 0.05$). This indicates that the weight loss of refrigerated apricots was suppressed by the effect of CS and CS-GA.

Firmness is the main basis for judging the texture of apricot fruit, and it decreases continuously as the fruit ages (Anastasiou et al., 2014). As shown in Figure 7B, the hardness of apricot fruit tended to decrease during storage. At the beginning of the experiment, the fruit hardness was 24.9 ± 0.39 N, which decreased to 11.07 ± 0.39 N in the control fruit treated with distilled water after 35 days of refrigeration. the hardness of chitosan and chitosan derivatives-treated apricots also decreased during storage, but these treatment groups were significantly ($p < 0.05$) more effective in maintaining fruit hardness than the control group. At the end of the storage period, the fruit hardness of CS-0.5% treatment was 13.79 ± 0.15 N 1.24 times higher than that of the control, and the highest hardness of CS-GA-0.5% treatment was 16.54 ± 0.68 N 1.49 times higher than that of the control. Thus, CS-CGA maintained relatively higher fruit hardness compared to the same concentration of CS.

TSS is a group of soluble compounds, including sugars, acids and vitamins, which are also the main substrates of respiration. As shown in Figure 7C, the TSS of apricot fruit in each treatment group increased and then decreased with the extension of storage period. During the first 0-14 d of storage, the soluble solids of apricot fruits in the control group were significantly higher than those in the other three treatment groups, and the TSS of apricot fruits in the control group reached a maximum value of 15.30% at 14 d. This indicates that the TSS can be reduced by CS-GA. This indicates that TSS can be maintained at a certain level by CS-GA. TSS content is an indicator of ripeness and fruit ripening, and our results showed that edible coatings slowed the ripening process, consistent with the findings of Gol et al. (2013). There is evidence that the film-forming properties of chitosan create an excellent semi-permeable membrane around the fruit, which alters the internal atmosphere by reducing O_2 and/or elevating CO_2, inhibiting ethylene production and reducing respiration rates (Yaman & Bayoındırlı, 2002) .

The changes of titratable acid (TA) in apricot fruits are shown in Figure 7D. TA showed a decreasing trend during storage, where TA in control fruits decreased the fastest, from the initial 1.14% to 0.44% at 35 d of storage, while the treatment group decreased slowly, while CS-0.5% and CS-GA-0.5% treatment groups decreased the most slowly, with TA at day 35 of storage being 0.49% and 0.55%, respectively, at day 35 of storage. The gradual decrease in TA content during storage may be due to the consumption of organic acids during fruit respiration, while CS-GA coated fruits could maintain relatively high TA content probably related to the lower respiration rate. Therefore, GA-g-CS treatment effectively delayed the decline of TSS and TA during storage. The higher the TSS and TA, the higher the acceptability quality. This result is supported by previous studies (Shi et al., 2018).

Respiration rate is an important indicator of fruit ripening and senescence. The respiration rate reflects the nutrient and energy consumption of the fruit (Giménez et al., 2016) .The changes in the respiration rate of the fruits during storage are shown in Figure 7E. The respiration rate of the control fruit showed an increasing trend from day 7 to 14 of storage and peaked at day 14, followed by a decrease. The treatment group significantly suppressed the increase in respiration rate and delayed the peak of respiration until 28 days, which was significantly lower than that of the control group ($p < 0.05$). The trend of respiration rate in CS-treated fruits was the same as that of CS-GA, which reached the peak of respiration at 28 days, but was significantly higher than that of CS-GA group ($p < 0.05$). Therefore, the CS-GA group could effectively reduce the respiration rate during fruit storage and could delay the arrival of the respiration peak. As can be seen from Figure 7F, the relative conductivity of apricot fruits showed a gradual increase with the increase of storage time under low temperature storage. The relative conductivity of apricot fruits was the smallest at the 7th day of storage and peaked at the end of storage, reaching 49.53 ± 0.58 in the control group and 25.20 ± 0.28 in the CS-GA-0.5% treatment group at 35 days of storage, which showed that CS-GA treatment could reduce the relative conductivity of fruits.

In view of the above experimental results, we conclude that CS-GA have good preservation effect, which provides a new idea for fruit preservation in Xinjiang. If conditions permit, we will cooperate with local fruit farmers and fruit supermarkets for practical application.

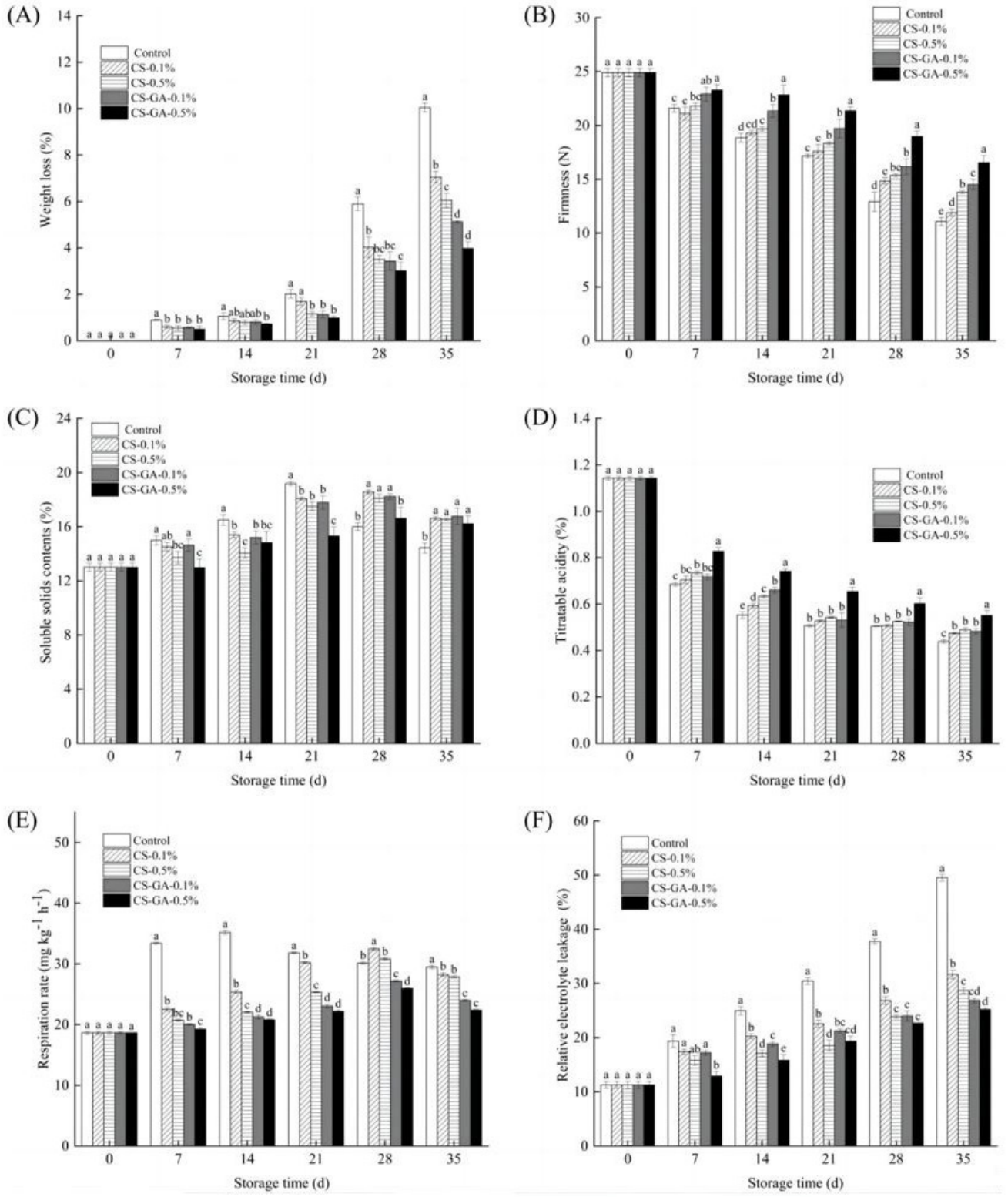

Figure 7. Effects of CS and CS-GA treatment on weight loss (A), firmness (B), SSC (C), TA (D), respiration rate (E) and relative conductivity (F) in apricot fruit. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

4 Conclusions

In this study, two phenolic acids were successfully grafted with chitosan by free radical induction and two chitosan-phenolic acid couples were synthesized. It was shown by sample characterization that the phenolic acids have been grafted onto chitosan and the CS-GA were more substituted than the CS-

SA. on the other hand, both chitosan-phenolic acid derivatives had stronger biological activities than chitosan after grafting compared to chitosan, but the comparison of the two derivatives clearly showed that the CS-GA had better antioxidant and antibacterial properties, which indicated that that CS-GA have better bioactivity. Therefore, in vivo experiments using CS-GA in fruit preservation demonstrated that CS-GA could better maintain the hardness, SSC, and TA of apricot fruits, and also significantly inhibit the weight loss rate of apricot. respiration rate and relative electrical conductivity. These results suggest that CS-GA is expected to develop new application prospects in the food industry in the future.

Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

All the data of the study can be obtained in this manuscript.

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Author contributions

Formal analysis, writing—original draft preparation, Y.Y.; supervision, writing—review and editing, J.C.; validation, F.L., J.S.,Y.H., F.S. , Z.H and .C.G; At the same time, Mr. Gu Chengzhi provides us with a chemistry laboratory. All authors have read and agreed to the published version of the manuscript.

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